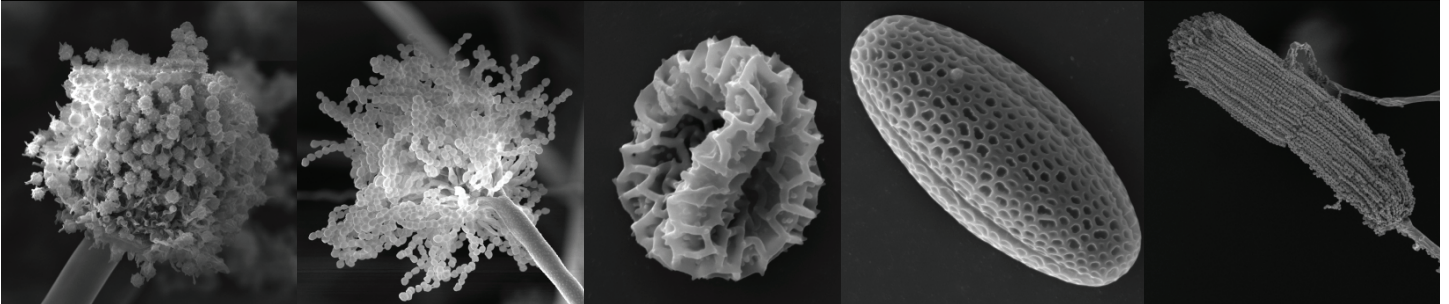
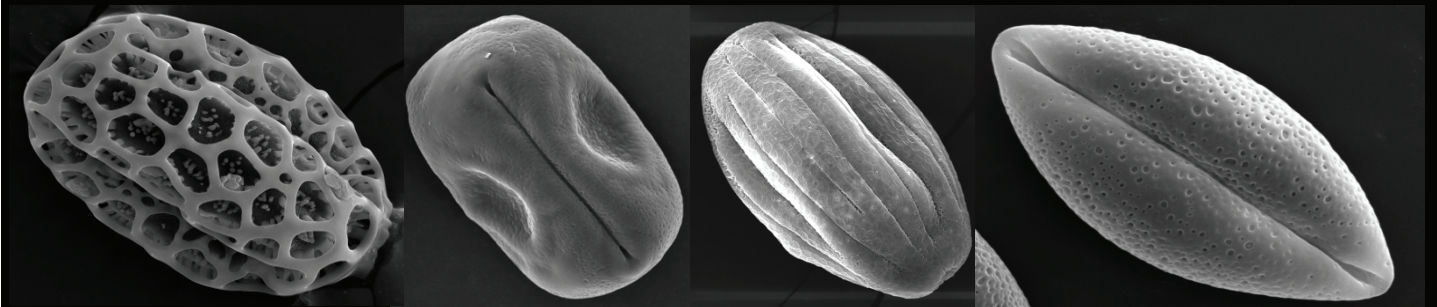


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DETECTION AND RECOGNITION OF AIRBORNE POLLEN GRAINS AND FUNGAL SPORES USING ARTIFICIAL INTELLIGENCE

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Airborne fungal spores are also of interest because of their potential to affect the health of both plants and human beings. So, it is of utmost importance to detect these agents in the air, in order to warn patients for any allergic infections and farmers for plant diseases. The existing methods to detect these agents in the air involves sampling and observing them manually which is time consuming and labour intensive. So, we proposed to perform their detection automatically with minimal human intervention to make it effective as it is not feasible to engage human experts for these tasks.

In this study, our team proposed an automated method to detect pollen grains and fungal spores in the air. This method would be realised using IoT based hardware and machine learning technology. Raspberry Pi High Quality Camera will be utilised to capture the microscopic images by mounting it on top of a microscope. It has 12 megapixels and greater sensitivity (approximately 50% greater area per pixel for improved low-light performance) than the existing Camera Module v2 and is designed to work with interchangeable lenses in both C- and CS-mount form factors in order to consolidate datasets containing pollen grains and fungal spores samples. We train three different Neural Network Models (NNM) to achieve three different tasks viz. 1. Classifying a sample into fungal spores or pollen grains, 2. Detecting the type of pollen grains, and 3. Detecting the type of fungal spores.

Custom Convolutional Neural Network architectures can be designed or utilised through existing good architectures such as YOLO v3, ResNet, etc. AlexNet can be utilised for the classification task and ResNet for the detection task. Samples running for five fungal spore types viz. *Cladosporium*, *Verticillium*, *Mucor*, *Rhizopus* and *Helminthosporium* can be performed to detect and identify the type of fungal spore with accuracy of 90 to 95%. Similarly, pollen grain types viz. *Brassica*, *Cannabis*, *Parthenium*, *Ricinus* and *Callistemon* can be run to detect and identify type with accuracy of 90 to 95% which can also be improved upto 99%. The technique used in our study were able to successfully segregate these samples into their respective classes with commendable accuracy. It is further proposed to deploy the present models in an edge system eliminating the need to send samples to the cloud system for further analysis. Jetson Nano Developer kit was used to run the models for prediction. It delivers the compute performance to run modern AI workloads at unprecedented size, power and cost. The present method will significantly reduce the cost and effort required to realise this task with very little sacrifice in the prediction accuracy. The System can detect and recognize more pollen grains and fungal spores if we provide more data of pollen grains and fungal spores to the present AI System.

Key Words: Automatic Identification of Pollen Grains and Fungal Spores, IoT Based Hardware, Raspberry Pi High Quality Camera, Convolutional Neural Network (CNN), Jetson Nano Developer Kit.

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INTRODUCTION

Artificial Intelligence and Automation

Artificial intelligence (AI) refers to the simulation of human intelligence in machines that are programmed to think like humans and mimic their actions. Traditionally, AI focused on making the machines smart by optimizing search methods in the solution space. With the increase in volume of data generation and powerful computation methods, AI is taking new form which is data centric in nature. Now, it tries to find an optimal solution of a problem by leveraging the data samples present in the solution space of that problem. Recently, Deep Learning is gaining popularity in AI which uses Neural Network and its variants such as Convolutional Neural Network and Recurrent Neural Network to solve

complex solutions such as automatic language translation and autonomous driving.

Computer Vision

It is the field of artificial intelligence that enables computers to derive meaningful information from digital images, videos and other visual inputs and take actions according to those information. It uses various image processing algorithms as well as convolutional neural network to perform tasks such as object detection, facial recognition, etc.

Convolutional Neural Network

A Convolutional Neural Network (ConvNet/CNN) is a Deep Learning algorithm which can take in an input image, assign importance (learnable weights and biases)

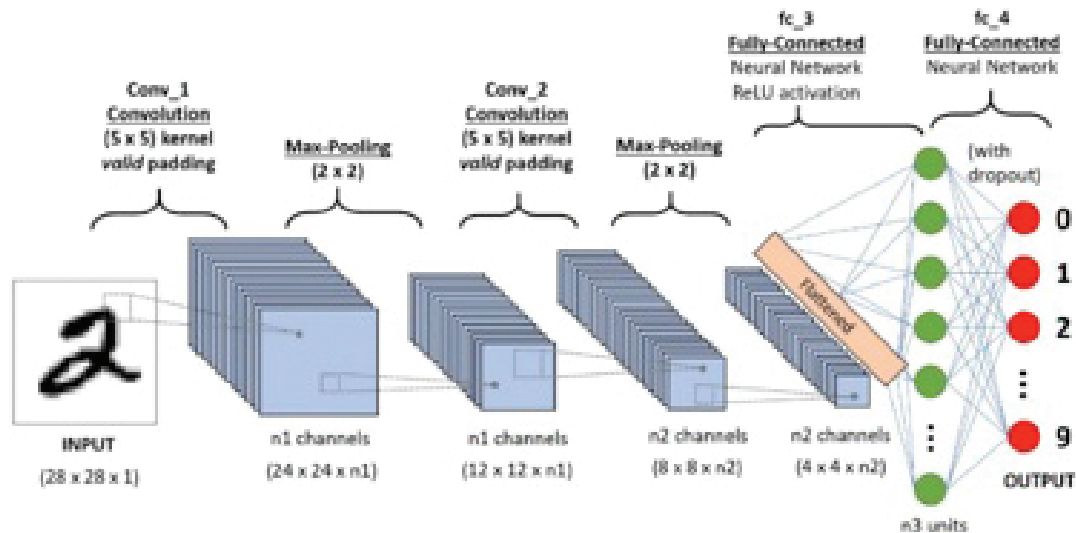


Fig. 1.1: A CNN (Convolutional Neural Network) sequence to classify handwritten digits. (Towards Data Science).

to various aspects/objects in the image and be able to differentiate one from the other (Fig. 1.1). The pre-processing required in a ConvNet is much lower as compared to other classification algorithms. While in primitive methods filters are hand-engineered, with enough training, ConvNets have the ability to learn these filters/characteristics.

The architecture of a ConvNet is analogous to that of the connectivity pattern of Neurons in the Human Brain and was inspired by the organization of the Visual Cortex. Individual neurons respond to stimuli only in a restricted region of the visual field known as the Receptive Field. A collection of such fields overlaps to cover the entire visual area.

Edge AI computing

It is the task of deploying AI software on edge devices like microcontrollers which has low computational power instead of deploying them on large servers.

Advantages of Edge AI computing

1. **Data Security and Privacy:** Since data processing occurs at the edge devices, the need to send them on servers is omitted making it less vulnerable for hackers to access the data. This increases data security and privacy.
2. **Real time analytics:** With the need to send data to servers omitted, the data is processed in the edge itself thus reducing latency in the processing.
3. **Lower internet bandwidth:** Since processing occurs

at edge, fewer data are sent to the servers thus reducing the bandwidth consumption.

4. **Low power consumption:** Processing occurs at edge thus removing the need to send data at servers. This saves energy thus reducing the power consumption.
5. **Better responsiveness:** Edge AI solution increases the response rate of smart devices as there is no need to send data to cloud for computation.

Detection and Recognition of Airborne Pollen Grain and Fungal Spore using AI

Air quality and composition significantly influence human health, with specific constituents such as bioaerosols (i.e., airborne pollen grains and fungal spores) comprising a dire environmental agent often held responsible for a variety of allergic diseases^{1,2}. Hence, there have been approximately 400 monitoring networks across the world that have traditionally measured the abundance and occurrence of pollen grains and fungal spores³, mainly aiming to inform allergic individuals and medical practitioners. While the above has been taking place for several decades, the methodological approach of Hirst-type volumetric sampling is practically 70 years old⁴. On the other hand, it is currently considered as the ‘gold-standard’ of biomonitoring, with the occurring pollen and spore concentration datasets comprising some of the longest and most valuable biological time-series in the world. Such data have repeatedly contributed to identification long-term trends in abundances, alterations in biodiversity of

fungi and plants, and quantification impacts of climate change^{5,6}. During the recent COVID-19 pandemic, the importance of such monitoring networks has been highlighted even more, given that higher airborne pollen concentrations have been proven to be positively correlated with higher numbers of SARS-CoV-2 infections⁷.

Despite the above mentioned increase, need for longer time-series, higher-resolution real-time data, upto now biomonitoring of airborne pollen and fungal spores is still conducted with labour intensive, temporally low-resolution measurements, which usually deliver measurements with a delay of at least a week. Nonetheless, it is well known⁸, that there is an immediate, and the strongest, effect of airborne pollen grains on the same day as the actual exposure, when the most intense respiratory symptoms are observed having the strongest immune reactions. Any delay in the dissemination of pollen grain exposure information beyond a day is, as a matter of fact, considered obsolete in terms of practical allergy management and medical treatment. Concentration–response associations are essential to understand when and how much pollen concentration will cause significant health risks to the population, and previous evidence suggests that these interrelations are complex, non-linear and taxon-specific^{9,10}. This variability highlights the necessity for research across different pollen taxa and locations to provide consistent and locally significant evidence on the clinical relevance of pollen data and forecasts.

While the techniques followed in Hirst⁴, to the best of their ability, attempt to simulate a normal respiratory function of a human in calm conditions (10 L min⁻¹), this might be far from the real-life situation: in at least two thirds of our lives we work, walk, converse, laugh, eat or exercise, which altogether immediately increase this rate to much higher levels. Moreover, this kind of traditional low-volume sampling technique may reduce the accuracy of concentration estimates for rarer particles. This practically means that we may still be far from the assessment of the genuine exposome and even farther from the definition of concentration thresholds for pollen grains and fungal spores, the quantification of the allergic (among others) reactome and symptomatology, and the optimal management of bioaerosol-associated diseases.

Having said this, the most consistent obstacle to the

technological advancement of bioaerosol research and biomonitoring techniques has been, in contrast to the detection of chemical pollutants, the lack of continuous public funding (if at all). For this reason, even though bioaerosol time-series are some of the longest modern biological data-series worldwide, they are usually not open-access, as they reflect the effort and expenses of research institutes, universities and of private initiatives. Therefore, an urgent switch is needed towards faster, online and more accurate reporting of airborne pollen and fungal spore concentrations, a requirement repeatedly noted as a hot topic in the aerobiological community^{11,12}.

Recent efforts have focused on developing automated sampling devices in real time, which have been developed on the borderline of the sciences of microbiology, engineering and informatics. Such approaches have already improved the efficiency of particle capturing and recognition, as well as data flow and dissemination, with results obtained allowing for larger-scale ecological interpretations. These new techniques refer to air-flow cytometry, such as the Yamatronics KH-3000¹³, Plair PA-300^{14,15}, Pollen SenseTM¹⁶ or the WIBS sensor¹⁷. DNA metabarcoding using trnL and nrITS2 have also shown highly improved taxonomic resolution for pollen from aerobiological samples^{18,19}, even though not at a (near-) real-time temporal resolution. Still, there is a huge gap in the application of automation techniques regarding other bioaerosols, i.e., fungi²⁰ or bacteria²¹, as well as development of such methods in indoor environments, as has been traditionally elaborated^{20,21}.

Apart from these recent advances in biomonitoring systems, palynology (including aerobiology) traditionally relies on image-based identification of particles²². Hence, automating this process has attracted attention for more than a decade now and is still one of the central pillars of technological progress^{3,23}. The high-throughput flow that, in any case, improved the efficiency of these devices compared to the conventional Hirst-type, has been complemented by automated microscopy for particle identification per pollen/spore type assisted with the use of image recognition algorithms²⁴⁻²⁷. If, to the above, advanced deep learning methods are integrated, the results already seem outstanding²⁸⁻³¹.

The first and most prevalent of such biomonitoring systems, based on automatic image recognition, is the

BAA500 (Hund GmbH), established and running fully operationally in Bavaria, Germany, which has reported significant preliminary performance results when using pollen recognition algorithms²⁶. It has been reported that more than 90% of the pollen identified was correctly recognized²⁶. However, under such rapid technological advancements, there are some common pitfalls at the beginning, such as reliability of the devices after longer-term monitoring and experimental repetitions, comparability of techniques and datasets, and reporting biases.

METHODOLOGY

HARDWARE USED:

Jetson-Nano-Developer-Kit

The NVIDIA Jetson Nano Developer Kit (Fig. 1.2) delivers the compute performance to run modern AI workloads at unprecedented size, power, and cost. The developer kit can be powered by micro-USB and comes with extensive I/Os, ranging from GPIO to CSI. This makes it simple for developers to connect a diverse set of new sensors to enable a variety of AI applications. It is incredibly power-efficient, consuming as little as 5 watts by Nvidia.



Fig. 1.2: Image of Jetson-Nano-Developer-Kit

Jetson Nano (Fig. 1.2) is also supported by NVIDIA Jet-Pack, which includes a board support package (BSP), Linux OS, NVIDIA CUDA, cuDNN, and TensorRT

software libraries for deep learning, computer vision, GPU computing, multimedia processing, and much more.

Raspberry Pi High Quality Camera

The Raspberry Pi High Quality Camera (Fig. 1.3) is a camera accessory by Raspberry Pi. It offers higher resolution (12 megapixels, compared to 8 megapixels), and sensitivity (approximately 50% greater area per pixel for improved low-light performance) than the existing Camera Module v2, and is designed to work with interchangeable lenses in both C- and CS-mount form factors. Other lens form factors can be accommodated using third-party lens adapters.



Fig. 1.3: Image of Raspberry Pi High Quality Camera

Hardware Working

Our Solution Consist of 4 parts of Hardware that is Microscope, Camera, 3D printed Camera mount and a microcomputer. The microscope will be used to help in capturing samples of image with the help of a Camera that is mounted on a microscope with the help of custom 3D printed Camera mount. The captured image is sent to a microcomputer where our ML Model is trained to detect and recognize pollen grains and fungal Spores. Then the result will be sent through our IoT platform through Wi-Fi or Ethernet (Fig. 1.4).

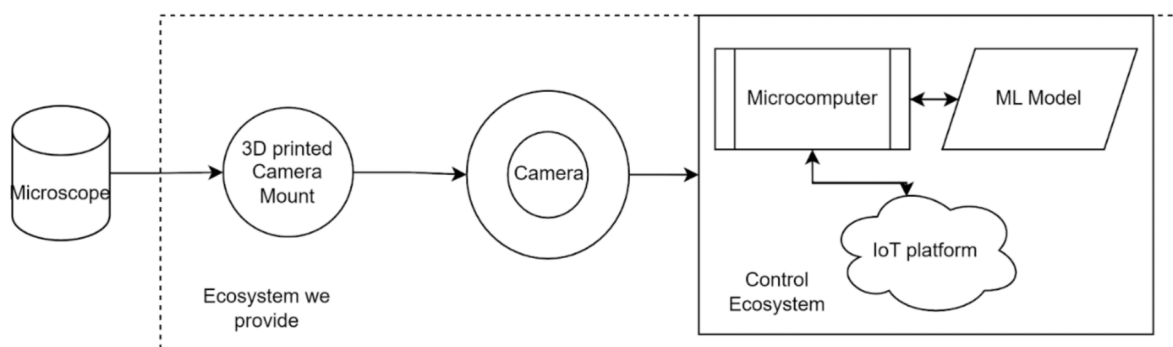


Fig. 1.4: Hardware Flow Diagram

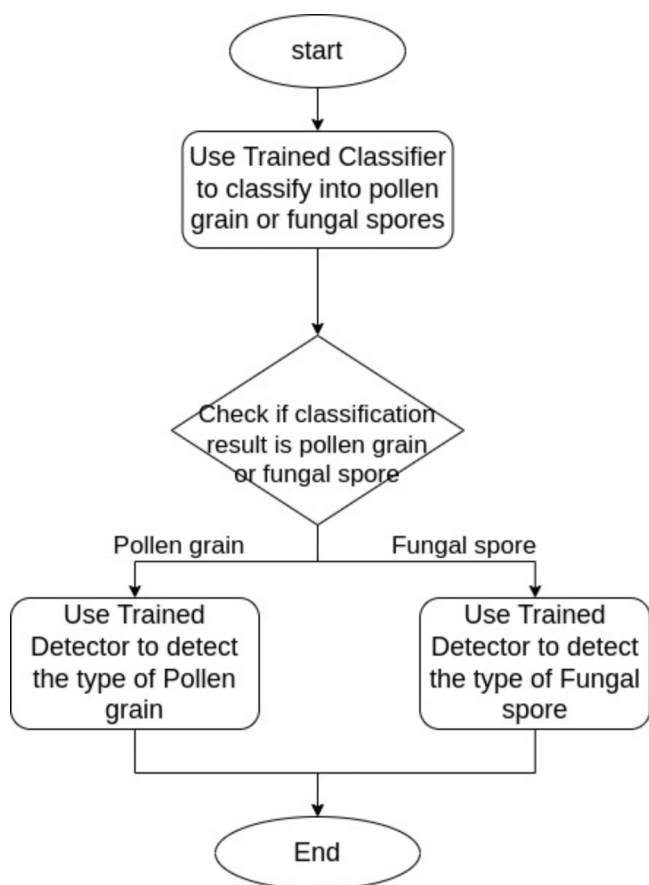


Fig. 1.5: Flowchart of the present software system

Software Working

The solution involves training three different neural network models. The first model will classify an image sample into either pollen grain or fungal spore. Based on the classification of this model, the other two models will determine the type of pollen grain or fungal spore

respectively. The workflow of the present model has been given in Fig. 1.5.

Data collection

The entire workflow involves training three different neural network models for three different purposes. For the neural network to classify fungal spore and pollen grain samples collections were made from various publicly available datasets^{32,33} and compiled dataset for training and validation purposes. These samples need to be further pre-processed to make them uniform for training and testing purposes. If the sample size is unsatisfactory, additional samples via augmentation can be generated.

For detection of types of pollen grain and fungal spore, there are some publicly available datasets. But further populate the dataset with samples collected and annotated manually. After collecting enough samples for each pollen grain and fungal type, pre-processing and augmentation can be performed on them to create database. The sample collected manually will be less, so it is very important to augment the dataset properly. Moreover, care must be taken while annotating the samples, as mis-sampling will lead to high bias during training.

AI Framework Used

The model is trained using an AI framework called Teachable Machine. It is a web tool that makes it easy and fast to create machine learning models without the

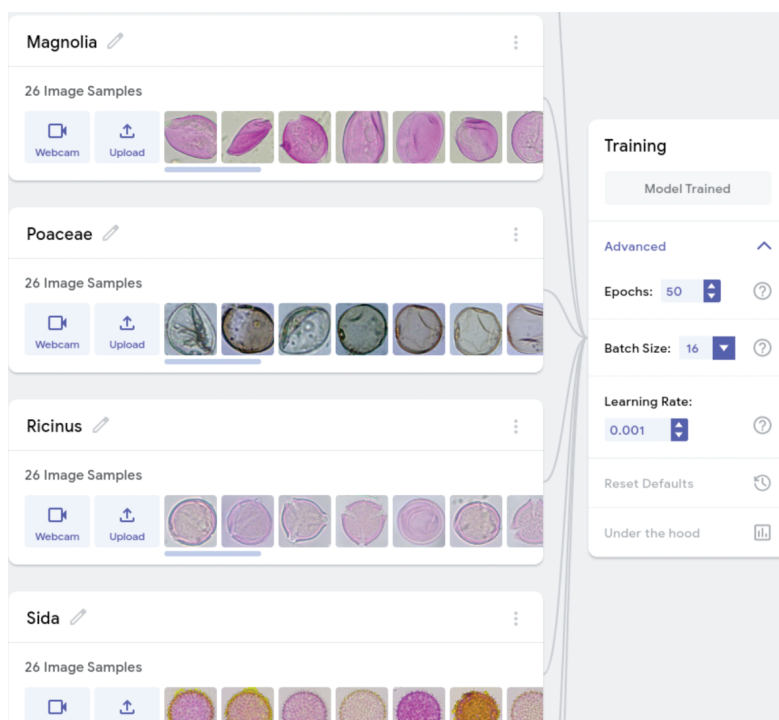


Fig. 1.6: Training of pollen detection model using Teachable Machine platform

need for programming. It can either be uploaded for training samples or captured them using the device cameras through a graphical interface (Fig. 1.6). It uses transfer learning with pretrained weights to train the models, eliminating the need to gather large amounts of data for training purposes.

Trained the present model for 50 epochs, with a learning rate of 0.001. The batch size of the model is set to 16.

RESULTS AND DISCUSSION

Trained a model to detect 7 types of pollen grains vastly available in Manipur viz. *Eucalyptus*, *Magnolia*, *Poaceae*, *Ricinus*, *Sida*, *Solanum* and *Zea mays*. Further, tested the model's performance on a test dataset containing pollen image samples belonging to each type. Employed accuracy, Area under the Receiver Operator Characteristic Curve (AUC) (Fig. 1.7) and F1 score as performance metric. Model performance (Table 1.1) is given below:

Table 1.1: Model performance on the test dataset

Metric	Value
Accuracy	92.06%
F1 score	91.75%
Area Under ROC curve	0.9938

The Receiver Operator Characteristic Curve of the present model performance and its confusion metric are plotted (Fig. 1.8). The model is strong enough in classifying the microscopic pollen image samples into their respective types. It has difficulty in classifying samples belonging to *Zea mays* and *Poaceae* which can be attributed to their similar physical appearance.

CONCLUSION

An automatic detection of microscopic pollen grain image samples has been proposed into different types using AI algorithms. This algorithm detects airborne pollen grains and fungal spores through their characteristic features such as colour, lightness and surface roughness. The automated sampling devices in real time offers a spectacular scope in plant disease control such as in tea with all its attendant benefits in improving crop productivity, product quality and cost effectiveness.

Future Scope:

Researchers can collect samples of airborne pollen grains and fungal spores which are native to their location to train and pre-process them. Air borne pollen grains and fungal spores have close linkage to disease epidemiology in public health and agriculture. Automated methods to separate pollen grains and fungal spores through use of high-quality cameras and IOT

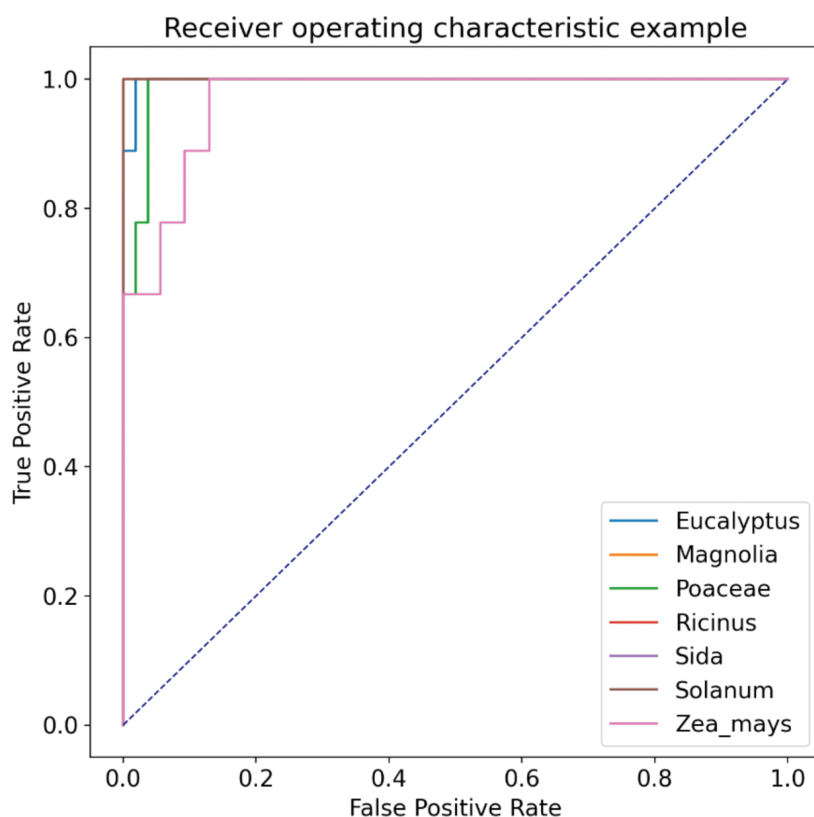


Fig. 1.7: The Receiver Operator Characteristics Curve of the pollen types

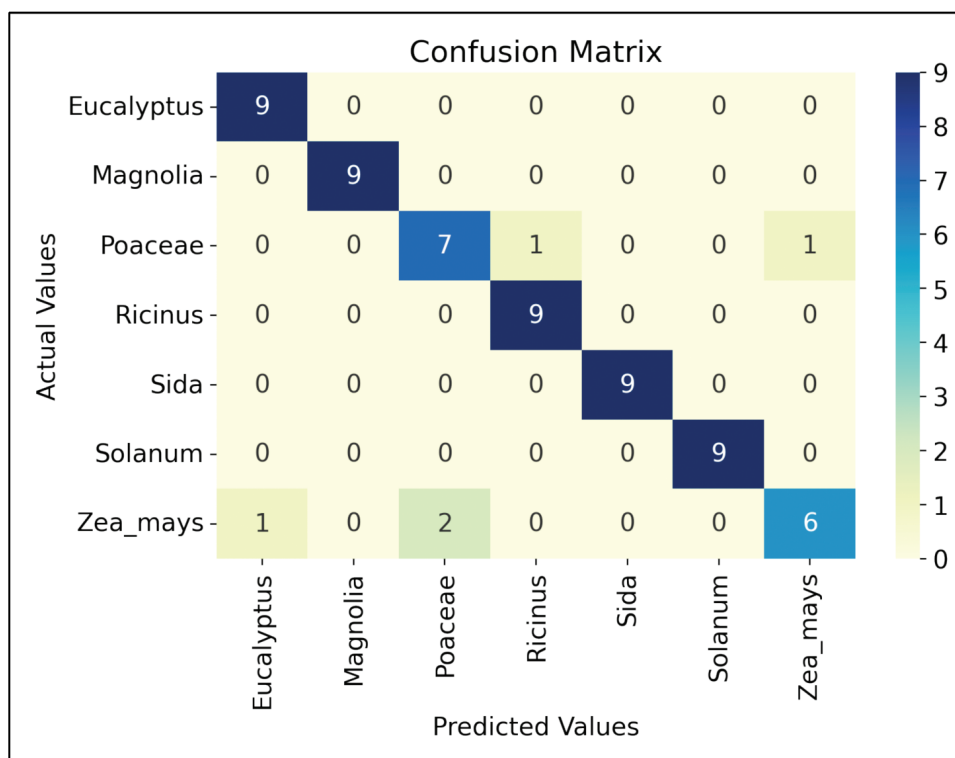


Fig. 1.8: Confusion Matrix of the model's prediction of the pollen types

based hardware coupled with machine learning techniques have reportedly, the potential to detect the type of pollen grains and fungal spores.

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EFFECT OF METEOROLOGICAL PARAMETERS ON AIRBORNE CYPERACEAE POLLEN IN WEST BENGAL OVER A TWO DECADE INTERVAL (1997-2017)

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Cyperaceae pollen grains are frequently airborne and have the potential to cause IgE mediated respiratory allergy. A study was conducted to observe the effect of meteorological parameters on airborne Cyperaceae pollen for three different years (1997, 2007 and 2017), over two decades interval (1997-2017) at Madhyamgram, West Bengal, India. Airborne Cyperaceae pollen was recorded using Burkard 7-day volumetric sampler. Meteorological data were collected accordingly and statistical analyses were done to observe the effect of climatic factors on Cyperaceae pollen. Perennially airborne Cyperaceae pollen exhibited two peaks in March and September for all the three years in two decades. The concentration depicted a gradual rise from 1997 to 2017. During the peak in March, the average level was 60.30 pollen/day/m³ air in 1997, 83 pollen/day/m³ air in 2007 and 87.88 pollen/day/m³ air in 2017. Compared to the year 1997, there was 37.64% rise of pollen level in 2007 and 47.74% rise in 2017. Regarding meteorological parameters, mean daily temperature had showed a rise in trendline in two decades period, with no such change in relative humidity as well as wind speed and slight change in precipitation. In correlative aspect, three hypotheses were analyzed and it was confirmed that only daily average mean temperature rise had a significant effect on the increased level of atmospheric Cyperaceae pollen in the study area. Such rise of atmospheric pollen level may trigger the increase the chance of specific pollen allergen exposure to susceptible respiratory allergic population of the study area.

Key Words: Airborne Cyperaceae pollen, meteorological parameters, temperature rise, long term effect.

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INTRODUCTION

Cyperaceae, commonly called sedges, are grass like weeds growing in all environments associated with moist places and wetlands, comprising the third largest family of monocot plants¹. Its pollen grains are stenopalynous in nature, which can be identified up to family level only². The members of this family are anemophilous and produce a large number of airborne pollen grains. Hence, pollen grains of this family contribute a fair percentage of total airborne pollen grains of a particular geographical area^{3,4}.

Airborne pollen grains are important inducers of IgE mediated respiratory allergy in susceptible individuals. Airborne pollen grains of Cyperaceae were found to be allergenic in different studies from different parts of the

world⁶⁻⁹. Different reports of aerobiological survey indicate the omnipresence of Cyperaceae pollen all over the Indian subcontinent¹⁰⁻¹².

It has been observed that the occurrence of pollen grains in the atmosphere of a particular place varies time to time with the meteorological parameters¹³. In order to have some idea about the exposure level of allergenic pollen grains, their seasonal pattern of occurrence, relationships with the climate factors have to be analysed and studied in detail. In the present scenario, some changes in global climate were also observed in previous decades, resulting into the rise of allergenic pollen grains level in atmosphere, which leads to the eventual increase of respiratory allergic problems like rhinitis, allergic asthma and related disorders¹⁴.

In the present study, the main objective was to estimate the trend of correlation between airborne Cyperaceae pollen concentration and the meteorological parameters over ten-year intervals in a location at Gangetic plane of West Bengal, India, in two decades in the period from 1997 to 2017.

MATERIALS AND METHODS

Aerobiological Monitoring

The daily data of three different years with a decade interval (1997, 2007 and 2017) for atmospheric concentration of Cyperaceae pollen (number/m³ of air) were recorded¹⁵ by Burkard 7-day volumetric sampler (Burkard Manufacturing Co., UK), at Madhyamgram, a suburban town, 19 km north from Kolkata city, India. The place was located in North 24 Parganas district, situated in the Gangetic plain of West Bengal, India.

The volumetric sampler was placed on a concrete base (1 meter high from ground level in a field covered with grasses and wild vegetation).

The flow rate of suction (10l/min) of the sampler was confirmed to be constant throughout. At the end of the sampling week, the tape was cut into seven segments, 48 mm each (corresponding to 1 day). They were mounted and fixed with Gelvatol to make permanent slides. Pollen grains of Cyperaceae, trapped from the air, were studied microscopically by high-resolution light microscope (Olympus) at 400X, to record the occurrence of Cyperaceae pollen grains.

Meteorological Data Collection

The meteorological data [daily average temperature (°C), relative humidity (%), wind speed (km/h) and precipitation (mm)] were recorded from Netaji Subhas Chandra International airport, Dumdum, 3 km south from the sampling site.

Statistical Analyses

The dataset of the observations on pollen concentration and meteorological parameters was split into subsets of seasons. For the purpose of analysis, four seasons were considered as per the Indian climatic cycle:

- Summer
- Monsoon
- Post Monsoon
- Winter

Each of the four subsets were broken down into 3 smaller sets of observations as per the year periods (1997, 2007 and 2017). Separation of year periods was meant to minimize drastic climatic changes that have occurred from 1997 to 2017.

All three smaller sets were using the Pearson correlation coefficient method to understand the pattern of relationships demonstrated by Cyperaceae pollen with the following parameters:

- Mean temperature
- Relative Humidity
- Precipitation
- Wind Speed

Hypothesis Testing for Causation

The statistical analysis was tested for causation between the pollen grain concentration and intensity of meteorological parameters through hypothesis testing and regression methods. As a part of this study, three hypotheses were tested based on results from correlation analysis (Table 2.1):

- Hypothesis 1: Temperature and Wind Speed inversely affect the concentration of pollen grains in the atmosphere during summer seasons
- Hypothesis 2: Temperature positively affects the concentration of pollen grains during the monsoon season
- Hypothesis 3: Meteorological parameters have a significant effect on concentration of pollen grains during Post Monsoon season.

The results from multiple regressions were used to estimate the probability of causation between the parameters and findings were confirmed.

RESULTS AND DISCUSSION

Airborne pollen of Cyperaceae was found to be present in the air almost round the year in 1997, 2007 and 2017. The pollen type was absent in air for the last two weeks of December in all the three years in the two decades span (Fig. 2.1a). On the basis of ecofloristic survey of the study area, the principal sources of flowering plants of Cyperaceae were identified as *Cyperus rotundus*, *Kyllinga nemoralis*, *Scirpus* spp. and others were also growing in the locality. Compared to present observation, an aeropalyngological study at two sites of south-

Table 2.1: Year wise correlation of meteorological parameters with Cyperaceae pollen and standard deviation

Year	Seasons	Mean temp. (°C)	PPtn. (mm)	WS (km/h)	RH (%)	Standard deviation
1997	Summer	-0.68349263	-0.34012235	-0.780763169	-0.38940165	19.13641618
	Monsoon	0.796916553	0.216407679	0.615063413	0.111957442	0.530188253
	Post monsoon	0.343076011	0.316541793	0.308038784	0.339506849	0.053452248
	Winter	0.226715044	0.458392873	0.445426179	-0.15939153	10.3623298
2007	Summer	-0.767519859	-0.217459497	-0.683016512	-0.1760111	27.49181696
	Monsoon	0.71955424	0.127742701	-0.067157404	-0.488815243	0.558605033
	Post monsoon	0.884924136	0.842190625	0.677674565	0.820869114	0.12721607
	Winter	0.288167718	-0.051514367	-0.356989291	-0.714648985	14.85001467
2017	Summer	-0.85010677	-0.280350003	-0.728076753	-0.383582232	29.43236726
	Monsoon	0.045086986	0.292668353	0.713929979	-0.64011992	0.773713987
	Post monsoon	0.915022548	0.505214732	0.679227727	0.475145154	0.131087484
	Winter	0.356679921	0.419194338	0.500241283	-0.054515221	18.74297709

west Spain¹⁶, presence of airborne pollen was volumetrically quantified for a decade, where the major contributor of Cyperaceae pollen spectrum was confirmed to be *Scirpus holoschoenus*, whose pollen could be distinguished from rest Cyperaceae members.

The weekly average count of the pollen grains/day/cubic meter air ranged from 0.05/day/m³ (3rd week of July, 1997) to 87.88/day/m³ (1st week of March, 2017). The seasonal periodic pattern of Cyperaceae pollen in atmosphere depicted two distinct peaks during March and September for all the cases. In this regard, Basak

*et al*¹⁷. reported the peak of Cyperaceae pollen grain, which was attained in May (2014), in the air of Santiniketan, of Birbhum district of West Bengal. The peak concentrations of March (up to 87.88/day/m³) were 4.10-6.58 times greater than that of September (up to 21.4/day/m³). During the periods in between the higher concentrations, (May-August and November-January), the concentration was within the range of 0.2-3.7/day/m³.

As observed from the linear trend line (which is the best fit straight line) for each year that the concentration of

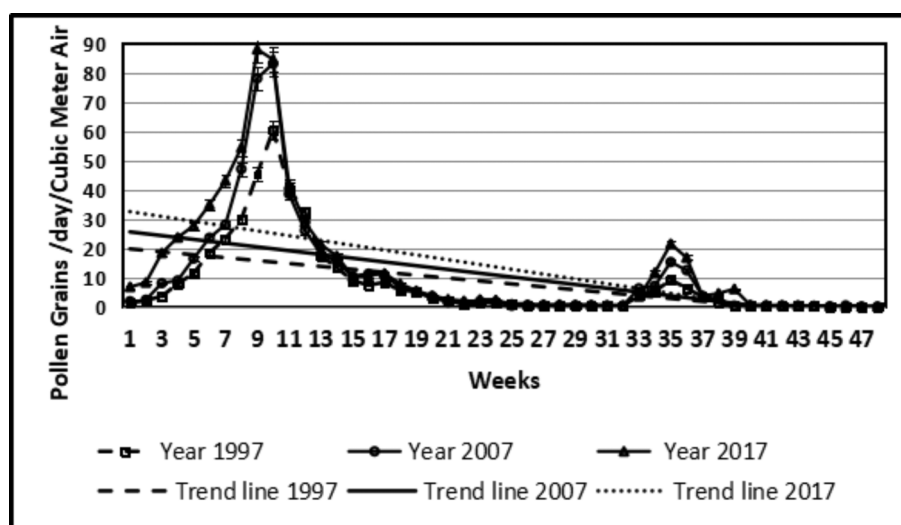


Fig. 2.1a: Seasonal periodicity of airborne Cyperaceae pollen grains in the study area during 1997, 2007 and 2017 along with respective linear trend line. The bars indicate standard deviation.

Cyperaceae showed a gradual rise from 1997 to 2017 concentration was up to 60.3/day/m³ in 1997, 83/day/m³ (Fig. 2.1a). During the major peak of March, the concentration was up to 60.3/day/m³ in 1997, 83/day/m³ in 2007 and 87.88/day/m³ in 2017. Hence, in com-

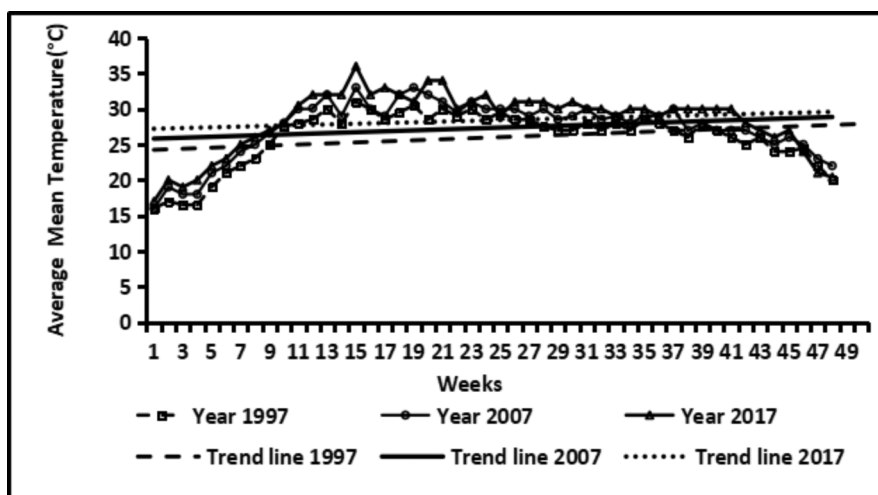


Fig. 2.1b: Mean daily temperature variation in the study area during 1997, 2007 and 2017 along with respective linear trend line.

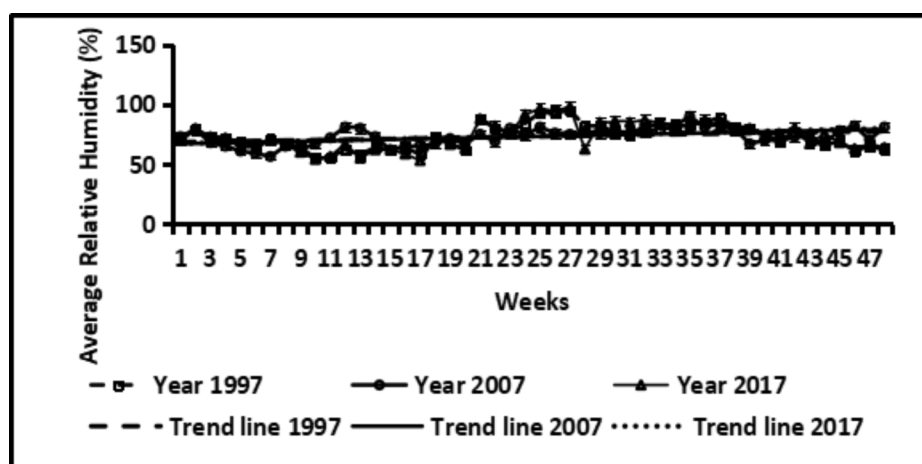


Fig. 2.1c: Variations in the relative humidity in study area during 1997, 2007 and 2017 along with respective linear trend line. The bars indicate standard deviation.

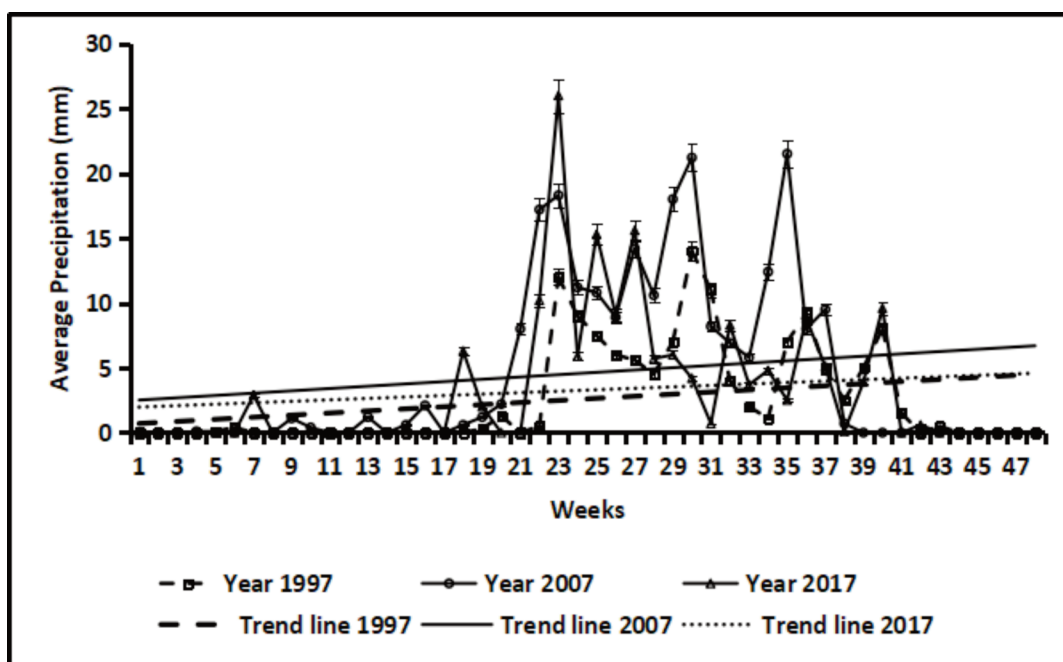


Fig. 2.1d: Variations in the precipitation levels in study area during 1997, 2007 and 2017 along with respective linear trend line. The bars indicate standard deviation.

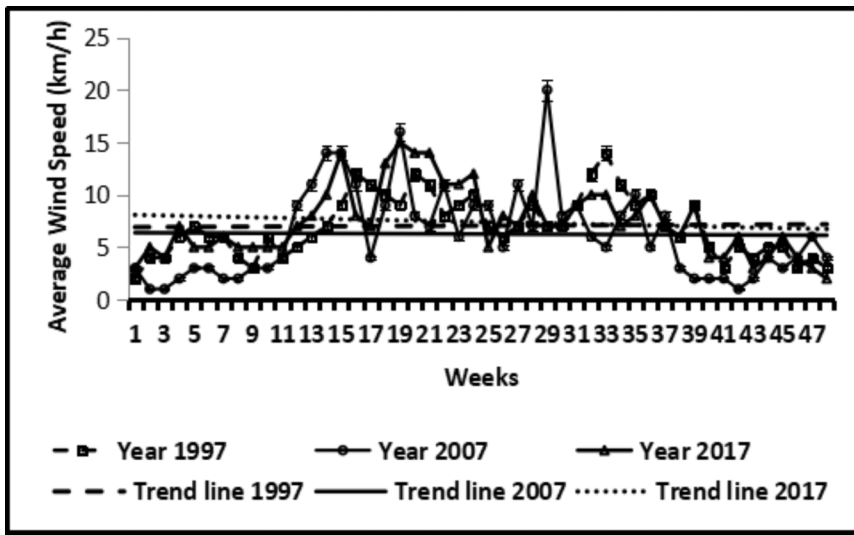


Fig. 2.1e: Variations in the wind speed level in study area during 1997, 2007 and 2017 along with respective linear trend line. The bars indicate standard deviation.

parison to 1997, there was 37.64% rise in 2007 and 45.74% rise in 2017 during peak. However, the peak concentrations of the year 2017 showed only 5.88% rise, in comparison to 2007, that is, in the span of second decade.

Mean daily temperature level depicted a rise in trend line in the twenty years span (Fig. 2.1b) throughout the year, except October. In case of relative humidity (Fig. 2.1c), it was observed to be less in summer and winter (lowest level of 53%), high in monsoon and post mon-

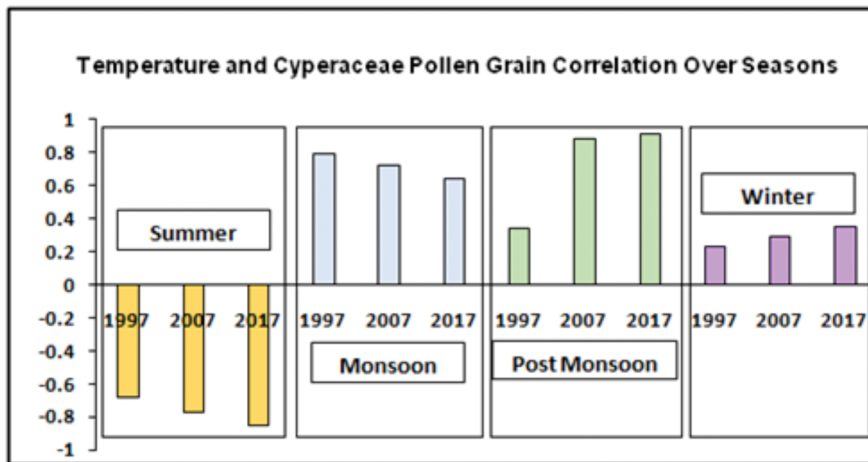


Fig. 2.2a: Correlation between average temperature and airborne Cyperaceae pollen over seasons of 1997, 2007 and 2017 in the study area.

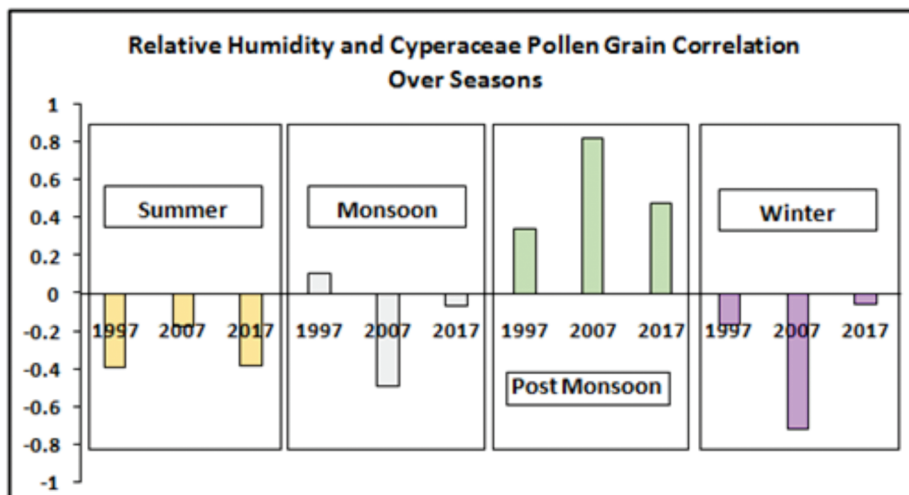


Fig. 2.2b: Correlation between relative humidity and airborne Cyperaceae pollen over seasons of 1997, 2007 and 2017 in the study area.

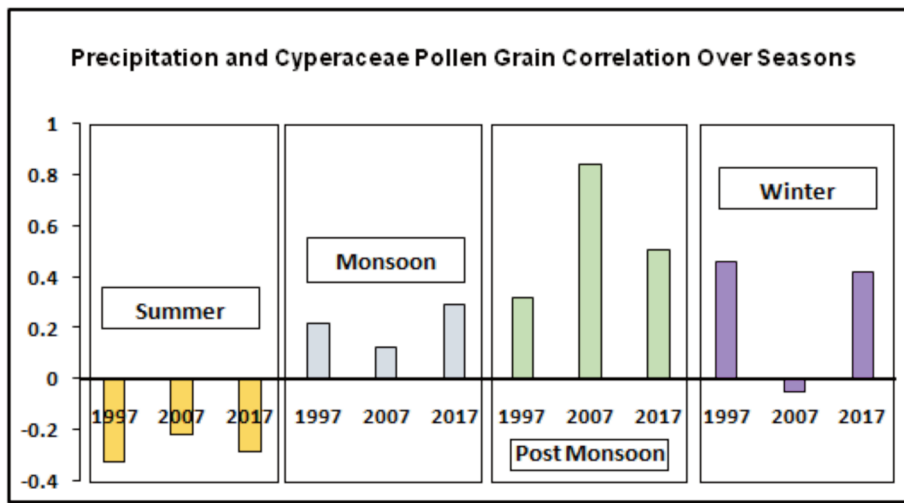


Fig. 2.2c: Correlation between precipitation and airborne Cyperaceae pollen over seasons of 1997, 2007 and 2017 in the study area.

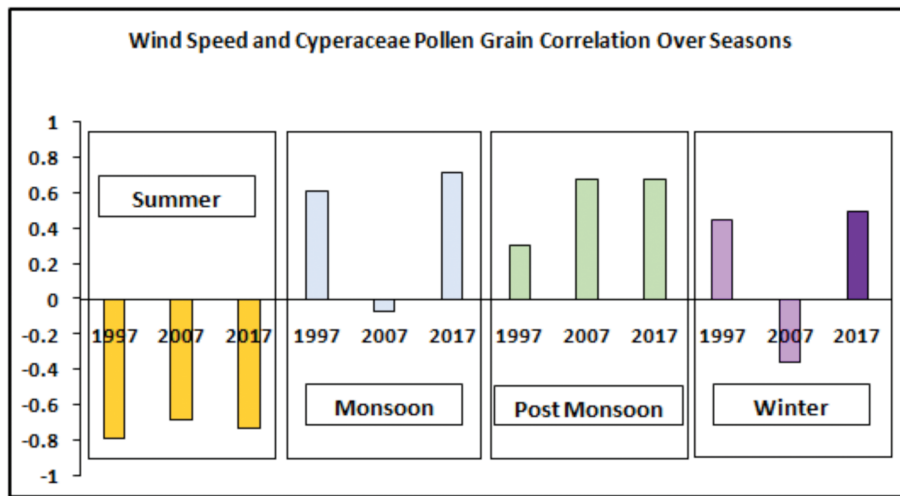


Fig. 2.2d: Correlation between wind speed and airborne Cyperaceae pollen over seasons of 1997, 2007 and 2017 in the study area.

soon (up to 94%). Sometimes in post monsoon period, it crossed 90% level. However, there is no such contrasting trend of rise/fall in of relative humidity within twenty years (1997-2017).

Regarding mean daily precipitation, it was up to 20-28 mm/day during monsoon and post monsoon (Fig. 2.1d). In the linear trend line, precipitation had a rise in 2007 in comparison to 1997. The trend line was found to be lowered again in 2017.

In case of mean wind speed, it was at high level in April-October period in the range of 10-20 km/h speed (Fig. 2.1e). Compared to 1997, there was no clear-cut change in the trend line, except slight fall in 2007 and consequent rise in 2017 during January-March period.

The seasonal correlation between airborne pollen and meteorological parameters were observed (Table 2.1, Fig. 2.2a-d). Among the meteorological factors, mean temperature showed positive correlation during monsoon, post monsoon and winter.

However, the correlation was negative in winter (Fig. 2.2a). Relative humidity showed positive correlation in post monsoon and monsoon of 1997 (Fig. 2.2b). It was negatively correlated with pollen count in summer, winter and monsoon of 2007 and 2017. Precipitation and wind speed mostly found to have positive correlation, except summer (Fig. 2.2c).

Cyperaceae pollen (mostly contributed by *Cyperus rotundus*, *C. kyllinga* and *Scirpus tuberosus* as per nearby vegetation) contributed 7.61% of total airborne pollen in Khairpur of Sindh province, Pakistan, another country of the Indian subcontinent¹¹. In this study a positive correlation was observed between the airborne Cyperaceae pollen and relative humidity ($p < 0.05$).

From the correlative study, there were three hypotheses, which were analytically tested:

Hypothesis 1: Temperature and Wind Speed inversely affect the concentration of pollen grains in the atmosphere during summer seasons:

	Coefficients	Standard Error	t Stat	P-value	Lower 95.0%	Upper 95.0%	Lower 95.0%	Upper 95.0%
Intercept	157.7454222	41.49982806	3.80111026	0.00059	73.3133872	242.1774573	73.3133872	242.1774573
Mean Temperature	-3.384026029	1.498192629	-2.258738939	0.030631	-6.432121852	-0.335930206	-6.43212185	-0.33593021
Wind Speed	-3.383706007	0.875125329	-3.866538764	0.000491	-5.164161876	-1.603250138	-5.16416188	-1.60325014

Hypothesis 2: Temperature positively affects the concentration of pollen grains during the monsoon season. The Regression results showed the R square vale 0.46. It represents the proportion of the variance of the dependent variable (pollen count in atmosphere) as 46% in the equation: Pollen grain concentration = -8.5 + 0.31 *Mean Temperature.

Hypothesis 3: Meteorological parameters have a significant effect on concentration of pollen grains during Post Monsoon season

Regression results R square Value: 0.75

Pollen Grain Concentration = -1.95 + 0.07 *Mean Temperature - 0.0021 *Precipitation - 0.007 *Wind Speed + 0.005 *Relative Humidity.

Finally, it was confirmed that only temperature has a significant effect on pollen grain concentration from regression analysis.

Corroboration to the present observation, in a recent study on atmospheric Amaranthaceae pollen¹⁸, it was found that after 19 years, the pollen season began two weeks earlier and the end of the season had an acceleration of 24 days. Amaranthaceae is another stenopalous pollen family along with Chenopodiaceae. Here, the plant response against climate change was reflected by a decrease of up to 35% of average daily pollen concentration and up to 60% decrease in annual total amount. The study indicated that atmospheric temperature had an effect of the release of pollen grains, i.e., anthesis; whereas relative humidity had a probable effect on pollen concentration in air. Airborne pollen grains are well-known trigger of IgE mediated respiratory allergy for the susceptible individuals and Cyperaceae pollen types are not the exception¹⁹⁻²¹. It was also reported that elevated atmospheric temperature and dryness have some important effect on different agricultural grass pollen grains²². The rise of atmospheric pollen level may lead to the increase of the level of aeroallergen exposure for the local people, suffering from respiratory allergy.

CONCLUSIONS

In the present study, daily average temperature has been recorded as the meteorological factor, having significant effect on the rise of the local atmospheric Cyperaceae pollen level over a long-term period of two decades between 199-2017. Such rise of atmospheric pollen, which are often allergenic in nature, may trigger the increase the chance of specific pollen allergen exposure to susceptible respiratory allergic population of the study area.

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COMPARATIVE AERO-PALYNOLOGICAL AND METEOROLOGICAL INVESTIGATION OF BOLPUR-SANTINIKETAN AND THEIR IMPACT IN DISEASE TRANSMISSION

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As per the 'Aerobiological triangle' environmental factors impart each stage of bioparticles transmission. The present study focuses on aero-palynological and meteorological investigation with regard to respiratory problem related hospital admission of the local inhabitants in and around Santiniketan and Sriniketan of West Bengal. The aero-palynological investigation was carried out for a period of one year (July 2019 - June 2020) with the help of seven day Burkard volumetric air sampler. A total of 38 pollen types were recorded from the ambient air, of which highest pollen concentration was retrieved in November with lowest value in July. The meteorological factors such as average temperature, rainfall, relative humidity, wind-speed and environmental pollutants like NO₂, SO₂, PM10 were studied simultaneously. All four environmental factors were found to be positively and/or negatively correlated with the dispersion of pollen grains. An ascending pattern is observed for both atmospheric pollen load and PM10 concentration as their peak seasons coincide with each other. The air quality index indicates a positive correlation between air quality index and monthly pollen count. It reveals that meteorological parameters have significant impact on the concentration of airborne pollen which in turn influence the respiratory problem related hospital admission.

Key Words: Aeropalynology, meteorology, air pollutant, Air Quality Index, respiratory problem related hospital admission, Santiniketan-Srineketan.

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INTRODUCTION

According to International Society of Biometeorology, 2013, 'Biometeorology' is "an interdisciplinary science that considers the interactions between atmospheric processes and living organisms (plants, animals and humans)". This multifaceted approach associates with human well being like the use of air quality indices to evaluate the atmospheric concentration of various pollutants with emphasis to human health. On the contrary, 'Aerobiology' investigates the nature and impact of airborne biological material such as pollen grains, fungal spores, bacteria and viruses on the transmission of airborne diseases like allergies, asthma etc^{1,2}. Both fields were established quite a same period and shares some common components indeed, of the two, biometeorological forecasting is considered to be comprehensive as it covers bigger scenario of welfare like climate change and their impact on living world, whilst aerobiology only focuses on airborne bioparticles and disease forecasting³.

Respiratory tract related problems include acute infec-

tion of respiratory tract or chronic respiratory diseases (CRDs) where patients with chronic respiratory diseases exhibit low pulmonary function. CRDs are incurable and could be fatal if untreated, nevertheless long-term treatment and appropriate management intend to control the advancement of the disease, thereby help to increase the chances of a better lifestyle for the patients⁴. Tobacco smoking, outdoor as well as indoor air pollution, combustion of fossil fuel and other factors together contribute to a high percentage of suspended particulate matter (PM) in the atmosphere. PM along with different aeroallergens and other risk factors adversely affect the human health as they can induce chronic respiratory diseases such as allergy and asthma, chronic obstructive pulmonary disease (COPD), pulmonary hypertension, occupational lung diseases and lung cancer⁵.

More than 25% world population is known to suffer from various types of allergenic disorders⁶. Predominant form of allergy in India is respiratory allergy, which includes bronchial asthma as a common disease phenotype and in most of the cases it is due to inhalant air-



Fig. 3.1: Google map showing sampling site for aero-palynological and meteorological survey

borne allergens such as pollen grains and spores⁷. The severity may vary from person to person and also from place to place and the airway obstruction is reversible with inhaled medicines such as bronchodilators which helps relieve symptoms by opening the airways⁸. Although anyone at any age can develop asthma and the recent research have shown that environmental factor might play some role as well⁹. Therefore, the present paper focuses on aero-palynological, meteorological and pollution data of the study areas to understand the influence of such factors in causing respiratory tract related problem or allergenic asthma among the local inhabitants^{10,11} in Bolpur subdivision of West Bengal.

MATERIALS AND METHODS

The present survey was conducted for over a period of twelve months from July, 2019 to June, 2020 in Santiniketan-Sriniketan of Birbhum district, West-Bengal (Fig. 3.1) which is highly valued for its cultural and educational heritage centering the Visva-Bharati, a Central university founded by Nobel Laureate Poet R.N.Tagore and also has rich tropical diversity. The meteorological data were collected from Sriniketan meteorological station. During the survey no data could be obtained for the months of April, 2020 and May 2020 due to COVID-19 pandemic outbreak.

Aero-palynological survey

Continuous air monitoring was done with the help of Burkard seven-day volumetric sampler to record the

concentration of airborne pollen grains from the ambient air. The volumetric sampler was placed at the roof of a one-storied building (3 m height) in the Department of Botany, Visva-Bharati University, Santiniketan (Fig. 3.2) and operated at fortnight interval in each month. The glycerine jelly coated exposed cellophane tape was cut into seven pieces and each piece (representing one day) was scanned under microscope to quantify different pollen load in the air (pollen calendar) based on the conversion factor ($0.6Nc$ spores/ m^3) retrieved from The British Aerobiology Federation¹².



Fig. 3.2: Burkard seven day volumetric sampler placed in the Department of Botany, Visva-Bharati

Meteorological and pollution data

Environmental factors such as temperature, rainfall, humidity, wind speed and/or pollutants like SO₂, NO₂ and particulate matters (PM10) play an important role in each step of disease transmission. Thus, to render scientific result of disease forecasting, the correlation study is very much useful to understand the impact of these factors in association with the concentration of airborne pollen grains¹³. In this context, the weather reports comprise of average values of rainfall (mm), relative humidity (%), wind speed (kmph), temperature (°C) were collected from the meteorological station of Sriniketan and Government websites^{14,15}.

Health survey

The health survey was conducted for a period of twelve months from July, 2019 to June, 2020 to understand the impact of different factors such as meteorological parameters, air pollutants and airborne pollen grains on allergic disease transmission¹⁶. The survey was conducted using a modified questionnaire of

European Academy of Allergy and Clinical Immunology (EAACI). A total of 13,940 patients' data (O.P.D.) were obtained from Bolpur Subdivisional hospital (Sian, Bolpur) and Bolpur Primary Health care centre, which are located in the vicinity of aerobiological survey site.

RESULTS AND DISCUSSION

A total of 12,931 pollen grains (No./m³) categorized into 38 pollen types were recorded from the ambient air through the annual aero-palynological survey. Among all the different pollen recorded, grass pollen grains showed its highest annual contribution (24.97%), while among the dicots, *Acacia auriculiformis* (5.63%) and *Eucalyptus* sp. (5.48%) were the dominant contributors followed by other plant families such as Acanthaceae (5.48%), Fabaceae (5.41%) etc.

On the basis of monthly abundance of pollen of different plant species and /or families an annual pollen calendar has been prepared for a period of one year from July, 2019 to June, 2020 (Fig. 3.3). The highest counts of

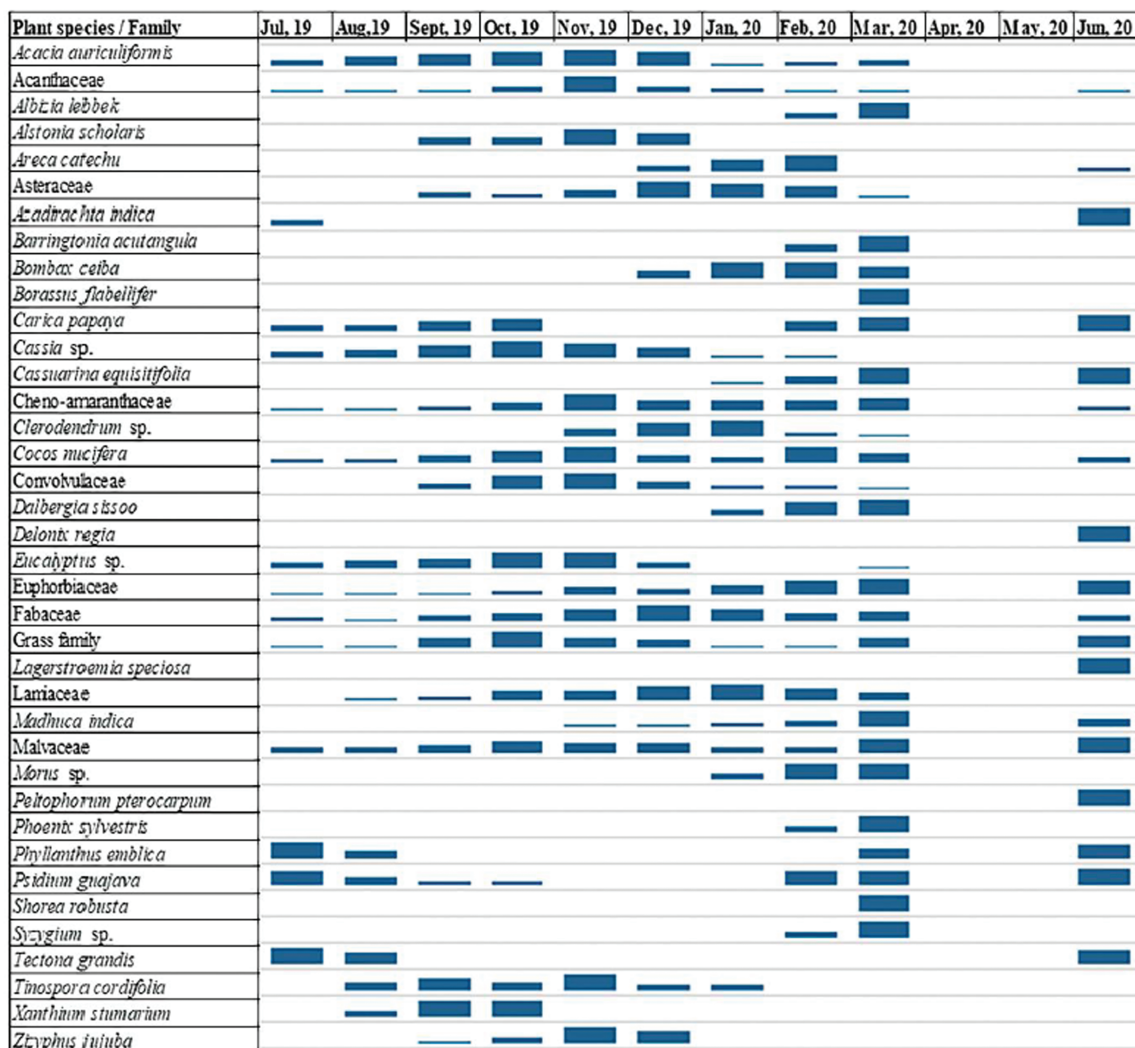


Fig. 3.3: Annual Pollen calendar of Bolpur-Santiniketan (July, 2019 - June, 2020)

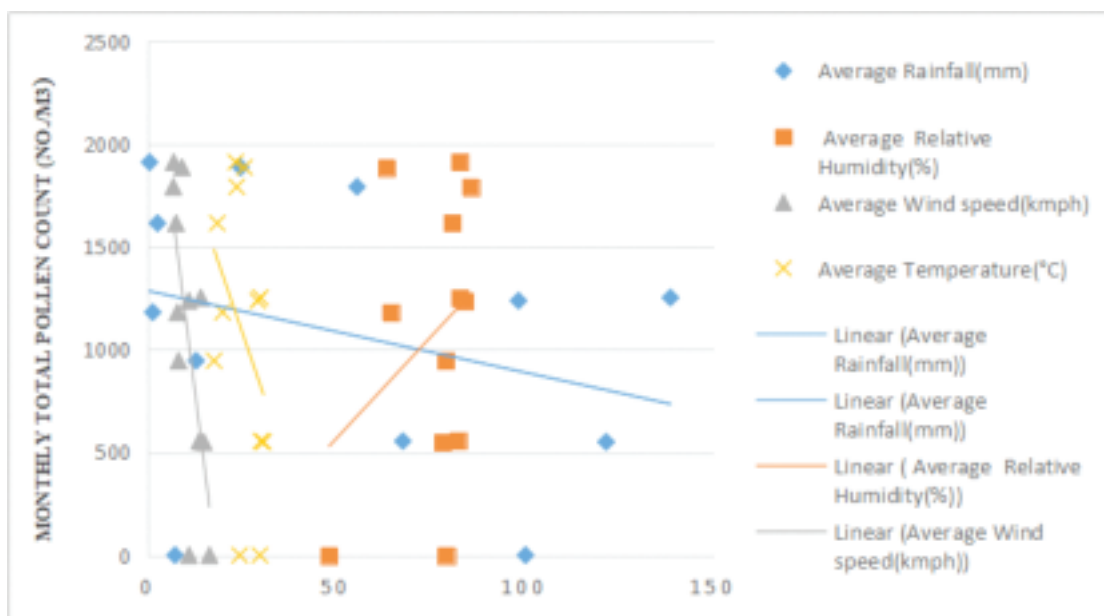


Fig. 3.4: Scattered plot of monthly total pollen count (No./m³) with respect to Average values of rainfall (mm), relative humidity (%), wind speed (kmph) and temperature (°C).

pollen load in the ambient air were recorded from the month of November, 2019 (1912/m³), followed in the degree of prevalence by March, 2020 (1887/m³), October, 2019 (1792/m³), June, 2020 (1253/m³) and the lowest values were recorded from the month of July, 2019 (551/m³), August, 2019 (555/m³) and December, 2019 (1615/m³). Perennial pollen species of Bolpur-Santiniketan are *Cocos nucifera*, *Cassia* sp., *Acacia auriculiformis*, followed by Acanthaceae, Chenopodiaceae, Eupobiaceae, Fabaceae and Malvaceae.

Based on the annual weather report (July, 2019 - June, 2020) the highest average values of temperature was recorded in July (30.81°C), rainfall in June (138.8 mm), relative humidity in October (85.72%) and wind-speed in May (16.5 kmph). The monthly aero-palynological data and different meteorological data (average rainfall, average relative humidity, average wind speed, average temperature) were observed in a scattered plot. The direction of the trend-lines of monthly total pollen count against monthly average values of rainfall, wind-speed and temperature indicate a negative association i.e. with

the increase in the values of these meteorological factors, the pollen load of the ambient air decreases, while the direction of the trend-line between the monthly pollen count and the average value of relative humidity suggests a positive association (Fig. 3.4).

Further interpretation of the Pearson correlation of the same confirms the forms and strength of the association, e.g. very weak positive association (0.3) of monthly pollen load and average relative humidity, very weak negative association (-0.3) with average rainfall, weak negative association (-0.4) with average temperature, moderate negative association (-0.7) with average wind-speed respectively (Table 3.1).

The annual air-pollution data (concentration of NO₂, SO₂ and PM₁₀) were considered to calculate air quality index of Bolpur-Santiniketan. The highest concentration of NO₂ were found in the months of July, November, December of 2019 and January, February, March of 2020, while the lowest concentration were recorded in the month of June, 2020. Monthly concentration of PM₁₀ ranged between 63.07µg/m³ (June, 2020) to

Table 3.1: Correlation study of Monthly total pollen count and different meteorological factors

The dependent variant	R ² value	Pearson correlation	The independent variants
Total Pollen count (No./m ³)	0.090	-0.3	Average Rainfall (mm)
	0.107	0.3	Average Relative Humidity (%)
	0.527	-0.7	Average Wind Speed (kmph)
	0.142	-0.4	Average Temperature (°C)

86.95 $\mu\text{g}/\text{m}^3$ (December, 2019), although the months of November, December, 2019 and February, 2020 showed highest values for both NO_2 and PM_{10} , but monthly concentration of SO_2 remained 2 $\mu\text{g}/\text{m}^3$ round the year. Three highest peaks of pollen load in the ambient air (October - November, 2019 and March, 2020) coincided within the peak of PM_{10} indicating a positive association between PM_{10} and monthly abundance of the pollen in the ambient air (Fig. 3.5).

Out of 13,940 patients' admission (OPD) in Bolpur sub-divisional hospital and Bolpur primary Health care center, a total of 1,722 hospital admission were asso-

ciated to respiratory tract infection related problems that contributes 12.35% of annual patient admission (OPD). Although hospital admission was highest in March and May, 2020, and November, 2019, while highest respiratory tract infection related hospital admission was observed in November, 2019 and March, 2020 (Fig. 3.6).

The air quality index (63-87) round the year indicates a satisfactory air quality range as per the Indian AQI guideline¹⁷. On the other hand, the peaks of RPRHA coincides within the peak months of pollen concentration ($\text{No.}/\text{m}^3$) in the ambient air indicating a positive

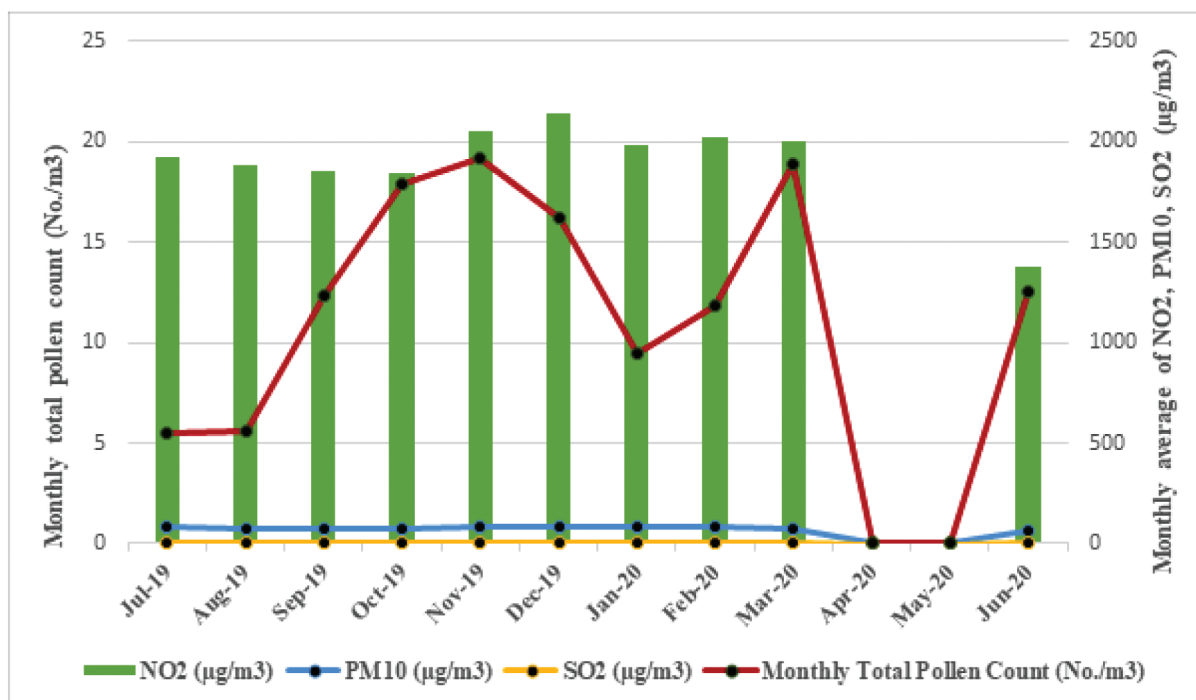


Fig. 3.5: Monthly correlation of inorganic pollutant level (SO_2 , NO_2 , PM_{10}) and total pollen count of Bolpur-Santiniketan (July, 2019 - June, 2020).

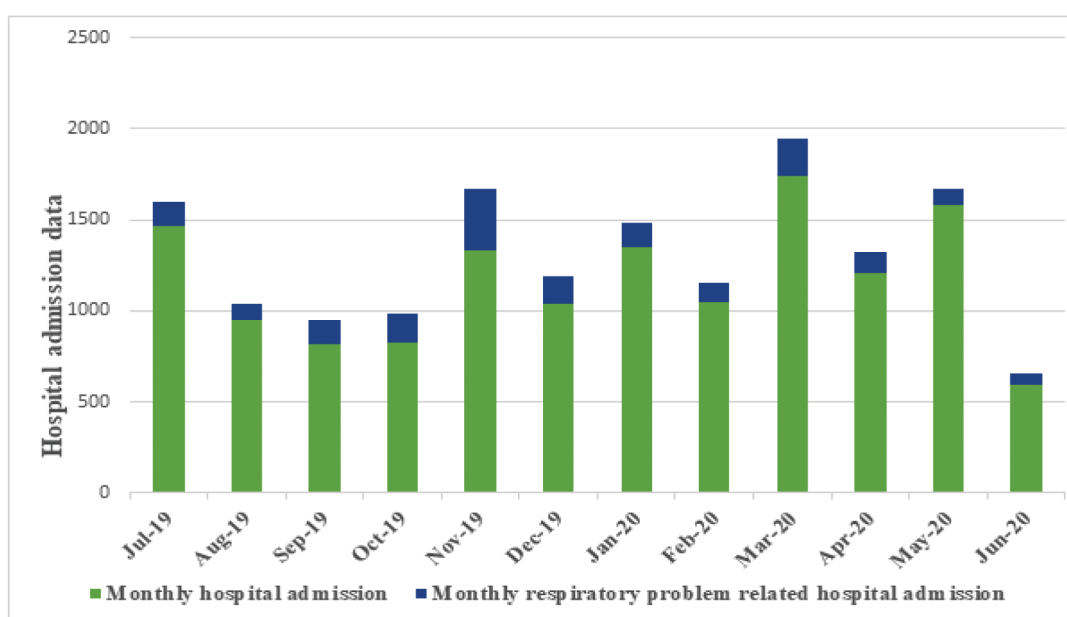


Fig. 3.6: Monthly data of total and respiratory problem related hospital admission data of Bolpur subdivision (July, 2019-June, 2020).

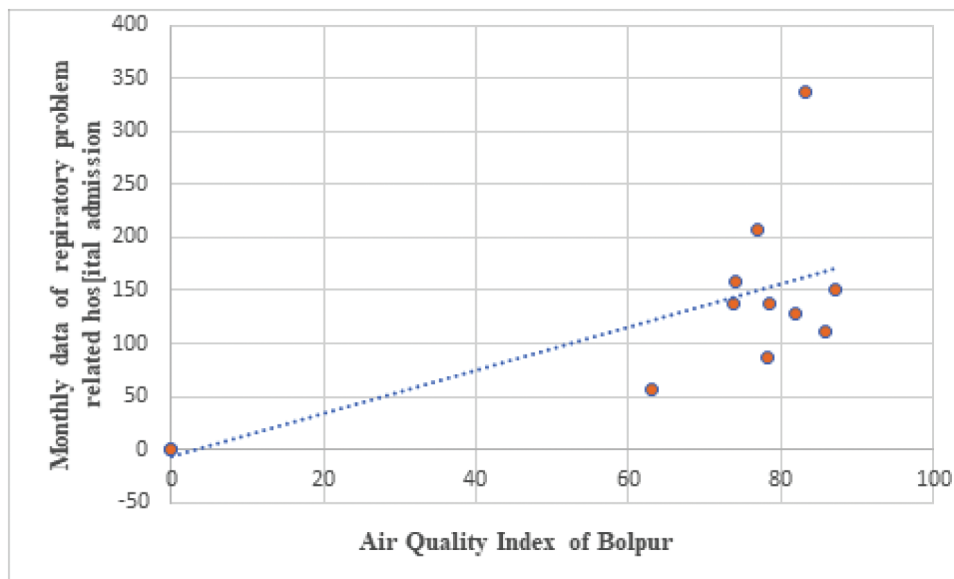


Fig. 3.7: Scattered plot of respiratory problem related hospital admission and air quality index of Bolpur-Santiniketan (July, 2019 - June, 2020).

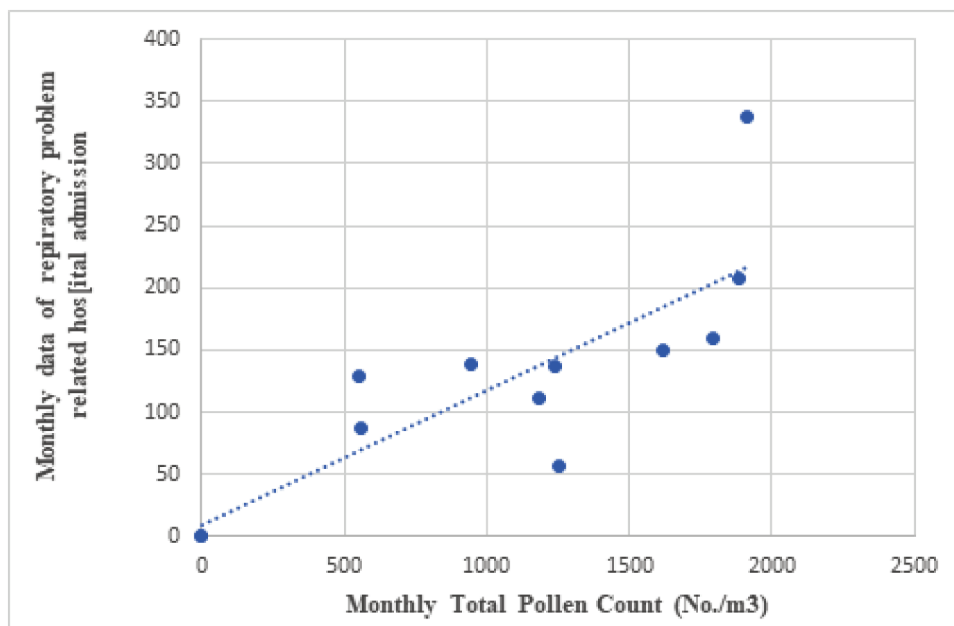


Fig. 3.8: Scattered plot of respiratory problem related hospital admission and monthly total pollen count(No./m³) of Bolpur-Santiniketan (July, 2019 - June, 2020).

association between air quality index and monthly pollen count (No./m³) (Fig. 3.8) as well as between air quality index and respiratory problem related hospital admission (RPRHA) (Fig. 3.7). Of the two parameters, monthly pollen count (No./m³) have a significant correlation with respiratory problem related hospital admission (Fig. 3.9).

CONCLUSION

The occurrence of chronic respiratory diseases like, bronchial asthma and COPD are common in low- and middle-income peoples of developing countries as they fail to provide promising solutions for hygiene and health measures. Hence a better disease management and awareness programs are highly recommended for

the prevention and care of acute and chronic respiratory diseases. To design the best programs, the primary steps include a scientific and research-based database to understand the impact of several factors on disease transmission. Bolpur-Santiniketan - a semi-urban area of West-Bengal - have been developing rapidly over the years and becomes a tourist spot for its cultural heritage and/or for its natural diversity. The present study contributes a significant impact for the preparation of awareness programs for respiratory disease forecasting and management¹⁸. Present study exclusively focuses on a continuous aero-palynological and meteorological investigation with respect to health survey of local inhabitants to understand the impact of pollen grains and other environmental factors on respiratory tract infec-

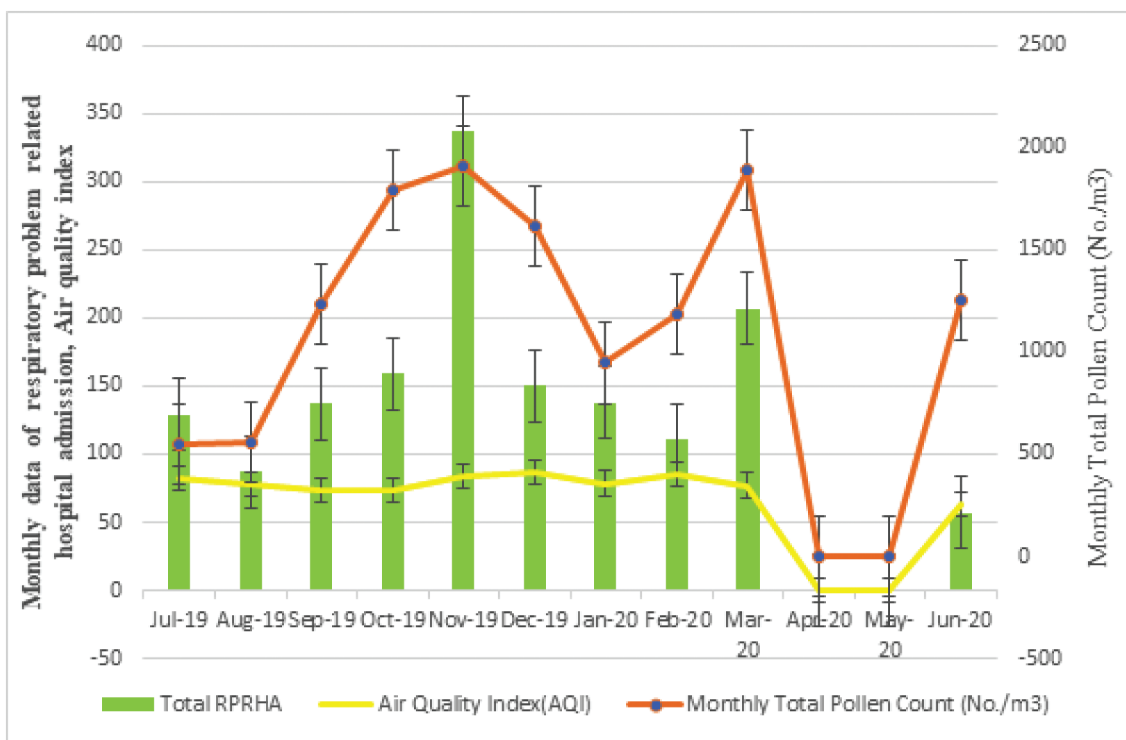


Fig. 3.9: Monthly data of respiratory problem related hospital admission, AQI, total pollen count (no/m³) of Bolpur-Santiniketan (July, 2019 - June, 2020).

tion related problems. An annual pollen calendar (July, 2019 to June, 2020) comprises pollen of grasses, *Cocos nucifera*, *Eucalyptus* sp., *Alstonia scholaris*, *Madhuca* sp., *Cassia* sp., *Acacia auriculiformis* etc., has been prepared conveying the possible contributors of respiratory tract related problems among the local community. Thus respiratory related hospital admission can significantly be correlated with airborne pollen load. The air quality index of Bolpur-Santiniketan is found to be satisfactory as per the Indian guideline of AQI indicating a positive association between air quality index and monthly pollen count. The correlation study further revealed the nature of the association of different meteorological factors and concentration of airborne pollen of the locality. It reveals that meteorological parameters have significant impact on the concentration of airborne pollen which in turn influence the respiratory tract related hospital admissions. Although aerobiological and meteorological surveys are quite common in different parts of West Bengal, but the present study would be very useful as a reference tool for future scientific works, disease forecasting, awareness programs, etc.

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AEROMYCOLOGICAL STUDY OF STORAGE PLACE WITH REFERENCE TO FUNGAL SUCCESSION AND DETERIORATION OF RICE GRAINS

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Rice is one of the most important crops having third-highest worldwide production to feed the world population especially in Asia and Africa. Rice is mainly infected with wide array of fungal species that reduce the yield of the rice seeds. In the present study emphasis was given on the seasonal variation of fungal spores in the air of storage place as well as to find out the succession of mycoflora of rice grains during natural storage conditions along with the deterioration of their stored food in the southern part of West Bengal. Carbohydrate, protein, oil content, germination percentage and moisture content of stored grains were monitored at monthly intervals during one year of storage. Both petriplate exposure method and Burkard personal volumetric air sampler were used for air sampling. *Aspergillus niger* was found to be dominant in petriplate culture, while 'Aspergilli' was most prevalent in Burkard sampler throughout the study period. In search of seed borne mycoflora, different species of *Aspergillus* (*Aspergillus terreus*, *A. flavus*, *A. ruber*, *A. niger*, *A. candidus*, *A. sulphureus*, and *A. versicolor*) were found to be dominant. Some other fungi like *Penicillium citrinum*, *Curvularia lunata*, *Rhizopus oryzae*, *Cladosporium cladosporioides*, and *Alternaria alternata* were also recorded. The dominance of storage fungi was superseded over the field fungi with the progression of storage. Gradual loss of carbohydrate, protein and oil content of rice seeds were noticed during storage. Simultaneously, the rate of germination was declined gradually, however, the moisture content was reduced up to three months of storage followed by gradual increase in subsequent months.

Key Words: Rice grains, airborne and storage fungi, deterioration, carbohydrate, protein, oil.

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INTRODUCTION

Seeds are the most important propagules for about 90% of food crops on the earth¹. Rice is one of the major carbohydrate sources that fulfill our daily needs. A large world population has depended upon rice for their major food in everyday life. China, India, Indonesia, Pakistan, Bangladesh, Vietnam, Thailand, Myanmar, Philippines and Japan are the major sources of rice cultivar². Generally, the small household farmers store their seeds in houses to propagate next year and these seeds are gradually contaminated with pathogens and simultaneously the quality of seeds is also decreased^{3,4}. Contamination of seeds with pathogens at storage conditions reduces the vigor, viability and induces chemical changes in negative ways and leads towards deterioration⁴. Over 100 seed borne fungal species have been reported from

rice, however, severity of pathogens depends on sampling time, geographic location and varieties of rice^{4,5,6,7}. Basically, three categories of seed borne fungi have been reported, (a) obligate parasite with strict parasitic life cycle, (b) facultative saprophyte - actually parasite but can live as saprophyte, and (c) facultative parasite - generally saprophyte but often changes to parasitic life cycle which categorizes into field fungi and storage fungi^{8,9}. Storage fungi are most insidious in nature that infect the seeds under storage condition and make over serious damage before tracing their presence¹⁰.

Being a tropical country the climatic conditions of India such as temperature, humidity and rainfall create a conducive atmosphere for the pathogens like fungi and bacteria during harvesting time¹¹. Due to the severe infection by such seed borne fungi, the seeds get con-

taminated producing mycotoxins, thus seeds become useless, even unsuitable for animal fodder¹². Fungi associated with the seeds may induce several health-related issues among the farmers such as aspergillosis, mycoses, etc. Consequently in stored condition, the carbohydrate, protein and total oil content of seeds are gradually decreased due to enhancement of moisture content within the seeds¹³. Seed borne fungi are also reported to reduce the germination rate of the seeds with increase in storage time^{12,14}.

The importance of the aeromycoflora study has increased over the last few decades in view of health-related issues by the activity of spores or metabolites including mycotoxins and volatiles compounds derived from fungi¹⁵. It is now established that inhalation of fungal allergens during childhood provokes sensitization to allergy¹⁶. The allergenic fungal spores present in the ambient environment may enter into the human body through inhalation and often evoke the immune system for manifestation of Type I allergic reaction like rhinitis, urticaria, asthma, etc.^{17,18}.

The present study mainly focuses on the seasonal variation of indoor fungal spores in the air of storage places vis-a-vis the seasonal variation of fungal invasion in the storage seeds of rice. The study also includes the changes in nutrients (carbohydrates, proteins and oil) of the seeds over the storage period plus the effect on germinability and moisture content in rice storage seeds.

MATERIALS AND METHODS

Aeromycological study of storage place

Aeromycological survey of the storage place in Purulia, West Bengal was carried out from June 2021 to May 2022 using both petriplate exposure technique and Burkard personal volumetric air sampler (Burkard Manufacturing Co., UK). Potato Dextrose Agar with Chloramphenicol (Himedia, product code: M1941) and Rose Bengal Chloramphenicol Agar (Himedia, product code: M640) were used for isolation. Plates were exposed for 4 minutes at 10 days intervals in a month¹³. Exposed plates were then transferred to the incubator at 28°C for 4-5 days. The developing colonies were mounted with lactophenol-cotton blue solution and observed under microscope (LEICA DM 3000 LED). In Burkard personal air sampler, the glycerin jelly coated slides were exposed for 30 minutes to capture

the spores travelling through air. The identification was done by observing colony morphology, microscopic features with consulting standard literatures^{19,20}.

Collection of seed sample

Bulk amounts of rice seed variety 'Swarna' were collected from seed storage house and kept in a gunny bag (10 kg) in the room temperature for one year in a godown of Purulia, West Bengal throughout the study period. Seeds were randomly picked up for the study.

Study of seed mycoflora

Surfaces of seeds were sterilized with 1% sodium hypochlorite for 10 minutes²¹, followed by 3-5 times washing with autoclaved distilled water, and were placed them in petridishes containing 3.9% Potato Dextrose Agar media supplemented with Chloramphenicol (pH 5.6) and 3.2% Rose Bengal Chloramphenicol Agar media (pH 7.2). Five seeds were placed in each petriplate in triplicate under laminar air flow and placed in an incubator at 28°C for 4-5 days. Developed fungal colonies were mounted with lactophenol-cotton blue solution and observed under microscope for their characterization and identification. The identification of fungal colonies were done as per previous protocol^{19,20}.

Moisture content analysis

Seed moisture content was analyzed following the method of Roberts and Roberts²². 10g dried seed samples were cooled at a desiccator containing CaCl₂ for a period of 40-45 min and weighed. Seeds moisture content was calculated on the basis of wet weight. Obtained data were confirmed using the moisture analyzer (Metrix⁺ GMM 7002A).

Seed germination test

Seed germination tests were performed in triplicate by placing 30 seeds in each tray with a bed of moist tissue paper covered with another tray. The seeds were surface sterilized by 5% sodium hypochlorite for 1 minute and followed by 3-5 times washing with distilled water prior to placing in the tray²³. The parameters of seed germination were adjusted following Monajjem *et al*⁴. The seeds containing trays were placed in a plant growth chamber with 12 hours of light, 90% humidity at 25°C. Seed germination percentage was calculated following the formula of Islam and Borthakur¹², represented as Germination Percentage (%) = Germinated seeds/Total number of seeds tested × 100.

Carbohydrate content

Total soluble and insoluble sugars (starch) were estimated following the method of Chow and Landhauser²⁴. 100 mg powdered rice seeds were used for the extraction of soluble sugar and insoluble sugar with 2 ml of 80% ethanol, followed by heating at 90-95°C for 15 minutes. Subsequently centrifugation was performed at 3000 rpm at duration of 5 minutes. The process was repeated for three times and the supernatants in combined were used for the analysis of soluble sugar. Moreover, the oven dried pellets after adding 1.1% HCl were used for the estimation of insoluble sugar. Both soluble and insoluble sugar was estimated using anthrone reagent. For spectrophotometric estimation, using Shimadzu UV-1800 spectrophotometer, standard curves were prepared using glucose for soluble sugar and starch for insoluble sugar. The absorbance was taken at 630 nm and percentage was calculated separately using standard curve for both soluble ($y = 0.0013x + 0.1642$ and $R^2 = 0.9981$) and insoluble sugar ($y = 0.0024x + 0.0978$ and $R^2 = 0.9981$).

Protein content

100 mg powder of rice seeds were used for extraction of proteins with 1 M sodium hydroxide (NaOH) by incubating at 80°C for duration of 1 hour. Extract was centrifuged at 4000 rpm for 10 minutes and the total protein content was estimated following the method of Lowry *et al.*²⁵ with Bovine Serum Albumin (BSA) as standard. Absorbance was taken at 660 nm and the percentage was calculated using the standard curve of BSA ($y = 0.0025x + 0.1012$ and $R^2 = 0.9981$).

Oil content

Oil from seeds was extracted using non polar solvent hexane in soxhlet apparatus²⁶. 100 g of seeds were used for the continuous extraction for 6 hours. The total solvent with oil was then transferred to rotary evaporator (IKA RV 10 with chiller) at 40°C under reduced pressure to separate and recover the solvent from the extracted oil. The quantity of oil was expressed in percentage as dry weight basis²⁷.

Table 4.1: Month wise and season wise airspora of a storage place in Purulia, West Bengal using petriplate exposure technique (June 2021 - May 2022)

Fungal isolates	Months												Count	%
	Monsoon			Post Monsoon			Winter		Summer					
	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May		
<i>Curvularia lunata</i>	21	32	29	23	11	6	2	6	14	8	8	19	179	10.89
<i>Cladosporium cladosporioides</i>	12	-	-	-	-	10	26	34	29	12	11	14	148	9.00
<i>Aspergillus niger</i>	58	64	55	45	32	36	29	50	36	39	42	52	538	32.73
<i>Aspergillus flavus</i>	17	12	8	-	-	-	10	8	6	-	-	13	74	4.50
<i>Aspergillus candidus</i>	12	4	3	-	4	7	5	3	-	-	3	7	48	2.92
<i>Aspergillus fumigatus</i>	14	12	8	4	-	-	-	-	-	-	-	4	42	2.55
<i>Aspergillus terreus</i>	-	4	3	-	-	-	2	4	4	8	7	-	32	1.95
<i>Aspergillus ruber</i>	8	7	5	3	3	2	2	7	4	4	2	-	47	2.86
<i>Penicillium citrinum</i>	4	-	-	-	-	4	6	6	3	2	2	14	41	2.49
<i>Drechslera sp.</i>	-	-	4	5	8	11	7	5	3	1	-	-	44	2.68
<i>Alternaria alternata</i>	6	5	10	16	11	8	16	13	17	30	8	15	155	9.43
<i>Rhizopus oryzae</i>	17	20	16	28	23	15	7	6	6	4	12	15	169	10.28
<i>Cunninghamella echinulata</i>	-	-	-	-	-	-	4	4	6	3	-	-	17	1.03
<i>Fusarium oxysporum</i>	6	7	7	8	9	12	4	-	-	-	-	6	59	3.59
Others	3	5	7	6	4	3	3	2	3	4	6	5	51	3.10
Total count	178	172	155	138	105	114	123	148	131	115	101	164	1644	100.00

RESULTS

Air mycoflora of storage place

The calendars for monthly spore concentration through petriplate exposure technique (Table 4.1) as well as through Burkard Personal Volumetric air sampler study (Table 4.2) in the air of storage place were prepared. Total 14 fungal spores were identified through colony morphology and microscopic characterization along with some unidentified spore types isolated through exposure of culture media. The results revealed the dominance of *Aspergillus niger*, however other *Aspergillus* species like *A. flavus*, *A. candidus*, *A. fumigatus*, *A. terreus* and *A. ruber* were also recorded. The higher concentration of spore load of *A. niger* was followed by *Curvularia lunata* and *Rhizopus oryzae* which were the most prevalent during the survey period (Table 4.1). In monthly contributor fungal spores, the month of June hold the highest position followed by July and May. Study through Burkard personal volumetric air sampler has revealed that ‘Aspergilli’ were the most significant contributor in the air of storage place, followed by

Curvularia and *Alternaria* spores (Table 4.2). Month wise, August was the highest contributor in relation to the number of total spores count.

To investigate the seasonal variation of the fungal spores in the air, the whole year were divided into four seasons, viz. monsoon (June to August), post-monsoon (September to November), winter (December to February) and summer (March to May). In petriplate exposure method, *Aspergillus niger* was recorded as the most prevalent spore type in the air of the storage location throughout the seasons. *Curvularia lunata* and *Rhizopus oryzae* were the second and third most contributors respectively during the monsoon, while their position were reversed during post-monsoon. Though during winter the number of *Cladosporium cladosporioides* and *Alternaria alternata* spores were moved up to the second and third highest peak respectively. But in the summer *C. cladosporioides* and *A. alternata* changed their position in the reverse manner in terms of their dominance (Fig. 4.1).

In Burkard volumetric spore capturing method, the monsoon was dominated by ‘Aspergilli’ followed by

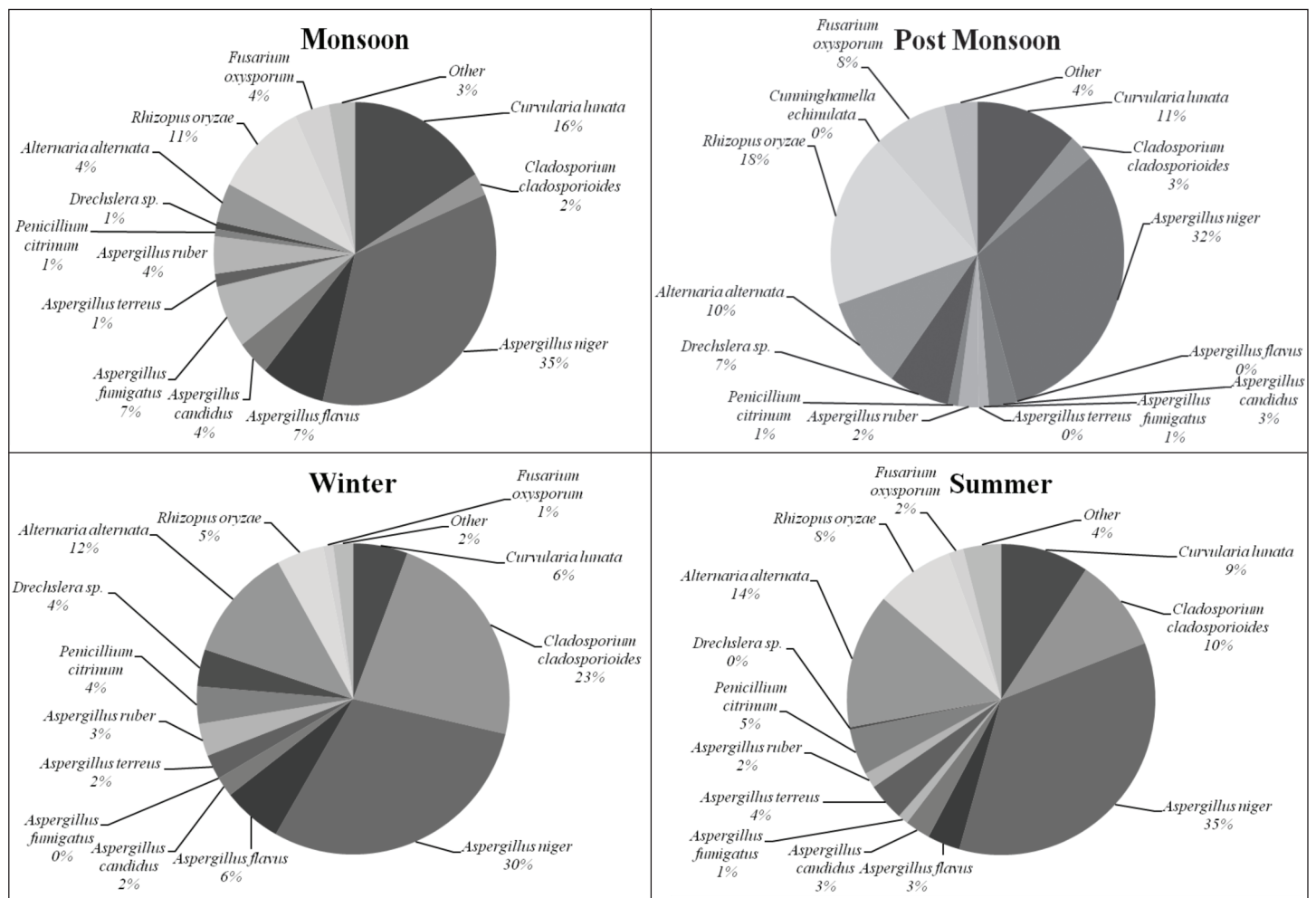


Fig. 4.1: Seasonal variation of fungal spores (expressed in %) in storage place in Purulia, West Bengal using petriplate exposure technique (June 2021 - May 2022)

Table 4.2: Study of airspora of storage place in Purulia, West Bengal using Burkard personal volumetric air sampler (June 2021 - May 2022)

Fungal isolates	Months												Count	%
	Monsoon			Post Monsoon			Winter			Summer				
	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May		
<i>Cladosporium</i> sp.	384	374	356	312	322	395	1126	1231	1168	594	382	376	7020	9.16
<i>Curvularia</i> sp.	1236	2896	2296	1968	696	244	302	622	890	322	396	866	12734	16.62
'Aspergilli'	4928	4925	4845	4510	3152	2612	2965	2906	2694	2429	2311	2226	40503	52.86
<i>Alternaria</i> sp.	180	642	980	1126	1368	712	418	672	972	652	260	140	8122	10.60
<i>Drechslera</i> sp.	-	-	324	184	440	496	246	180	116	-	-	-	1986	2.59
<i>Tetraploa</i> sp.	-	-	240	312	284	192	62	-	-	-	-	-	1090	1.44
<i>Fusarium</i> sp.	-	-	-	152	382	489	466	411	340	292	242	210	2984	3.89
Others	188	192	246	188	180	165	156	162	190	184	173	154	2178	2.84
Total count	6916	9029	9287	8752	6824	5305	5741	6184	6370	4473	3764	3972	76617	100.00

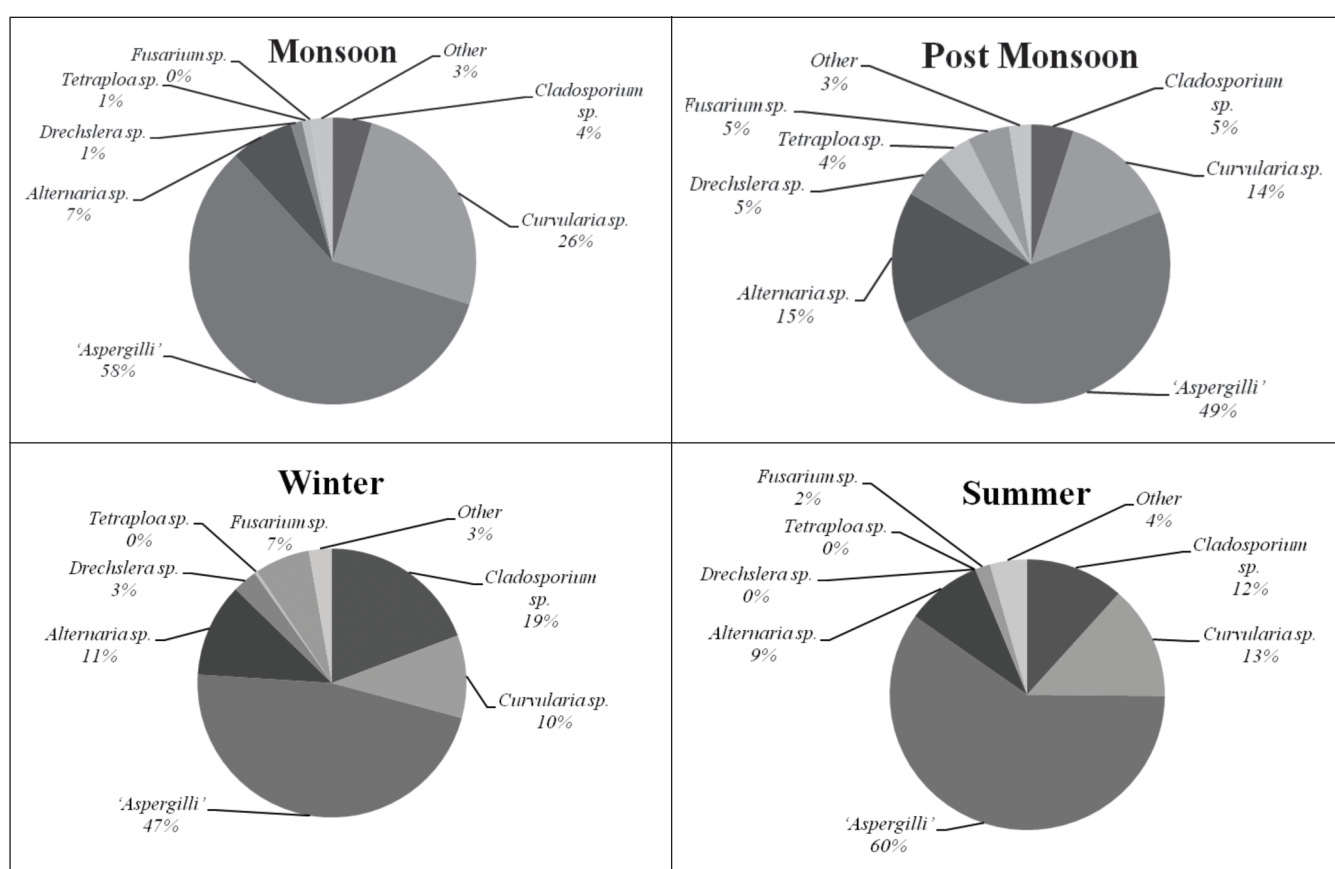


Fig. 4.2: Seasonal variation of fungal spores (expressed in %) of storage place in Purulia, West Bengal using Burkard Personal volumetric air sampler (June 2021 - May 2022)

Curvularia and *Alternaria*. 'Aspergilli' continued to show its dominance for the rest of the seasons (Table 4.2). *Alternaria* and *Curvularia* occupied second and third dominant spore types respectively during post monsoon period, however, *Cladosporium* replaced *Alternaria* as the second dominant spore type in winter and *Alternaria* came down to third position during this period. During summer, *Cladosporium* was replaced by *Curvularia* as the second dominant spore type, whereas

the third dominant position was occupied by *Cladosporium* replacing *Alternaria* (Fig. 4.2).

Seed mycoflora study

Seed mycoflora study was conducted for a period of twelve months (June 2021 to May 2022). The percentage of fungal colonies in each month of storage was noted (Fig. 4.3). Total 12 fungal isolates were identified during this timeline of study. The most dominant storage

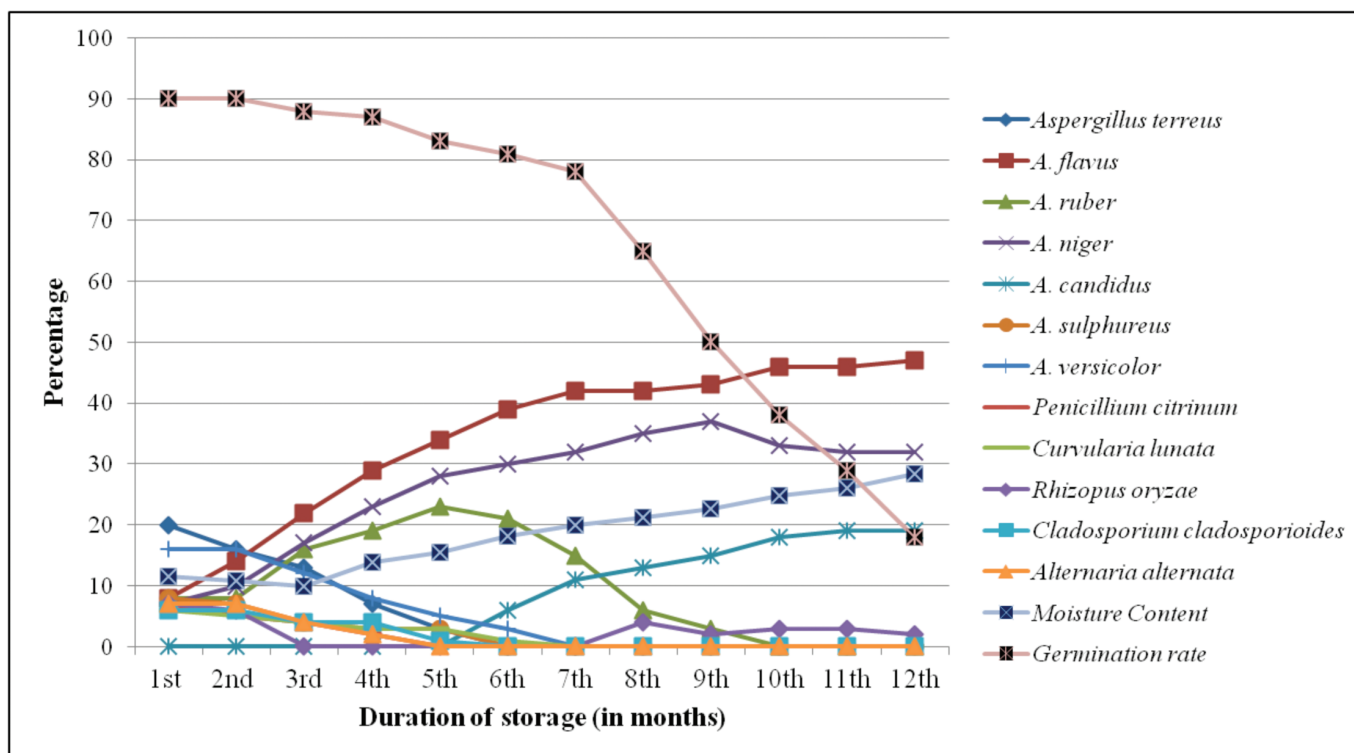


Fig. 4.3: Incidence of different fungal species and changes of moisture content and germination rate during the storage period (in %) [1st Month = June 2021 and 12th Month = May 2022]

fungus genus *Aspergillus* with a total seven species were isolated from the seed samples. Among them *A. flavus* and *A. niger* were found throughout the storage duration. However, *A. flavus* was found to be the most abundant among all isolates and reached its peak during the progression of storage (Fig. 4.3). The percentage of *A. niger* was gradually increased along the storage with a tenuous decrease after tenth month of storage. *A. terreus* was appeared during the early months of storage but disappeared after five months, on the other hand *A. candidus* was absent in the early months and came out after five months of storage. *A. ruber* was detected from the initial stage and gradually increased up to five months of storage, however, it seems to decrease and finally disappeared after ninth months (Fig. 4.3). *A. sulphureus* was noticed for the first four months and thereafter no trace was observed. *A. versicolor* was observed for the first six month of storage and then terminated. The number of *A. versicolor* was more for the first two months in comparison of the next four months of occurrence. Other fungi like *Penicillium citrinum*, *Curvularia lunata*, *Rhizopus oryzae*, *Cladosporium cladosporioides* and *Alternaria alternata* were also isolated in very low concentration during the storage time (Fig. 4.3).

Moisture content

Initially moisture content of the seeds was 11.5% and

gradually decreased along with the increase in storage time up to third month (10%), thereafter gradual enhancement of the moisture content was observed (maximum 28.3%) up to the twelve months of storage (Table 4.3, Fig. 4.3).

Germination rate

Germination rate was 90% in the initiation two months of storage (Table 4.3). After the second month of storage, the rate of germination was gradually reduced and finally decreased to 18% after 12 month of storage (Fig. 4.3).

Carbohydrate content

The amount of carbohydrate was initially 40.6%, of which soluble sugar was 3.2% and insoluble sugar was 37.4% (Table 4.3). During storage insoluble sugar was continuously reduced to 29.5%, while the soluble sugar was reduced to 1.0% after the period of twelve months. The total carbohydrate was reduced from 40.6% to 30.5% after 12 months of storage period.

Protein content

Protein content of rice seeds was continuously decreased from the initial 6.75% to final 4.27% during the storage condition (Table 4.3). The amount of reduction in terms of protein percentage was low for the first three months during storage, however from the fourth month of

Table 4.3: Physical and biochemical changes of rice seeds during storage [1st Month = June 2021 and 12th Month = May 2022]

Storage duration (Month)	Moisture content (%)	Germination (%)	Total carbohydrate			Total protein (%)	Oil (%)
			Soluble (%)	Insoluble (%)	Total (%)		
1st	11.5	90	3.2	37.4	40.6	6.75	7.0
2nd	10.8	90	3.1	36.1	39.2	6.67	6.8
3rd	10.0	88	2.9	35.8	38.7	6.55	6.5
4th	13.8	87	2.7	35.3	38.0	6.31	6.5
5th	15.5	83	2.3	34.9	37.2	6.03	6.3
6th	18.1	81	2.0	34.5	36.5	5.83	6.1
7th	19.9	78	1.8	34.1	35.9	5.67	5.8
8th	21.3	65	1.6	33.5	35.1	5.35	5.6
9th	22.6	50	1.5	32.9	34.4	5.11	5.3
10th	24.8	38	1.3	32.2	33.5	4.79	5.2
11th	26.1	29	1.1	31.3	32.4	4.59	5.1
12th	28.3	18	1.0	29.5	30.5	4.27	4.9

storage the amount of protein loss was quite faster from the earlier months.

Oil content

The amount of oil was measured initially to 7.0%, but during storage oil content gradually decreased up to 4.9% during the twelve months of storage (Table 4.3).

DISCUSSION

In storage conditions, seeds are the significant source of air mycoflora with *Aspergillus* and *Rhizopus* being the foremost constituents²⁸. The air mycoflora data of the present studied storage place exhibits the dominance of *Aspergillus* species, *Curvularia lunata* and *Rhizopus oryzae* in the air. The group ‘Aspergilli’ also showed its dominance in Burkard sampling. Occurrence of the miscellaneous fungi in seed mycoflora (*Curvularia lunata*, *Rhizopus oryzae*, *Cladosporium cladosporioides*, *Alternaria alternata*) were in the low level during the storage condition, however the occurrence of storage fungi (different species of *Aspergillus*) continuously rise as well as decline during storage. The reasons for the lower number of miscellaneous fungi may be due to predominance of storage fungi^{29,30}. Successions of different

species of *Aspergillus* were also observed during this study period. The number of *Aspergillus niger* was earlier reported to increase along the increase in storage period³¹ which corroborates with the present study. Seeds infected with *Aspergillus flavus*, *A. parasiticus* and *A. terreus* had shown the reduction of protein, lipid, and carbohydrate in comparison to the control³². It was reported that the protein content of green gram, black gram, chick pea and pigeon pea reduce because of seed borne fungi³³. The germination rate, carbohydrate content, protein content and oil content in maize, groundnut and soybean seeds were also reported to diminish during storage condition¹³.

The findings in the present study substantiates the earlier findings because it was revealed earlier that the carbohydrate, protein and oil content of rice seeds gradually decrease during storage period. It was also reported that rice seeds infected with *Aspergillus flavus* get maximum deterioration in comparison to *A. niger*, *Penicillium* sp. and *Fusarium* sp. due to having ability of *Aspergillus flavus* to produce aflatoxins, enzymes like alpha amylase, protease and induction for signaling of ageing process¹¹. In the present study, *Aspergillus flavus* was

found to be the most significant and dominant fungus during the storage, thus might be one of the main reasons for the deterioration of the storage rice seeds.

Moisture content is one of the major factors for infection by different fungal pathogens. Moisture content was found to reduce with the prolongation of storage duration. It has been also revealed that the germination percentage was also reduced with the storage time³⁴ may be due to degeneration of mitochondrial membrane and scarcity of energy³⁵. However, in this study it was observed that moisture content initially started to decrease, but later it increased, similar to the study of Paderes *et al.*³⁶ who reported that increase in moisture content in between 14.5-18.33% remarkably increased the infection with storage fungi³⁶. In the present study, a huge increase in the moisture content of the seeds was observed from 11.5% to 28.30%, may be due to high rainfall in late monsoon and inadequate storage conditions.

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MICROBIAL PROFILING OF STREET FOODS AT DIFFERENT LOCATIONS IN THE GONDIA CITY OF MAHARASHTRA, INDIA

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"Street foods" basically describes as a wide range of ready-to-eat foods and prepared in public places, notably streets. Foods and beverages which are prepared and sold by the sellers on places like streets, festival areas, and consumed by the consumers on the run are known as street food. These foods are alternatives to homemade food and are more affordable when compared with the food supplied at the restaurants. The final preparation of street foods occurs when the customer orders the meal which can be consumed where it is purchased or taken. The present study aims to establish the hygienic status of street vended food at various locations in the Gondia city of Maharashtra and its impact. It is recommended to bring your own/homemade food or choose a clean and hygienic place (hotel, restaurant, vendor) for having street food, and avoid street food in the rainy season. Vendors must adopt good handling practices and finally, local government or the respective authorities must check the quality of street food from time to time.

Key Words: Street foods, microbiological quality, hygienic practices, Gondia, Maharashtra.

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INTRODUCTION

The street food industry has played an important role in the cities and towns of many developing countries in meeting the food demands of urban dwellers. It feeds millions of people daily with a wide variety of foods that are relatively cheap and easily accessible but foods borne illnesses of microbial origin are a major health problem associated with it¹. The traditional methods of processing, improper handling, poor personal hygiene of vendors are the main causes of contamination in street-vended food and are subject to cross-contamination from various sources such as utensils, knives, raw foodstuffs, flies that sporadically landing on the foods, vendor's bare hand serving, and occasional food handling by consumers. Consumers 2002 who depend on such food are more interested in its convenience than in the question of its safety, quality, and hygiene². There are reports of food-borne illness associated with the consumption of street vended foods at several places in India and elsewhere^{3,4}. Thus, it is important to ensure food safety, as a public health measure toward reducing

the mortality rate⁵. Street vended foods as it is ready-to-eat foods are highly demanded both by the sellers and consumers because of their tastes, availability, low cost, cultural and social heritage connection, and serves to the major population those who are in the low-income group in the developing countries^{6,7}.

Street foods are frequently associated with diarrhoeal diseases, due to improper use of additives, the presence of pathogenic bacteria, environmental contaminants, and improper food handling practices based on good manufacturing practices (GMPs) and good hygiene practices (GHPs). Vendors are often from poor level education, unlicensed, untrained in food hygiene, work under crude unsanitary conditions, and lack of knowledge about the causes of food borne disease^{8,9}. Thus, potential health risks are associated with contamination of food by *Escherichia coli*, *Salmonella typhi*, *Pseudomonas* spp., *Staphylococcus aureus*, *Proteus* spp., etc., during preparation, post-cooking, and various handling stages^{10,11}. The serving utensils used at the vending site are often contaminated with *Staphylococcus* spp. which

perceives a major public health risk¹²⁻¹⁴. The present study deals with the microbiological analysis of the collected street food items, assesses the preparation, storage, and handling practices of the street food, safety aspects, and public awareness about street food consumption.

MATERIALS AND METHODS

Collection of Samples

During the study, 12 locations in the Gondia City, Maharashtra catering to different age groups and communities were chosen for the collection of samples and a total of 50 samples were analyzed in which Aloo bhajia/Bonda/Chaat (7), Bhel/Chaat (2), Bread Pakoda (5), Kachori (4), Kanda Bhajia/Vada (7), Mirchi Bhajia (1), Moong Vada/ Moongodi (4), Palak Vada (1), Pani Puri (3), Poha (6), Samosa (7) were included. The samples were collected in a sterilized container and transported to the laboratory within 1h on the same day.

Sample analysis

For analysis 1 g of food was diluted with 10 mL sterile distilled water. From this diluted sample, 0.5 mL was inoculated in 4.5 mL MacConkey broth and incubated for 4-5 h. The microbial growth observed as turbidity in broth was then subcultured on the Cysteine Lactose Electrolyte Deficient (CLED) agar and incubated at 37°C for 24 h. Tentative identification of isolates was made by Gram staining, motility, oxidase test, and cultural characteristics on CLED such as yellow-colored colonies of lactose fermenting *E. coli*, greenish color colonies of *Proteus* spp., greenish-blue or blue colonies of *Pseudomonas aeruginosa*, mucoid yellow to whitish-blue colonies of *Klebsiella* sp. and deep yellow opaque colonies of *Staphylococcus aureus*¹¹. Confirmation of various bacterial pathogens was made by subculturing on Xylose Lysine Deoxycholate Agar (XLD agar; M1108, Himedia, Mumbai), *Salmonella-Shigella*-agar (S-S agar M108, Himedia, Mumbai) for *Salmonella* spp, Mannitol salt agar for *Staphylococcus aureus*, Cetrinide Agar for *Pseudomonas* spp., MacConkey agar for other enteric pathogens along with various special biochemical tests. The confirmation of pathogens was made whenever necessary with standard methods shown in Himedia Laboratories manual, Mumbai, India. Along with street vended food samples, information, or data on season, place, and site of shop, time of collection, the

hygienic status of vendor and their servants, methods of food preparation, and hygienic condition of the vending site were collected and correlated these data with bacterial contamination in food.

RESULTS AND DISCUSSION

In the present study, a total of 50 street food samples were collected from different shops in Gondia City to isolate pathogenic bacteria (Fig. 5.1). A total of 102 microorganisms were isolated from street food samples collected. A total of 50 street foods samples were selected out of which 34 (68.0%) samples indicated the presence of pathogenic microorganisms, whereas 16 samples (32.0%) did not show presence of any pathogenic microorganisms in it (Table 5.1).

Table 5.1: Number of Contaminated food samples

Samples	Number of sample collected	Percent of contamination
Bacterial contaminated	34	68.0
Non-contaminated	16	32.0
Total	50	100

The most prominent bacterial pathogens isolated from 50 street vended food samples were *P. aeruginosa* (37.6%) followed by *S. aureus* (19.8%), *E. coli* (18.8%), *Salmonella* spp (11.9%), and *Proteus* spp. (11.9%)



Fig. 5.1: Various street food samples of Gondia city, Maharashtra

Table 5.2: Percentage of bacterial pathogens isolated from various street food samples

Type of sample	<i>S. aureus</i>	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>Salmonella</i> spp.	<i>Proteus</i> spp.	Total
Aloo Bhajia/ Bonda/Chaat	0	2(5%)	1(3%)	0	0	3(1%)
Bhel/Chaat	2(13)	1(5%)	6(17%)	1(9%)	1(9%)	11(12%)
Bread Pakoda	1(7%)	1(5%)	3(8%)	1(9%)	0	6(7%)
Kachori	1(1%)	0	2(2%)	0	0	3(1%)
Kanda Bhajia/ Vada	1(7%)	0	1(3%)	0	0	2(2%)
Mirchi Bhajia	1(7%)	0	2(3%)	1(9%)	0	3(3%)
Moong Vada/ Moongodi	4(27%)	6(32%)	7(19%)	2(18%)	23(18%)	21(23%)
Palak Vada	3(20%)	3(16%)	5(14%)	1(9%)	5(45%)	17(18%)
Pani Puri	4(12%)	2(11%)	2(6%)	0	1(9%)	9(5%)
Poha	1(1%)	2(11%)	1(3%)	2(9%)	0	5(4%)
Samosa	2(13%)	4(21%)	9(25%)	4(36)	2(18%)	21(23%)
Total	20 (19.8%)	19 (18.8%)	39 (37.6%)	12 (11.9%)	12 (11.9%)	102 (100%)

(Table 5.2). In earlier work, Tambekar *et al.*¹² reported high incidences of *Pseudomonas* spp. in street food samples of Amravati city. In the present study, percentage contribution of *Pseudomonas* spp. was found to be 37.6%, which might be due to poor personal hygiene of vendors and partially cleaned utensils. In addition, a slime layer or biofilm on the utensils might be an important source of street food contamination. The cloth used in cleaning dishes represents a hazard to the safety of foods⁹. In the present study, the presence of *E. coli* was 18.8% which might be through contaminated water supplies or by food handlers through poor hand washing or contamination of utensils and the absence of good manufacturing, handling, and serving practices⁸.

The *S. aureus* contamination was 18.8% (Table 5.2) in street foods samples which might occur through infected wounds, running hands through hair or scratching the scalp, cuts, burns, and dirty clothing of the vendors. In the present study, the contamination of *Salmonella* sp. and *Proteus* sp. contributed 11.9% each in street vended food (Table 5.2), which was due to contaminated water, sewage, soil, handling of food by infected workers, vendors, and consumers in the marketplace¹³.

The food, which was prepared already and kept, showed more contamination (75%) than food prepared on time (25%)¹¹. The demand for kachori and samosa was more, hence prepared them in larger numbers as well quite before serving. These foods were prepared at home and place in uncovered conditions at vending sites as a result of which more dust and soil particles adhered to them. Pohe, Bread Pakoda, Kachori, Kanda Bhajia/Vada, Mirchi Bhajia showed minimal bacterial contamination, as these food items were prepared freshly and consumed mainly in the morning. In the morning, there was less traffic and crowded in the market at the vending site. The cloth used in cleaning dishes represents a hazard to the safety of foods¹¹. The most dominating organism in the monsoon season was *E. coli*, which may occur due to human sewage or contaminated water as fecal matter gets mixed with water and causing more contamination of *E. coli* in the monsoon. This bacterial contamination might be due to dirty clothing, improper cleaning of glasses and dishes, unhygienic handling and serving practices, contaminated hands of the vendor, lack of knowledge of hygienic practices, and safety of food products^{8,14}.

CONCLUSION

The study demonstrated the unhygienic quality of the most popular types of street vended food. Hundred and two bacterial pathogens were isolated and identified, of which the most prominent organisms were *P. aeruginosa*, *S. aureus*, and *E. coli*. The contamination of food mainly occurred due to poor personal hygiene, environmental exposure, and improper handling and washing of utensils and lastly, foods are stored in uncovered conditions. Running water is not available at vending sites and dishwashing is usually done in the same buckets without soap. Materials used for wrapping are leaves, old newspapers, and reusable polythene bags that led to contamination of street food.

Though the street food is being prepared on daily basis and in some cases its preparation in hot oil may decontaminate the food (equals to sterilization) and could be safe for consumption, but the improper handling and unhygienic practices may lead to the contamination of pathogenic bacterial which are harmful to the consumer.

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Short Communication

PREVALENCE OF AEROMYCOFLORA IN THE LABOUR DELIVERY ROOM OF PATNA MEDICAL COLLEGE AND HOSPITAL, BIHAR

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The labour room of hospitals, often known as the Labour Delivery Room (LDR), is the place for the delivery of babies. Utmost importance should be given for maintaining the hygiene of these rooms. The present study was conducted in five LDRs of Patna Medical College and Hospital, Bihar during November, 2020 to March, 2021. Aeromycoflora of LDR was trapped both by using petriplate culture and Burkard Air Sampler. The study recorded 18 fungal species, of which *Cladosporium herbarum* was found to be the most prevalent genera followed by *Aspergillus flavus* with *Trichoderma* sp. as least preponderant species. Additionally, *Fusarium moniliforme*, *Penicillium* spp. were also found. Hence LDRs were not completely sterilized as well as were not safe for women and children. Therefore, constant monitoring for maintaining a clean and germ-free environment is needed.

Key Words: Aeromycoflora, labour delivery room, fungal colonies, Hospital, Patna, Bihar.

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INTRODUCTION

Patna Medical College and Hospital is one of the oldest medical colleges in Bihar. The present study was conducted in the labour delivery rooms (LDRs) of this hospital. Aeromycoflora is the causative agent of different diseases like allergy, bronchial asthma, pulmonary diseases¹. *Candida albicans* causes most neonatal infestations. *Rhizopus* sp., and *Mucor* sp. cause Mucormycosis disease² which is characterized by the growing hyphae surrounding the blood vessels, while *Aspergillus* sp. causes aspergillosis³.

Germ free environment is necessary in the LDRs for the safety of mother and newborn babies. Air sampling is the only way to know the fungal load in the air. The present study will provide necessary data regarding concentration and types of fungal spores present in the air of LDRs.

MATERIALS AND METHODS

Air sampling

Burkard Air Sampler was used to trap viable and non-viable fungal spores from the indoor environment of

LDRs in Patna Medical College and Hospital, Bihar, during November 2020 to March 2021. Spores were identified under microscope. Sampling was performed in weekly basis.

Trapping and culture of viable fungal spores

Petriplates containing sterilized Potato Dextrose Agar (PDA) medium were exposed for 10 to 20 minutes inside different corners of patient beds in the LDRs (Fig. 6.1). Then petriplates were incubated at 27°C for 3-4 days in incubator. After incubation fungal colonies were counted and percentage contribution of each species was calculated.

Identification of aeromycoflora

Identification of fungal species was made on the basis of micro- and macro-morphological characters. Colony characters and microscopic features were compared with literatures to identify the genera.

RESULTS AND DISCUSSION

Airborne fungal spores were recorded in the LDRs. They showed varying seasonal periodicities. In this study 333 colonies of 18 different fungal species were



Fig. 6.1: Exposed petriplates at different corners of patients' bed in the LDRs, Patna Medical College and Hospital, Bihar.

isolated. *Cladosporium herbarum* was found to be the most prevalent fungal genera followed by *Aspergillus flavus* (Fig. 6.2). Additionally, *Microsporium canis*, *Aspergillus fumigatus*, *Penicillium* sp. were also found.

Fusarium moniliforme, *Mortierella*, *Trichoderma* were the least dominant species. The number of colonies of each species and their percentage contribution have been given in Fig. 6.2.

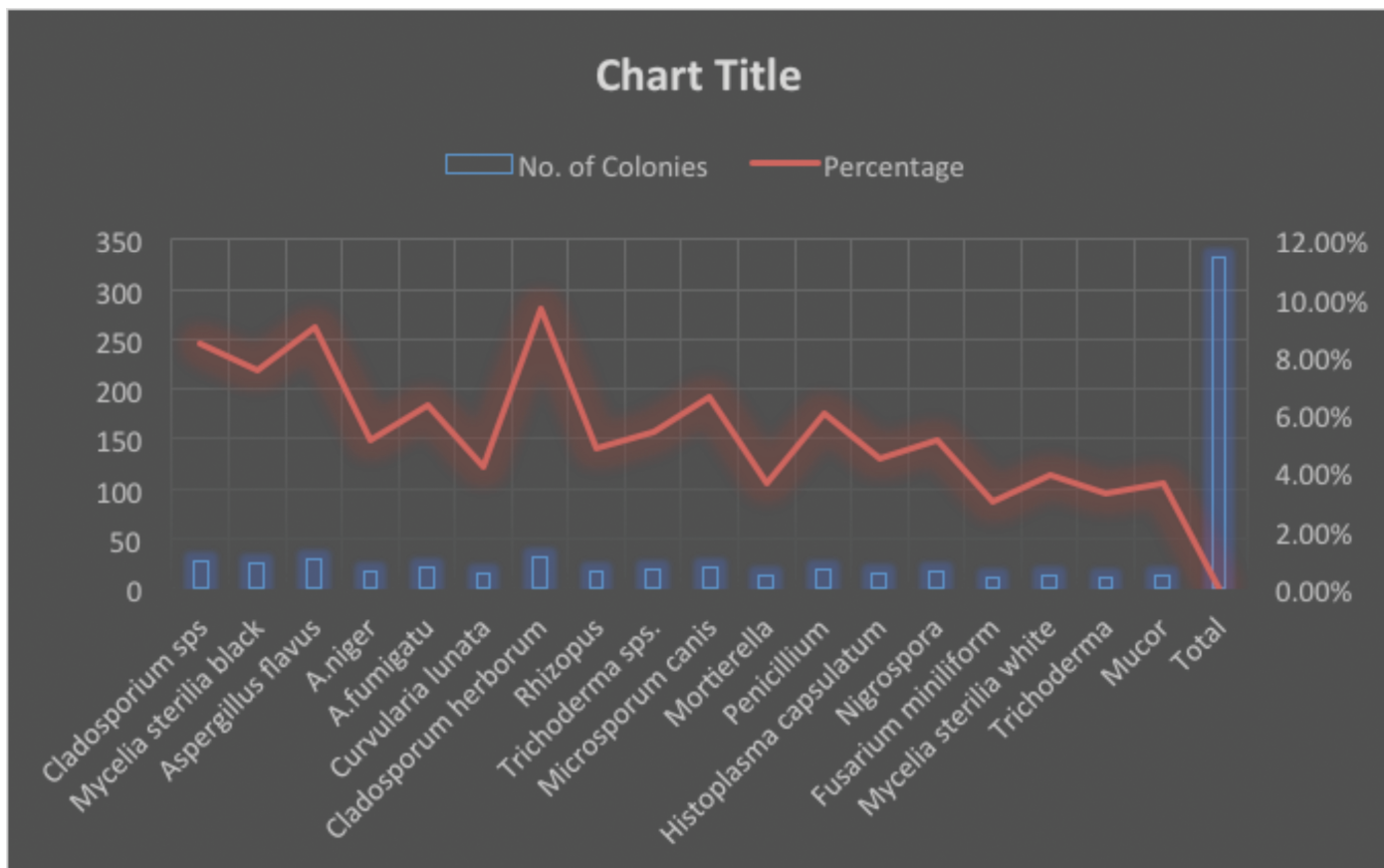


Fig. 6.2: Graph showing no.of fungal colonies and their percentage of contribution

Airborne fungal spores in various environments have already been reported from different parts of India and abroad^{4,5,6}, however, there is hardly any study in LDRs. Hospital based studies were also conducted. This study provided the scenario of indoor environment of hospital. Frequent monitoring and precautionary measures like regular cleaning, fumigation etc. will improve the indoor air quality of hospital and may increase patients' safety.

CONCLUSION

The present aeromycological survey in labour delivery rooms (LDRs) of Patna Medical College and Hospital (PMCH), Bihar showed a number of harmful fungi in LDRs. The indoor and outdoor areas must be kept clean always, proper ventilation is also needed. Beds, tables and other equipments and tools must be cleaned properly. ICU rooms and air conditioning machines should be cleaned properly in regular intervals. The maintenance of hygiene is very necessary, not only for patient, but also for the safety of other personnel like doctors, nurses and visitors.

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Short Communication

AEROMYCOLOGICAL ASSESSMENT OVER PADDY (*ORYZA SATIVA* L.) FIELDS IN RAIGAD DISTRICT, MAHARASHTRA

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Aeromycological study was carried out over paddy fields during rabi season (2022) at Raigad district in the Konkan region of Maharashtra. Paddy is a staple food crop in the Konkan region. For this study, Tilak's Air sampler was used. The objective of this study is to find out the impact of fungal spores on paddy crops. During this study, 22 airborne fungal spores were recorded which range from 4.3% to 4.9%. It was observed that most of the fungal spores cause diseases to paddy crops.

Key Words: Aeromycology, paddy fields, Raigad, Maharashtra.

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INTRODUCTION

For this study, paddy fields were selected at different places of Raigad district in the Konkan region of Maharashtra state with respect to the latitudes and longitudes. The different types of varieties are cultivated in Maharashtra states like Jaya, Pusa-2-21, Sabarmati, Vijaya, Ratna, Karjat-184, Ratnagiri 24, Sona, Pankaj, Jagannath, Surya, Satya, Suhasini, Ratnagiri-1, Karjat-35-1, Pusa-33, Ratnagiri-2, ACK-5, Kundlika, Karjat-1, Karjat-5, Karjat-6 and Sahyandri, HMT, Kolamba, Parag, etc. This study was carried out during rabi season and Karjat-5 variety was selected because this variety is cultivated at a large scale in the Raigad district of Maharashtra. The objective of the present study was to find out the impact of fungal spores on the paddy crop.

MATERIAL AND METHODS

For the sampling of fungal spores over largely cultivated paddy fields in the Raigad district Tilak's air sampler¹ was used. The sampler was run on an electric power supply with a continuous sampling of air for eight days. Air was sucked through the orifice of the projecting tube at the rate of 5 lit/min and it impinged on transparent

cello tape. Before the tape was mounted on a glass slide, at the end of eight days, it was divided into eight equal parts. Thus, each piece represented the one day or 12 hours sampling area. The tape was mounted on a slide with glycerin jelly, then scanning of slides was done under microscope.

RESULTS

After sampling and scanning of slides for one season, 22 different fungal spore types were recorded over the paddy fields (Fig. 7.1). These fungal spores caused different types of diseases to the paddy crop.

Brief description of 22 fungal spore types identified from the present study:

1. *Alternaria* Nees. The size of the spore ranges 5-11 × 4-5 μm. The conidia is dark, with transverse and longitudinal septa, variously shaped, ovoid, and frequently borne acropetally in long chains. Often it is borne singly having an apical simple or branched appendage of different sizes, causes leaf spots in the paddy crop².
2. *Beltrania* Penzig. Spores are one-celled smooth,

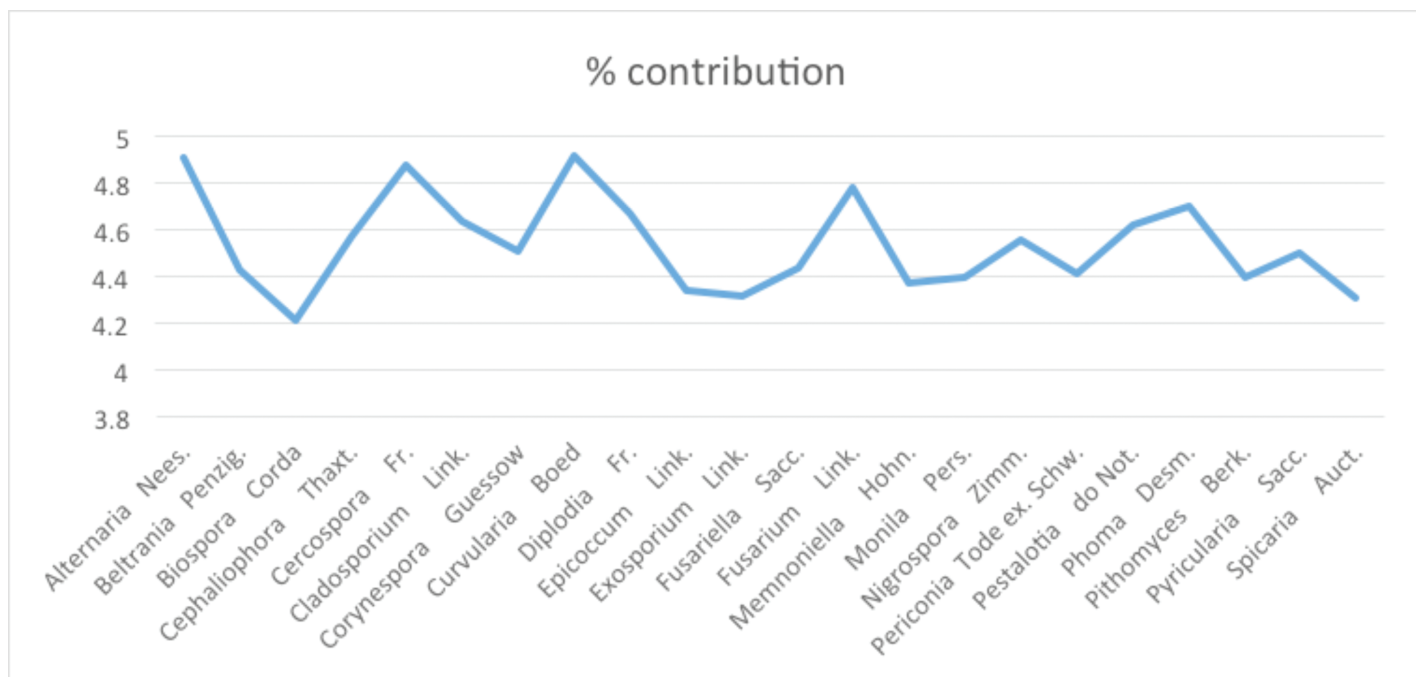


Fig. 7.1: Percentage contribution of 22 different fungal spores over cultivated paddy fields in the Raigad district of Maharashtra

bi-conic brown with a paler middle band. The size of the spore is $12-23.5 \times 8.5-11.5 \mu\text{m}$. Conidia is round with a scar or denticulate at the base, with a long conical, one-celled, hyaline to subhyaline septa at the apex

3. *Biospora Corda*. Conidia are mostly bi-celled, less often tri-celled, dark, oblong with thick black septa, catenulate measuring $5-2 \times 6-8 \mu\text{m}^3$.
4. *Cephalophora* Thaxt. Spores are ovoid to elongate, narrower at the base, the upper cell triangular, hyaline, relatively thin-walled, 3-more separate, $20-46 \times 14-2 \mu\text{m}$.
5. *Cercospora* Fr. Conidia are dark, 3-5 celled, fusiform, typically bent or curved with one or two central cells enlarged. Two types of spores were found, with $20-30 \times 7.4-17.5 \mu\text{m}$.
6. *Cladosporium* Link. Conidia are hyaline to dark brown, produced by the budding single or branched chain, easily separating, polymorphous, globular, oval, ovoidal to cylindrical, some are typical lemon-shaped, obtuse, or tapering at once or both the ends, smooth or finely verrucose, 1-4, length variable $4-4.8 \times 2-4.8 \mu\text{m}$ conidiophores⁴.
7. *Corynespora* Guessow. Spores are single-celled, terminal sometimes occurred in short-chain, obclavate or cylindrical, straight or curved smooth brown with 4-20 pseudo-septa with a thick colorless and prominent dark based scar, $55-0 \times 8-16 \mu\text{m}^5$.
8. *Curvularia* Boed. Conidia are dark, 3-5 celled, more or less fusiform, typically bent or curved with one or two central cells enlarged. Two types of spores were found, one with $20-30 \times 7.4-17.5 \mu\text{m}$ and another $30.5-49 \times 9-23.6 \mu\text{m}^6$.
9. *Epicoccum* Link. Conidia are globose to sub-globes dark brown, one to several celled, irregularly septate, 7-9 μm in diameter.
10. *Exosporium* Link. Conidia are apical, single, obclavate, broadest towards the base becoming much narrow tapering above, brown varicose, 3-7 septate, $55-74.5 \times 18-23 \mu\text{m}$.
11. *Fusariella* Sacc. Conidia are 3-celled, cylindrical, greenish dark, curved septate and borne in chains, and each conidium is attached at the side of the conidium below and not end to end, $18-25 \times 4-7 \mu\text{m}$.
12. *Fusarium* Link. Conidia are hyaline variable, macro-conidia several celled slightly curved or bent at the pointed ends, typically curved shape $20.5-26 \times 5-6.5 \mu\text{m}^7$.
13. *Diplodia* Fr. Conidia are dark brown 2-celled, ellipsoidal or ovoid, thick-walled, measuring $12-15.5 \times 3-7 \mu\text{m}$. The spores were trapped throughout the period of investigation. Spores cause tip blight disease⁸.
14. *Memnoniella* Hohn. Conidia are dark, one-celled, globose $6.6-9.0 \mu\text{m}$ in diameter³.

15. *Monila* Pers. Spores are 1-celled, short cylindrical to rounded, in acropetal branched chains; conidiophores branches, its cells differing little from the older conidia; spores hyaline, gray in mass, 15-25 μm long and 10-15 μm in width.
16. *Nigrospora* Zimm. Conidia are dark, smooth, opaque, black, one-celled lobe to somewhat flattened 12-23 \times 10-26 μm , spores occurred throughout the period of investigation. They belong to "Day spora"³.
17. *Periconia* Tode ex Schw. Conidia are dark, smooth, opaque, one-celled, globose to somewhat flattened, 12-23 \times 10-26 μm ³.
18. *Pestalotia*. Spores are 5-false-septate, 4-median cells brown, end cells hyaline, 3-9 apical, cellular, simple or dichotomously branched appendages, and 1-basal cellular simple or branched appendages, 14-23 \times 5-7 μm ⁶.
19. *Phoma* Desm. Spores are one-celled hyaline, globose to sub-globose with 6 μm in diameter, spores are found on dead and decaying material of plants near the surrounding crops⁹.
20. *Pithomyces* Berk. Conidia are single, broadly elliptical dark brown, oblong with pyriform or irregular doliform, many-celled with transverse and vertical septa 20-26 \times 9-17 μm ¹⁰. Spores are also found on dead leaves.
21. *Pyricularia* Sacc. Conidia are elongated, pyriform to obclavate to narrowed or tapering towards the apex and rounded at the base, 2-3 \times 6-10 μm ¹¹.
22. *Spicaria* Auct. Spores are 1-celled, mostly globose or sometimes globose elliptic, smooth, hyaline 2.5-3 \times 1.8-2.5 μm . Conidiophores erect branched; branches and phialides divergent and loose; conidia in dry basipetal chain.

DISCUSSION

During this study, 22 types of fungal spores were recorded belong to a diverse families that cause different types of plant diseases. Almost all the fungi occurred in air in moderate concentration showing their percentage distribution between 4.3 to 4.9. Some spores are soil-borne, some are endophytic and some of them are found

to be saprophytic. The present preliminary study will perhaps help paddy cultivators/farmers to take preventive measures against such fungal diseases.

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