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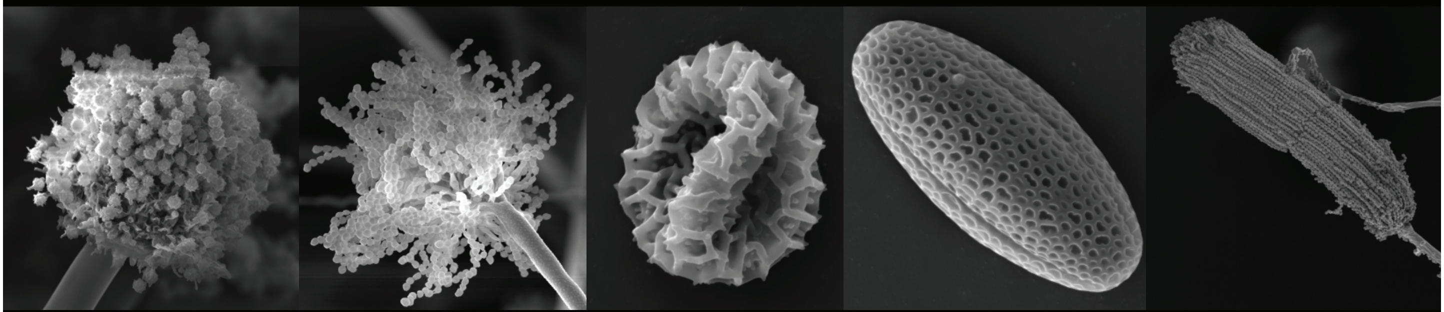
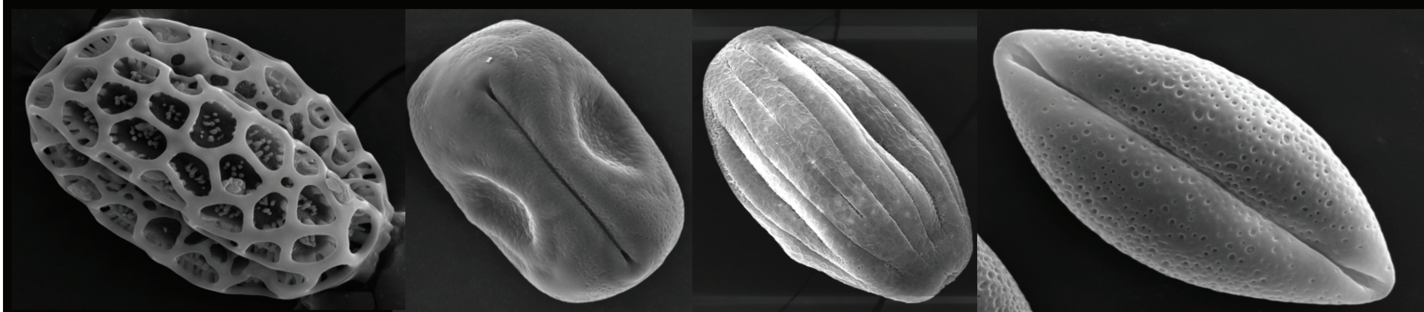


INDIAN JOURNAL OF AEROBIOLOGY



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Research Article

INTEGRATING AI AND BIOINFORMATICS FOR AUTOMATED DETECTION OF AIRBORNE POLLEN GRAINS AND FUNGAL SPORES

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Airborne pollen grains and fungal spores pose a significant health risk, particularly with seasonal allergies, which is being worsened due to climate change. Traditional methods of detection of airborne pollen grains and fungal spores are laborious and time-consuming, need knowledge of aerobiologists, which needs automated solutions. The present study gives an innovative approach utilizing IoT-based hardware and machine learning technology to automate the detection process.

The present method integrates Raspberry Pi High Quality Camera with a microscope for capturing microscopic images of pollen grains and fungal spores. Leveraging custom Convolutional Neural Network (CNN) architectures, we train models to classify samples into pollen grains or fungal spores, detect their types, and distinguish between different species. The deployment of models on edge systems such as Jetson Nano Developer Kit enables real-time prediction without reliance on cloud-based analysis, reducing both cost and processing time.

By leveraging pre-collected bioinformatics data, which includes information on pollen types prevalent in different regions, we use bioinformatics information from Manipur for building this AI model for pollen like *Eucalyptus, Brassica, Cannabis* etc. and we aim to enhance the training process of our machine learning models. This approach allows for the customization of detection algorithms to better suit the local pollen landscape, ultimately leading to more precise identification and classification. By consolidating datasets and employing state-of-the-art CNN architectures like YOLO (You Only Look Once) v5, our approach achieves high accuracy rates of 90-95% in identifying fungal spore types (e.g., *Cladosporium, Verticillium*, etc.) and pollen grain types (e.g., *Brassica, Cannabis*, etc.). With further data augmentation and refinement, accuracy rates can potentially reach up to 99%.

This automated detection system offers a promising tool for allergologists and healthcare professionals by providing timely warnings for allergic diseases and facilitating proactive measures. The integration of AI and bioinformatics not only streamlines the detection process but also enhances the scalability and accessibility of allergy management, ultimately contributing to improved public health outcomes.

Key Words: Machine learning, Raspberry Pi High Quality Camera, Microscopic image, pollen grains and fungal Spores, Convolutional Neural Network (CNN), YOLO v5, ResNet, AI, Bioinformatics.

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INTRODUCTION

In recent years, the integration of artificial intelligence (AI) with bioinformatics has opened up new avenues in various scientific fields. One promising application is in allergology, where the prevalence of airborne allergens like pollen grains and fungal spores presents significant challenges. Traditional monitoring methods are slow and limited in coverage. However, the marriage of AI and bioinformatics offers a revolutionary approach, automating allergen detection and providing faster, more comprehensive insights. This article/ lecture explores how AI algorithms, combined with bioinformatics tools, enable real-time analysis of environmental samples for rapid and precise allergen identification. Machine learning models ensure adaptability to different conditions, vital for capturing dynamic allergen patterns. The paper/ lecture delves into the system's key components and methodologies, highlighting benefits such as improved diagnosis and treatment strategies for allergologists. Ultimately, this convergence promises to revolutionize allergology, offering powerful tools to enhance understanding and management of allergies while advancing environmental health research.

Artificial Intelligence and Automation

Artificial intelligence (AI) involves machines programmed to emulate human intelligence and actions. Traditionally, AI focused on optimizing search methods, but with the rise of big data and powerful computing, AI has shifted to a data-centric approach. Deep Learning, utilizing Neural Networks like Convolutional and Recurrent Neural Networks, is gaining traction for solving complex tasks such as language translation and autonomous driving[1].

Computer Vision

It is an AI field focused on extracting useful insights from digital images and videos to guide actions. It employs image processing algorithms and convolutional neural networks to tasks like object detection and facial recognition.

Convolutional Neutral Network (CNN)

A Convolutional Neural Network (CNN) is a type of Deep Learning algorithm used for image analysis. It automatically learns to recognize important features in images through training, requiring minimal preprocessing. Inspired by the organization of the human visual cortex, CNNs use learnable filters to detect patterns in images. Each neuron responds to a specific region of the visual field, and their collective responses cover the entire image[1] (Figure 1.1).

Edge AI computing

Edge AI computing involves deploying AI software on low-power edge devices like microcontrollers instead of large servers. This offers several advantages:

1. Enhanced Data Security and Privacy: Processing data at the edge device reduces vulnerability to hacking, improving security and privacy.
2. Real-time Analytics: By processing data locally, latency is reduced, enabling real-time analytics.
3. Lower Internet Bandwidth Usage: Edge processing reduces the amount of data sent to servers, minimizing bandwidth consumption.
4. Reduced Power Consumption: Local processing eliminates the need to transmit data to servers, conserving energy and reducing power consumption.
5. Improved Responsiveness: Edge AI solutions enable faster response times for smart devices by eliminating the need to send data to the cloud for computation[1].

Role of AI in detection of and recognition of airborne pollen grains and fungal spores

AI plays a crucial role in the detection and recognition of airborne pollen grains and fungal spores, aiding in monitoring air quality and its impact on human health. Traditionally, monitoring networks have relied on methods like Hirst-type volumetric sampling, which are decades old but considered the gold standard. These networks provide valuable datasets for studying long-term trends in pollen and spore concentrations, biodiversity shifts, and climate change impacts. AI technologies enable more efficient and accurate analysis of these

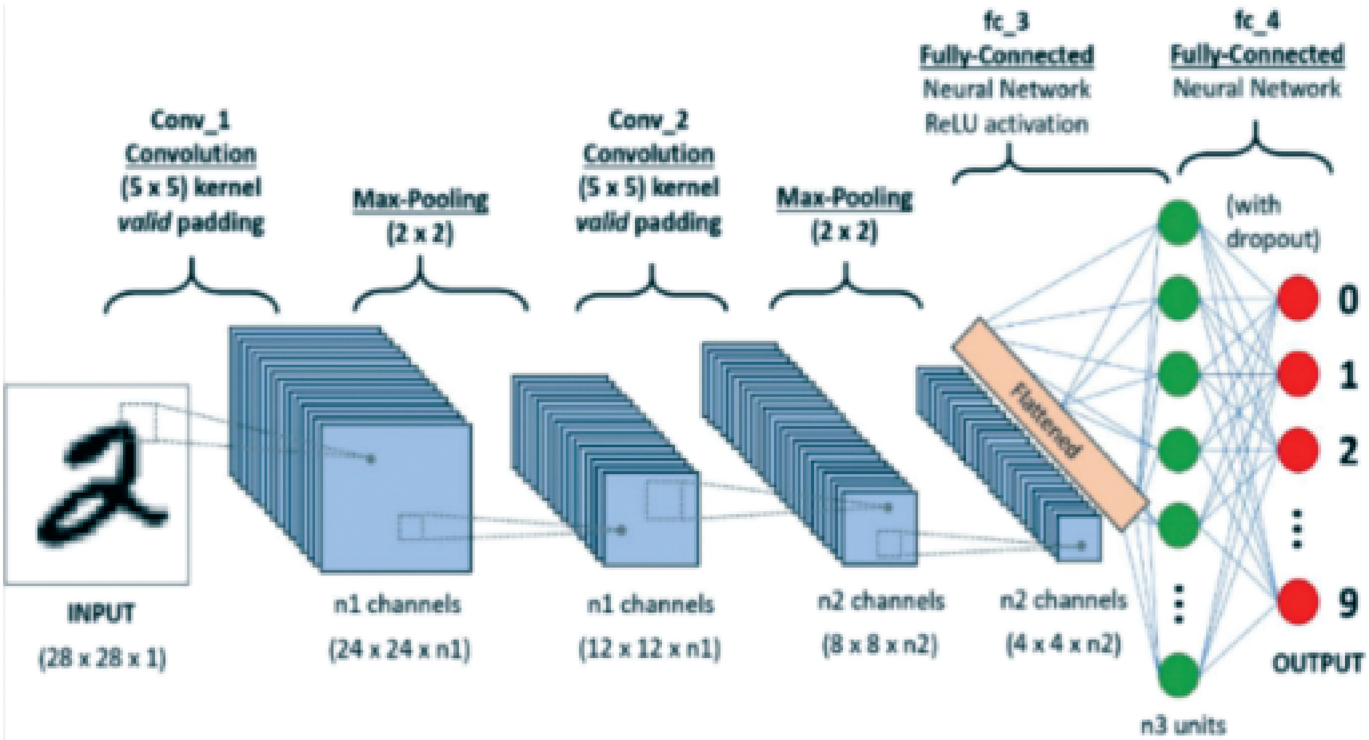


Fig. 1.1: A CNN (Convolutional Neural Network) sequence to classify handwritten digits. (Towards Data Science).

datasets, helping identify patterns, trends, and correlations with factors like SARS-CoV-2 infections during events like the COVID-19 pandemic.

Despite the growing demand for longer time-series and real-time data at higher resolutions, monitoring of airborne pollen and fungal spores remains labour-intensive and temporally low-resolution. Current methods often result in delays of at least a week in data delivery. However, immediate effects of pollen exposure are well-documented, with the strongest symptoms observed on the same day as exposure. Any delay beyond a day in disseminating pollen exposure information is considered impractical for allergy management and medical treatment. Understanding concentration-response associations is crucial for assessing health risks, but these relationships are complex, non-linear, and taxon-specific. Research across different pollen taxa and locations is necessary to provide consistent and locally relevant evidence on the clinical significance of pollen data and forecasts[2].

Current techniques for simulating human respiratory function during pollen and fungal spore sampling aim to replicate calm conditions but may not reflect real-life situations accurately. Activities like working, walking, and exercising increase respiratory rates significantly, affecting sampling accuracy. Traditional low-volume sampling techniques may also underestimate rarer particle concentrations. This limits our ability to assess the true exposome, establish concentration thresholds for pollen and spores, quantify allergic reactions and symptoms, and effectively manage bioaerosol-related diseases.

The primary obstacle hindering the advancement of bioaerosol research and biomonitoring techniques is the consistent lack of continuous public funding, unlike the detection of chemical pollutants. Despite bioaerosol time-series being extensive, they are typically not open-access due to the financial burden on research institutes, universities, and private initiatives. There is a critical need to transition to faster, online, and more precise reporting of airborne pollen and fungal spore concentrations, which has been highlighted as a pressing issue within the aerobiological community.

Recent efforts have concentrated on creating automated sampling devices in real-time, blending microbiology, engineering, and informatics. These advancements have enhanced particle capturing, recognition, and data dissemination, facilitating broader ecological interpretations. New techniques like air-flow cytometry[3] (e.g., Yamatronics KH-3000, Plair PA-300, Pollen Sense™ , WIBS sensor) and DNA metabarcoding (using trnL and nrITS2) have improved taxonomic resolution for pollen in aerobiological samples, though not yet achieving near-real-time temporal resolution. However, there remains a significant gap in applying automation to other bioaerosols like fungi or bacteria, and in developing such methods for indoor environments, which have been traditionally overlooked.

In aeropalynology, traditional methods rely on image-based identification of particles, which has garnered attention for over a decade. Automating this process remains a central pillar of technological advancement. High-through put flow has enhanced device efficiency compared to conventional methods, with automated microscopy aiding particle identification per pollen/spore type using image recognition algorithms. Integrating advanced deep learning methods further improves results, showcasing significant progress in the field[4-8].

The BAA500 (Hung Gumbh) biomonitoring system, utilizing automatic image recognition, is the foremost example, operating in Bavaria, Germany. Initial results demonstrate significant performance, with over 90% accuracy in pollen recognition. However, challenges such as device reliability over extended monitoring periods, experimental reproducibility, comparability of techniques and datasets, and addressing reporting biases, are common amidst rapid technological advancements.

In this study, a unique dataset was utilized, the largest known of its kind, comprising manually identified pollen images and a wide diversity of pollen taxa. The results from two different devices were presented, assessing the reliability, performance, accuracy, and comparability of the biomonitoring system. Comparison was made, using a commercial image library with an improved, manually classified version and evaluate against the 'gold standard' Hirst-type pollen measurements. The primary objective was to assess if the automatic, near-real-time biomonitoring system differs from conventional methods and to identify the advantages and limitations to enhance the development of this novel technique.

Aerobiology in India using Bioinformatics Tools

Bioinformatics is the application of computational techniques to comprehend and organize information related to biological macromolecules. It emerges from the recognition that biology functions akin to an information system, where genes serve as digital repositories dictating an organism's physiology and behaviour. Advances in data generation technologies, such as producing over 100 gigabytes of data daily in experimental laboratories, coupled with enhancements in CPU, disk storage, and Internet capabilities, have revolutionized data processing, storage, and exchange in the field[9].

Digitalization for Aerobiological data of Manipur state, India for development of a database has been initiated. A relational database has been rendered with a simple and robust web-based search facility developed by using PHP (Hypertext preprocessor), MySoL; HTML and CS. At first, the information was compiled from various primary sources and organized into folder/file metaphor (Microsoft Windows)[10]. Approximately 97 airborne pollen types have been uploaded into database from work done in the Manipur University, Canchipur. The database will act as a resource for the information about airborne pollen grains which give information about species found in the NE India especially in Manipur and toxin containing in 6(six) pollen family types[6]. We also collected primary resources from previous works done under Manipur University – data from already published/ unpublished articles, journals, Ph.D. theses, etc. and secondary resources – being constructed from the primary sources available – 97 airborne pollen grains types – Airborne Pollen Grains Database of Manipur state – APGDMAN database[11-12].

Bioinformatics with AI for diagnosis and treatment of allergic diseases

Bioinformatics empowered by AI offers allergologists predictive analysis capabilities to anticipate allergic susceptibilities. By integrating genomic and environmental data, AI algorithms discern patterns for personalized medicine approaches. This synergy expedites drug development, identifies novel targets, and enables early diagnosis and personalized interventions. AI-driven patient management tools facilitate optimized treatment strategies, remote monitoring, and research acceleration, promising improved outcomes and enhanced patient care in allergic conditions[13].

METHODOLOGY

HARDWARE USED

Jetson-Nano-Developer-Kit

The NVIDIA Jetson Nano Developer Kit (Fig. 1.2) offers high-performance computing tailored for modern AI tasks, all within a compact, cost-effective package. With versatile connectivity options and low power consumption (as little as 5 watts), developers can easily integrate various sensors and create diverse AI applications.

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

The Jetson Nano (Fig. 1.2) is backed by NVIDIA Jet-Pack, providing comprehensive support including a board support package (BSP), Linux operating system, and essential software libraries such as CUDA, cuDNN, and TensorRT. These libraries empower developers with tools for deep learning, computer vision, GPU computing, multimedia processing, and a wide array of other applications[1] (Fig. 1.3).

Rasberry Pi High Quality Camera

The Raspberry Pi High Quality Camera is an accessory boasting enhanced specifications compared to its predecessor, the Camera Module v2. With a resolution of 12 megapixels and improved low-light performance due to larger pixel area, it offers superior image quality. It supports interchangeable lenses in both C- and CS-mount formats, with options for other lens types through third-party adapters[1].

Fig. 1.3: Image of Raspberry Pi High Quality Camera

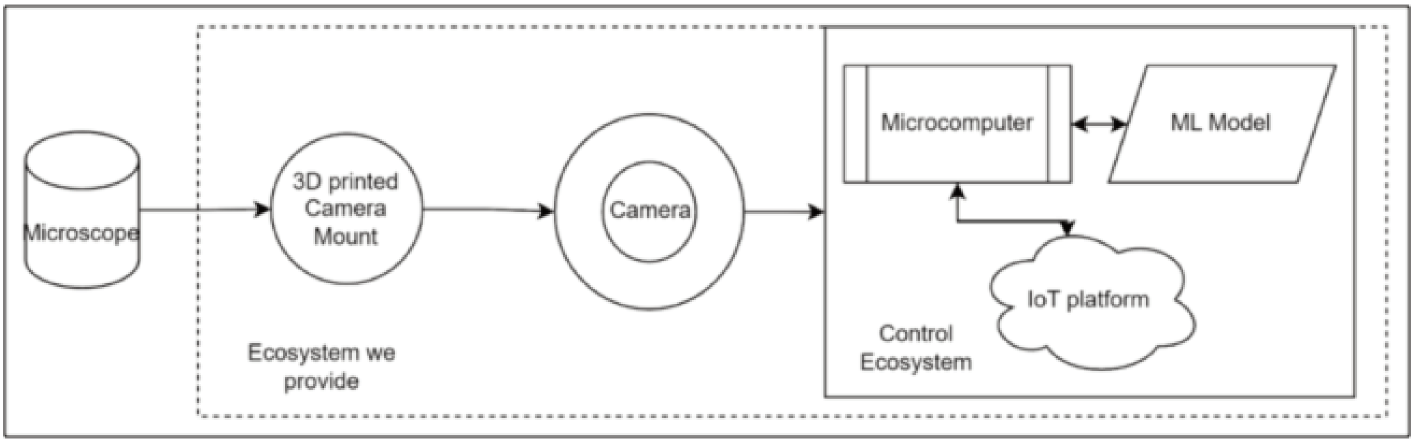


Fig. 1.4: Hardware Flow Diagram

Hardware working

The hardware setup comprises a microscope, camera, 3D printed camera mount, and a microcomputer. The microscope assists in capturing sample images, with a camera mounted on it using a custom 3D printed mount. These captured images are then transmitted to a micro-computer where a machine learning model is employed to detect and recognize pollen grains and fungal spores. Finally, the results are sent via an IoT platform using either Wi-Fi or Ethernet connectivity[1] (Fig. 1.4).

Software working

The software solution entails training three distinct neural network models. The initial model categorizes an image sample into either a pollen grain or a fungal spore. Subsequently, based on this classification, the remaining two models identify the specific type of pollen grain or fungal spore. This workflow facilitates comprehensive analysis and classification of image samples captured by the hardware setup[1] (Fig. 1.5).

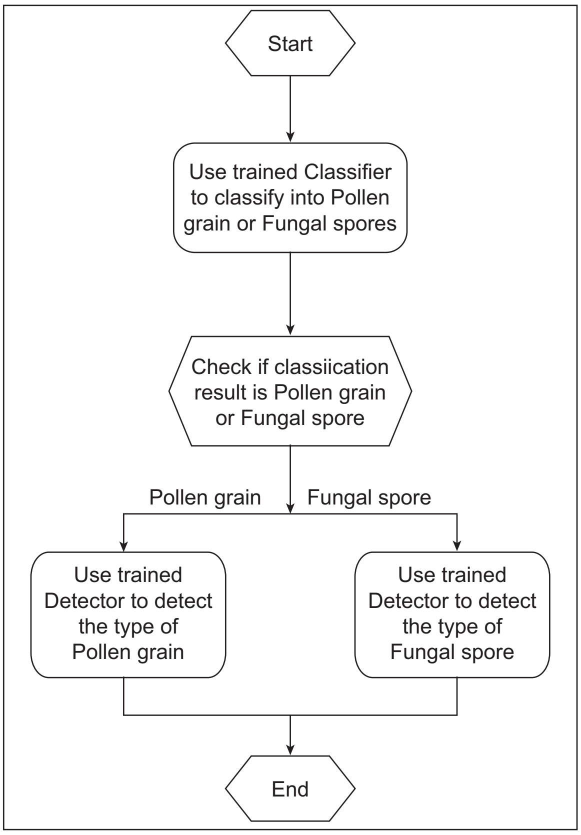


Fig. 1.5: Software Flow Diagram

Data Collection

The workflow includes training three distinct neural network models for classifying fungal spore and pollen grain samples. To facilitate this, collections were gathered from diverse publicly available datasets and combined into a compiled dataset for training and validation. These samples undergo preprocessing to ensure uniformity for training and testing. If the sample size is inadequate, augmentation techniques can be utilized to generate additional samples[14].

To enhance the detection of pollen grain and fungal spore types, publicly available datasets are supplemented with manually collected and annotated samples[15]. This process ensures a comprehensive dataset for training. Preprocessing and augmentation techniques are then applied to create a robust database. Given the limited number of manually collected samples, proper augmentation is crucial to adequately populate the dataset. Moreover, meticulous annotation is essential to prevent bias during training, highlighting the importance of accurate sample representation.

AI Framework Used

The AI framework used for training the model is Edge Impulse, which facilitates the development of AI models without coding. It employs the YOLO (You Only Look Once) Model Framework[16] and operates as a web-based tool, enabling quick and straightforward creation of machine learning models. Users can upload training samples or capture them using device cameras via a graphical interface. Utilizing transfer learning with pre-trained weights, Edge Impulse streamlines the training process, eliminating the necessity for extensive data gathering (Fig. 1.6).

Edge Impules

Edge Impulse is an advanced platform that sits at the forefront of merging IoT devices with machine learning. It enables developers to craft intelligent solutions that operate directly on edge devices, eliminating the necessity for continuous cloud connectivity[16]. This feature is especially crucial in contexts prioritizing real-time processing, low latency, and privacy, such as healthcare applications like allergology. By utilizing Edge Impulse, allergologists can efficiently gather, analyse, and respond to patient data, facilitating personalized and timely interventions tailored to specific allergic profiles.

Edge Impulse streamlines development through its user-friendly interface and a robust set of tools for data collection, model training, and deployment. This accessibility democratizes machine learning adoption in IoT applications, allowing healthcare professionals, including allergologists, of all technical backgrounds to utilize advanced analytics and predictive capabilities. With increasing demand for intelligent healthcare solutions, Edge Impulse emerges as a versatile platform, empowering allergologists to optimize patient care through the full potential of edge computing and machine learning.

YOLO

YOLO v5, the fifth version of the You Only Look Once (YOLO) object detection algorithm, represents a notable advancement in computer vision. Developed by Ultra-

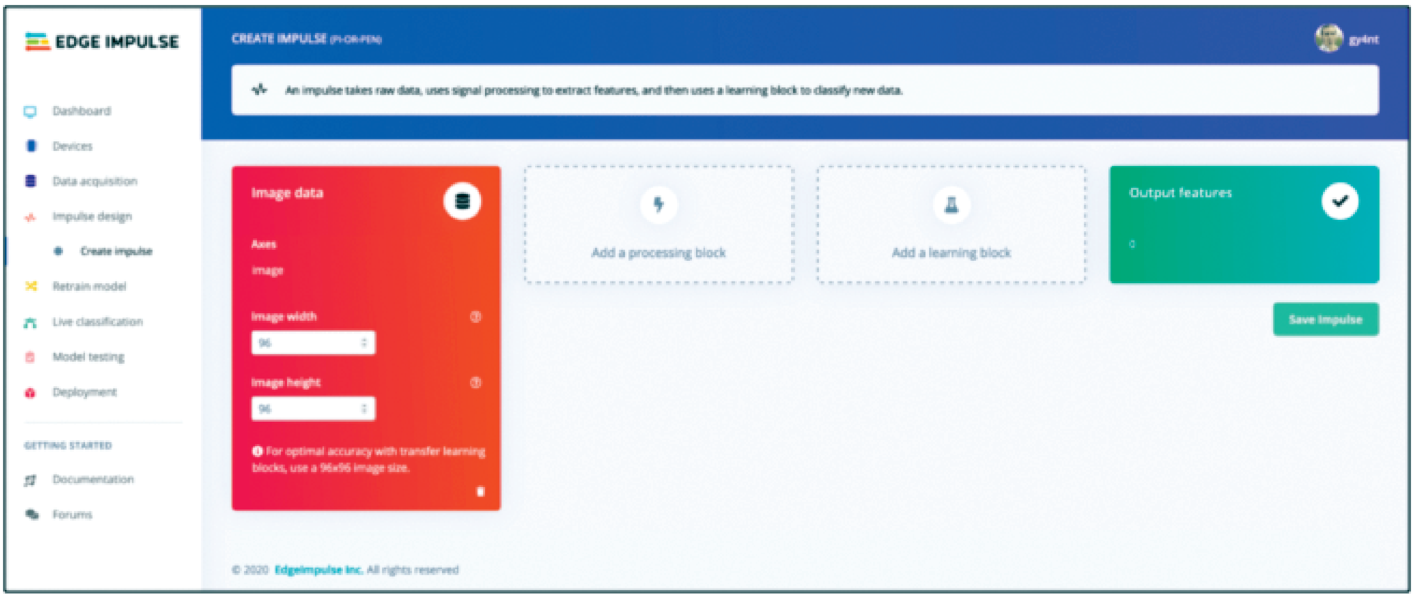


Fig. 1.6: Edge Impules IDE

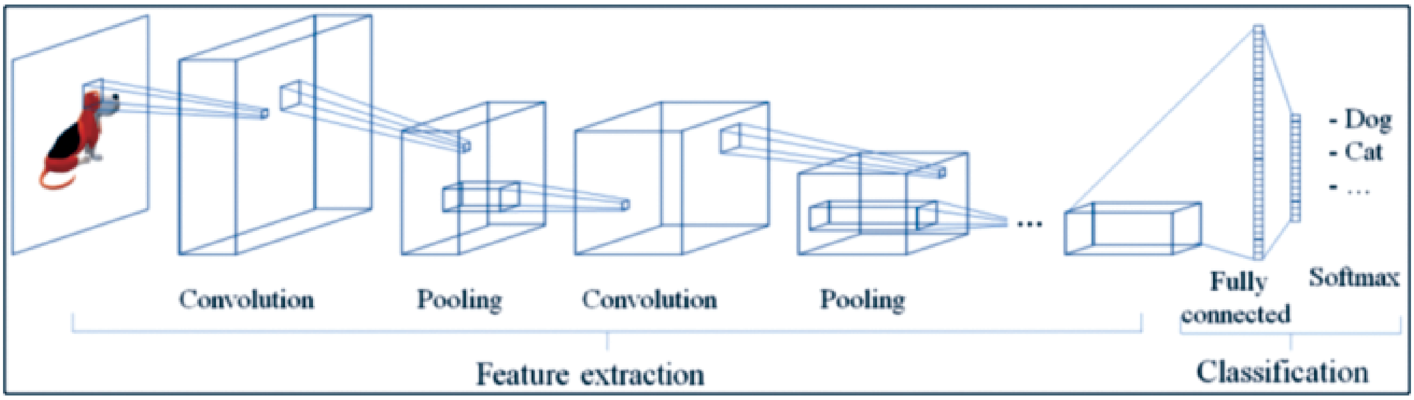


Fig. 1.7: How YOLO Work (Source Application of YOLO v5 and v8 for Recognition of Safety Risk Factors at Construction Sites Fig. 1.1)

lytics, it aims to enhance accuracy, speed, and ease of implementation compared to its predecessors. Key features include improved accuracy, real-time performance, simplicity in architecture, versatility across computer vision tasks, and its open-source nature. Operating on a single-stage detection principle, YOLO v5 predicts bounding boxes and class probabilities directly from grid-divided input images using convolutional neural networks. Trained on large datasets like COCO, it excels in detecting various objects across categories, with applications in autonomous driving, surveillance, robotics, healthcare, and beyond, signifying its potential for future advancements in computer vision[17] (Fig. 1.7).

IoT platform used for Collecting Data after Identification of Pollen Grains and Fungal Spores

The IoT platform utilized for collecting data post-identification of pollen grains and fungal spores is Thingsboard, provided by the company located in New York, USA. Thingsboard serves as the repository for data generated by the IoT device. Additionally, it counts the number of pollen present on a particular slide, storing this information for future projects focused on predicting pollen grain and fungal spore levels in the air. This platform facilitates predictive analysis to anticipate peak pollen levels, enabling proactive measures for allergy management (Fig. 1.8).

To train the AI algorithm for detecting and locating pollen grains, we begin by acquiring a pollen dataset from Kaggle, containing images of various pollen types. From this dataset, we select three specific pollen types, such as *Eucalyptus*, Poaceae, and *Sida* each comprising 25 images. These images are then placed randomly on a white background image to mimic microscope imagery. Additionally, we annotate the location of pollen grains within each image and extract features such as color, texture, and size. This annotated and feature-extracted data is then fed into our AI framework, YOLO. YOLO is trained on this data to learn the characteristics and locations of pollen grains. Subsequently, we evaluate the trained AI model's accuracy through testing, assessing its performance in accurately

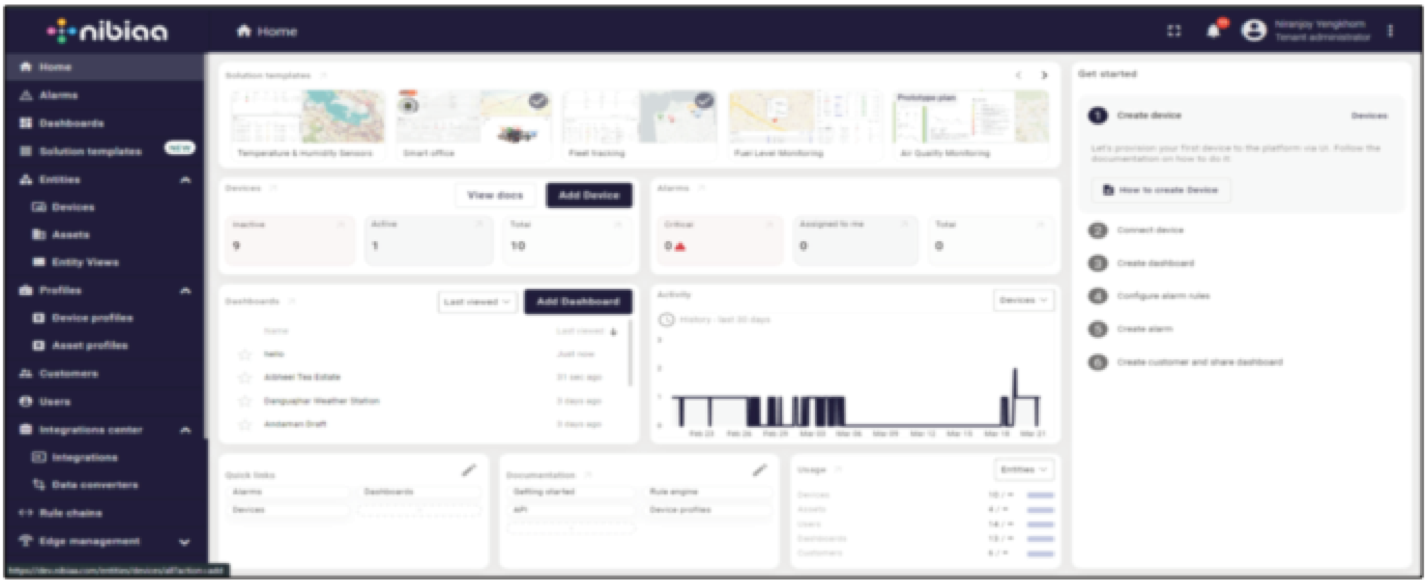


Fig. 1.8: IoT platform

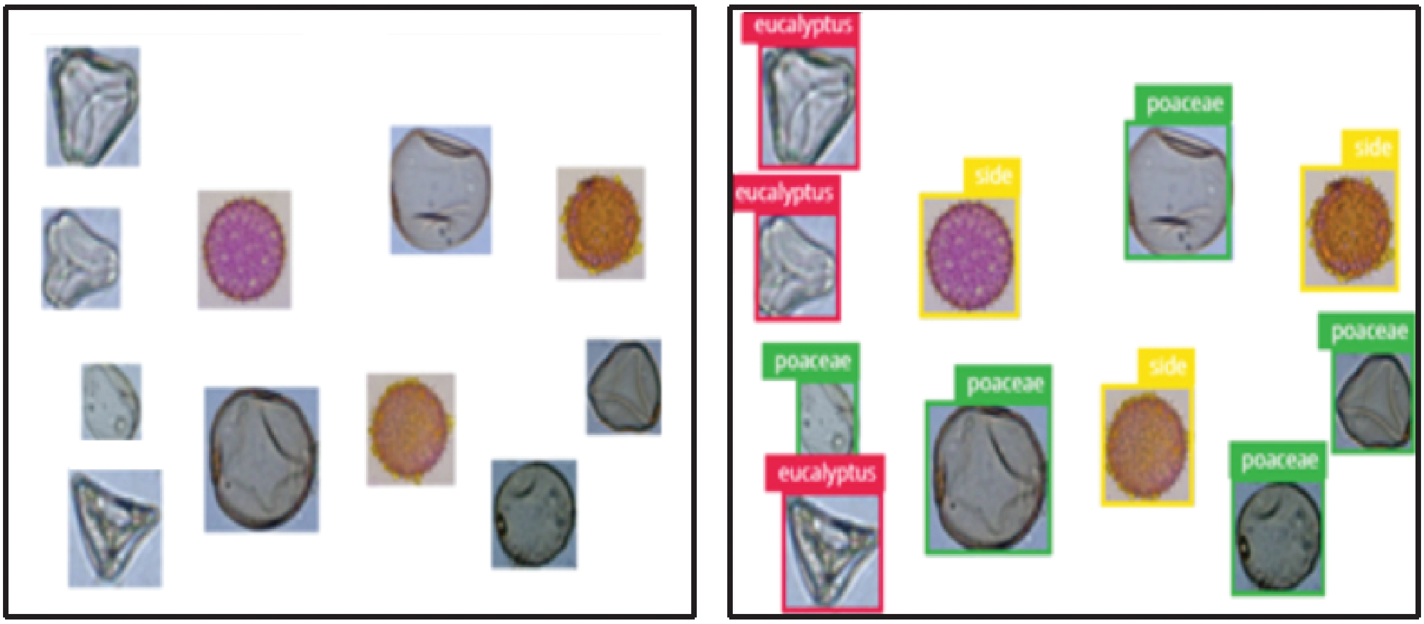


Fig. 1.9: (a) Pollen Grains Sample, (b) Annotation of pollen Grains

detecting and locating pollen grains within images[18] [Fig. 1.9(a)-(b)].

Feature Explorer

The Feature Explorer tool aids in visualizing the features extracted from a dataset, offering insights into outliers and the clustering and separation of classes. By plotting the features in a 3D space using the Flatten block, each axis represents a specific feature. For instance, if the features chosen are average acceleration X, average acceleration Y, and average acceleration Z, a sample with corresponding values would be plotted as a dot in the 3D viewer. This visualization helps assess the degree of separation between classes, indicating whether simpler machine learning models could achieve higher accuracy. Ultimately, the tool facilitates better understanding of the dataset's characteristics and informs the selection of appropriate ML models[19] (Fig. 1.10).

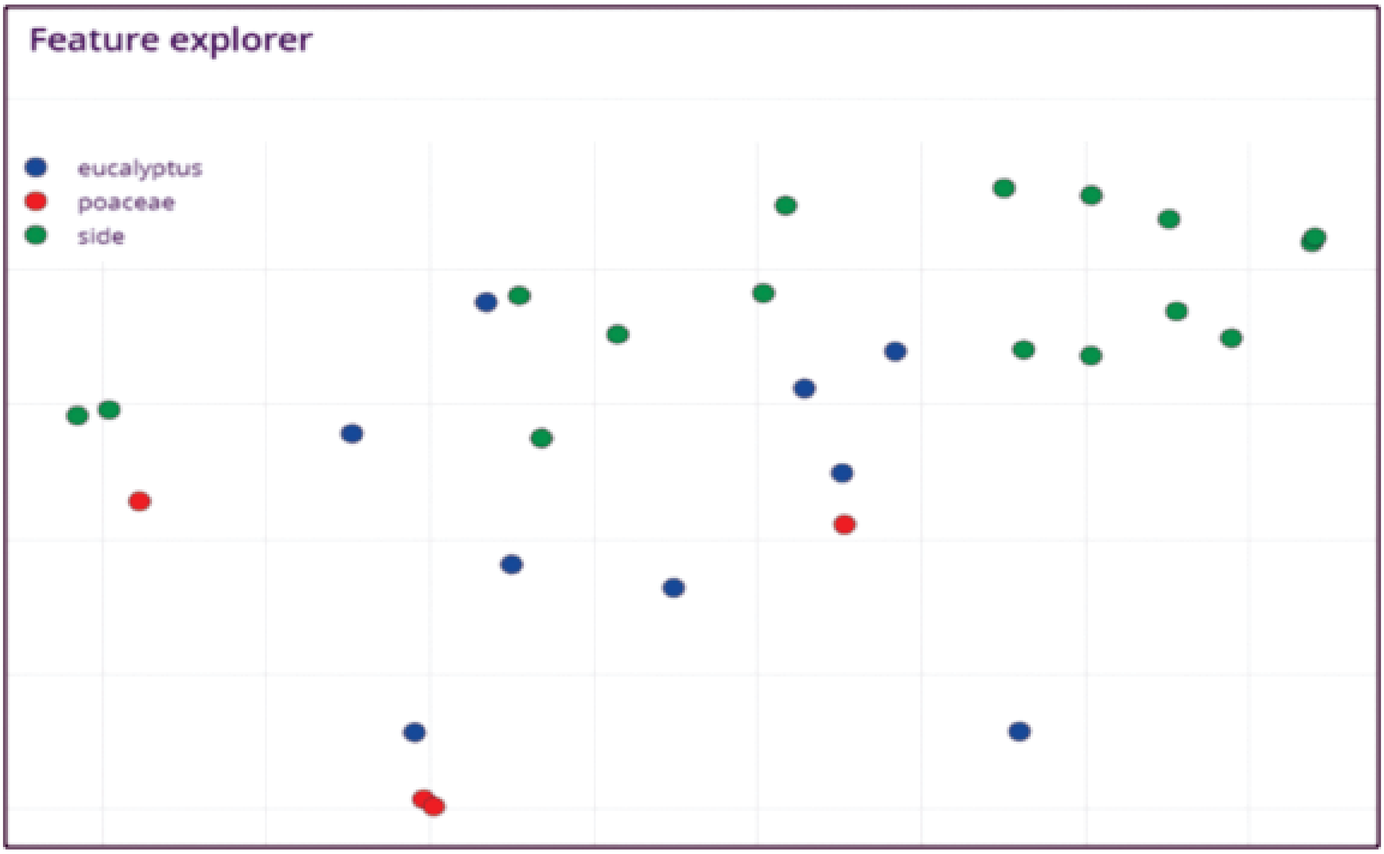


Fig. 1.10: Features Explorer

Avg accX = -0.24

Avg accY = 0.17

Avg accZ = -9.81

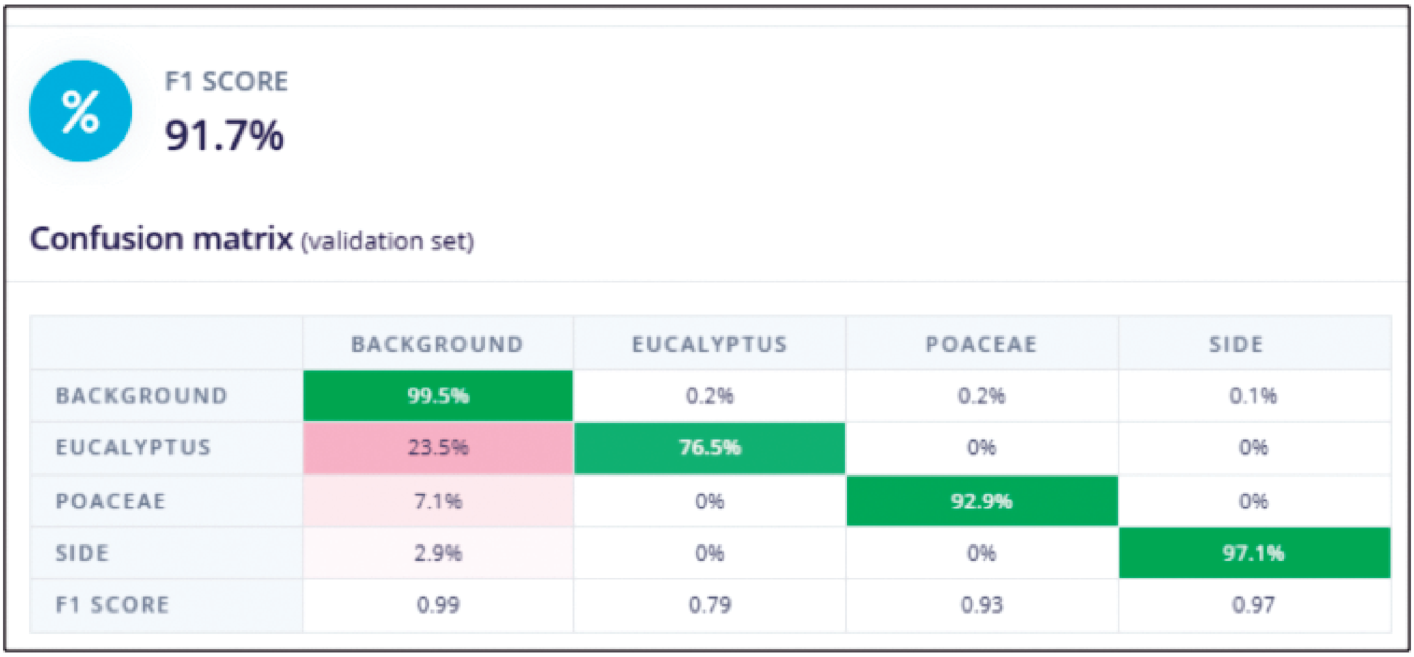
A dot would be plotted at (-0.24, 0.17, -9.81) in the 3D viewer.

RESULTS

A confusion matrix serves as a pivotal tool in evaluating the performance of a machine learning model, particularly in classification tasks. It provides a concise summary of the model's predictions on a test dataset by categorizing instances into four categories: true positives (TP), true negatives (TN), false positives (FP), and false negatives (FN). True positives denote instances where the model accurately predicts positive data points, while true negatives signify accurate predictions of negative data points. On the contrary, false positives occur when the model incorrectly predicts positive instances, and false negatives happen when negative instances are mispredicted. Through this matrix, analysts can gauge the model's accuracy, precision, recall, and other performance metrics crucial for assessing its efficacy in classification tasks (Fig. 1.11).

Implementation for Real Time Use

To upload AI code to our IoT hardware, we first download the AI model weights from our AI development



F1 SCORE

91.7%

Confusion matrix (validation set)

	BACKGROUND	EUCALYPTUS	POACEAE	SIDE
BACKGROUND	99.5%	0.2%	0.2%	0.1%
EUCALYPTUS	23.5%	76.5%	0%	0%
POACEAE	7.1%	0%	92.9%	0%
SIDE	2.9%	0%	0%	97.1%
F1 SCORE	0.99	0.79	0.93	0.97

Fig. 1.11: Confusion matrix and Accuracy level

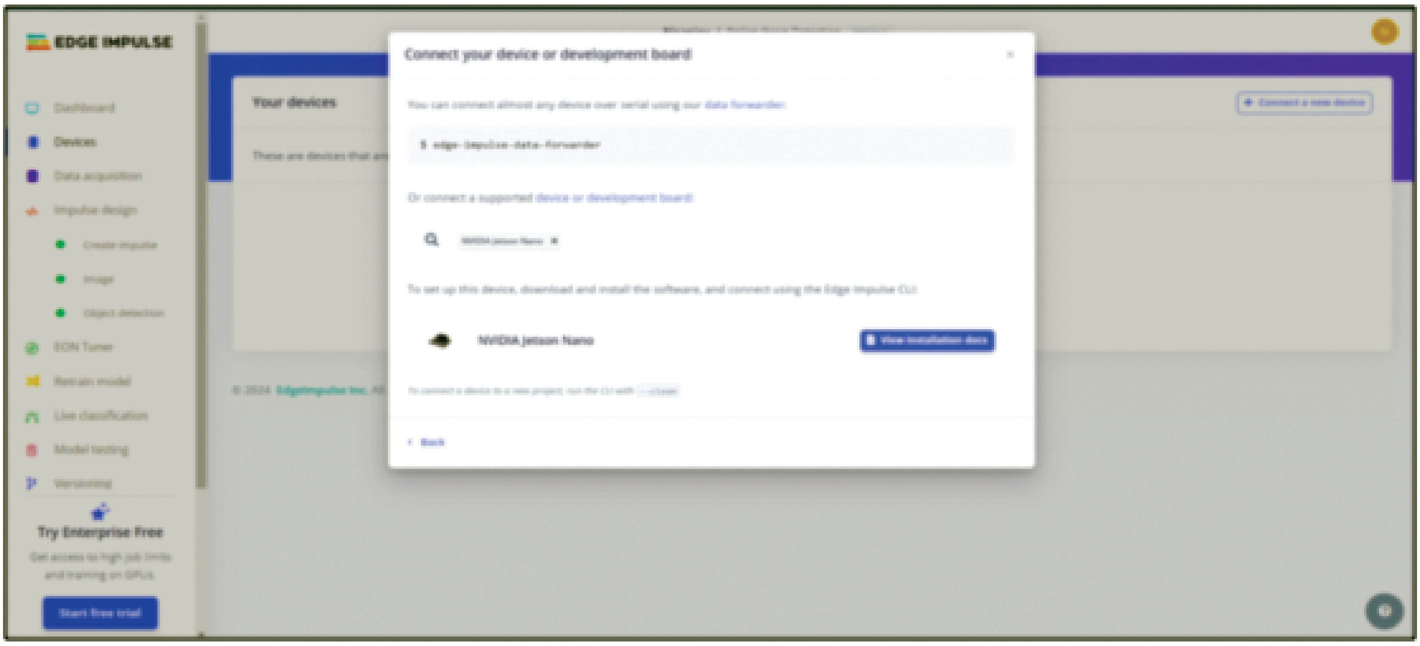


Fig. 1.12: Model Weight Download Page

IDE, ensuring compatibility with our specific IoT device. Additionally, we download the necessary firmware required to execute these AI models on our hardware. Subsequently, we upload this firmware to our IoT devices, ensuring to include credentials necessary for connecting with our designated IoT platform. Once the firmware is successfully uploaded, we activate the device in detection mode, allowing it to start processing data. The device then collects and transmits data to our IoT platform for further analysis and utilization. This streamlined process ensures seamless integration of AI capabilities into our IoT hardware, enabling efficient data processing and utilization for various applications (Fig. 1.12-14).

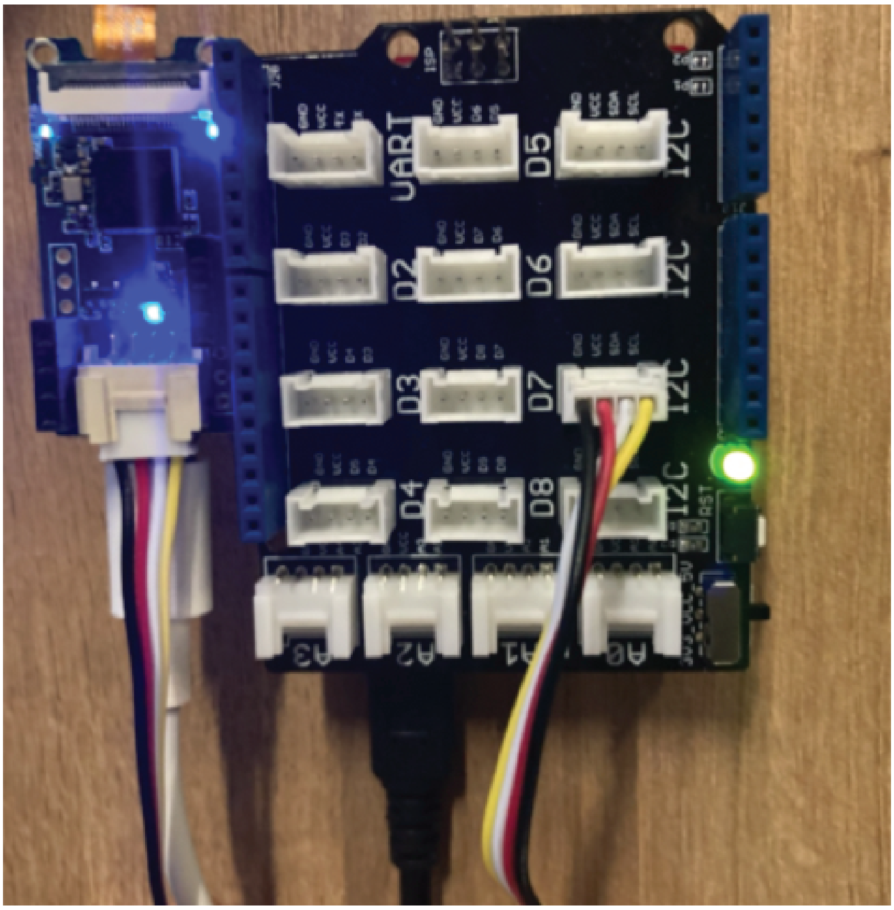


Fig. 1.13: Image of Firmware uplaoad to our Device

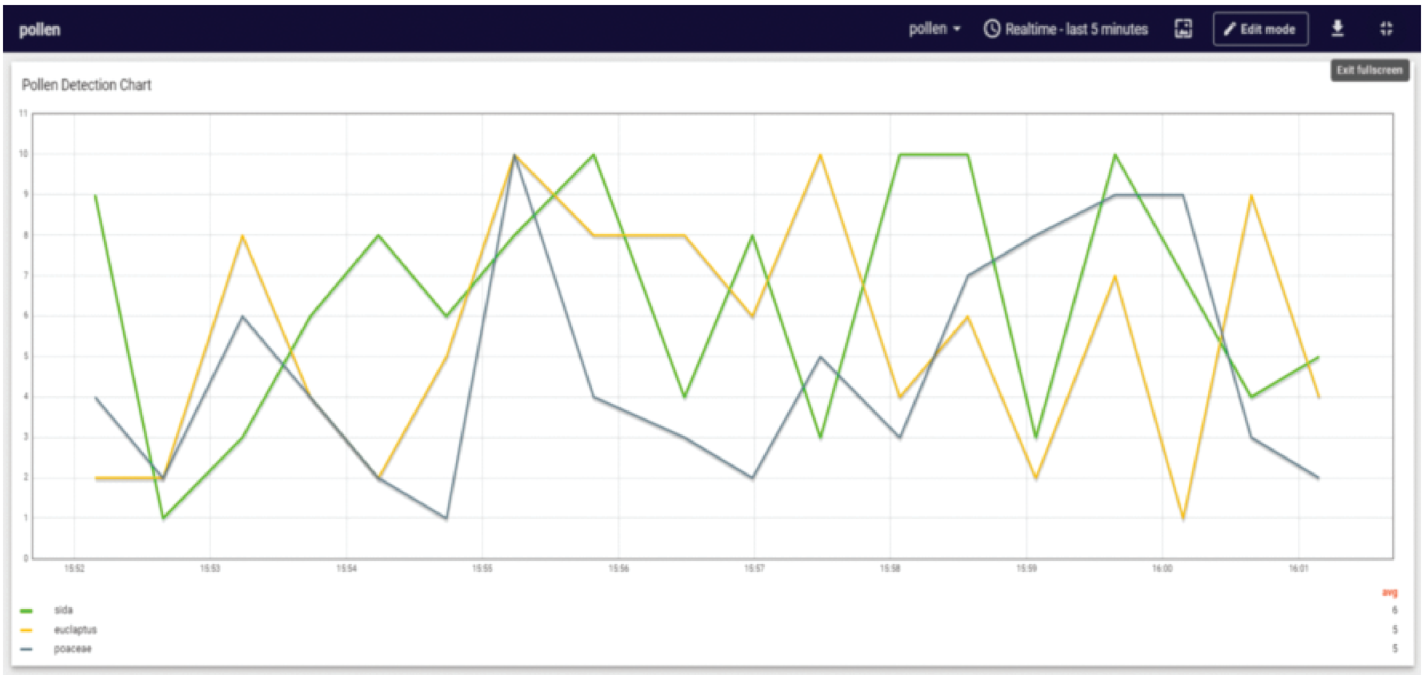


Fig. 1.14: Image of dashboard providing quantity of pollen present in the air.

CONCLUSION

An automated approach is done for detecting microscopic pollen grain image samples using AI algorithms. The algorithm leverages characteristic features such as color, lightness, and surface roughness to identify airborne pollen grains and spores. Additionally, the predicted data are saved to an IoT platform, along with the count of pollen present in each sample for every pollen type. Results demonstrate the algorithm's effectiveness, showcasing comparable performance to other similar algorithms in existing literature. Overall, this research offers a promising solution for automated pollen detection and classification.

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Research Article

DIDYMOCARPUS PYGMAEUS (GESNERIACEAE) – A NEW GENERIC RECORD TO THE FLORA OF TELANGANA, INDIA

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Didymocarpus pygmaeus C.B. Clarke (Gesneriaceae) is reported here as a new generic record for the state of Telangana. Detailed description, phenological data, photographs and herbarium voucher numbers have been provided here to facilitate easy identification and future references.

Key Words: *Didymocarpus, Gesneriads,* Kannaigudem RF, New record, Telangana state.

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INTRODUCTION

The genus *Didymocarpus* Wall., was redefined by Weber & Burtt[1], now it comprises around 100 species found across India, Nepal, Bhutan, southern China, Myanmar, Thailand, Vietnam, Laos, Cambodia, Peninsular Malaysia, and Sumatra[2]. According to phylogenetic research conducted by Palee[3], the probable ancestral origin of the genus *Didymocarpus* is suggested to be the Malay Peninsula with over half of the known species concentrated in the Northeast region of India and Southern China[4]. India comprises 25 species of *Didymocarpus*, primarily as narrow endemics, mainly confined to lesser-explored regions of Northeast India[5].

MATERIALS AND METHODS

Study area

The Bhadradri Kothagudem district, formerly part of Khammam district until its establishment as a separate entity in 2016, boasts a rich historical legacy, having been under the rule of various dynasties such as the Kakatiyas, Musunuri Nayaks, Reddy dynasty and others. It is the easternmost district of Telangana, it spans an area of 7,483 Sq. kms, lies between latitudes 17° 14' N to 18° 15' N and longitudes 80° 14' E to 81° 16' E. The district shares borders with Bijapur and Sukuma districts of Chhattisgarh to the North and northeast, East Godavari and West Godavari districts to the East, Khammam district to the South and southeast, Mahabubabad district to the West, and Jayashanker Bhupalapally district to the Northwest. The district comprises 6 territorial divisions, 23 Ranges, 101 Sections and 506 Beats, and encompasses diverse vegetation types including Semi-Evergreen Forest, Tropical Moist Deciduous Forest, Tropical Dry Deciduous Forest, Scrub, and Savanna.

Floristic survey

Over the course of the past three years, comprehensive floristic exploration endeavours have been conducted within the Bhadradri Kothagudem district of Telangana state. Notably, specimens of a newly recorded species were meticulously collected from the Kannaigudem Beat, Anantharam Section, Aswaraopet Range of Paloncha Forest Division.

After a critical study, the specimens have been identified as *Didymocarpus pygmaeus* C.B. Clarke, utilizing information derived from existing floras[6,7]. On scrutiny of related literature, it was revealed that the genus *Didymocarpus* Wall., has not hitherto been documented within the *Flora of Telangana state*[8,9,10]. Hence, it is with significance that the genus *Didymocarpus* is hereby reported as a new generic record to the *Flora of Telangana state, India*. In order to facilitate facile identification, detailed descriptions accompanied by photographs have been provided. Furthermore, adhering to the standard herbarium methods[11], the voucher herbarium specimens were meticulously prepared, these voucher specimens will be deposit at Osmania Univesity (HY), Hyderabad, Telangana state, India.

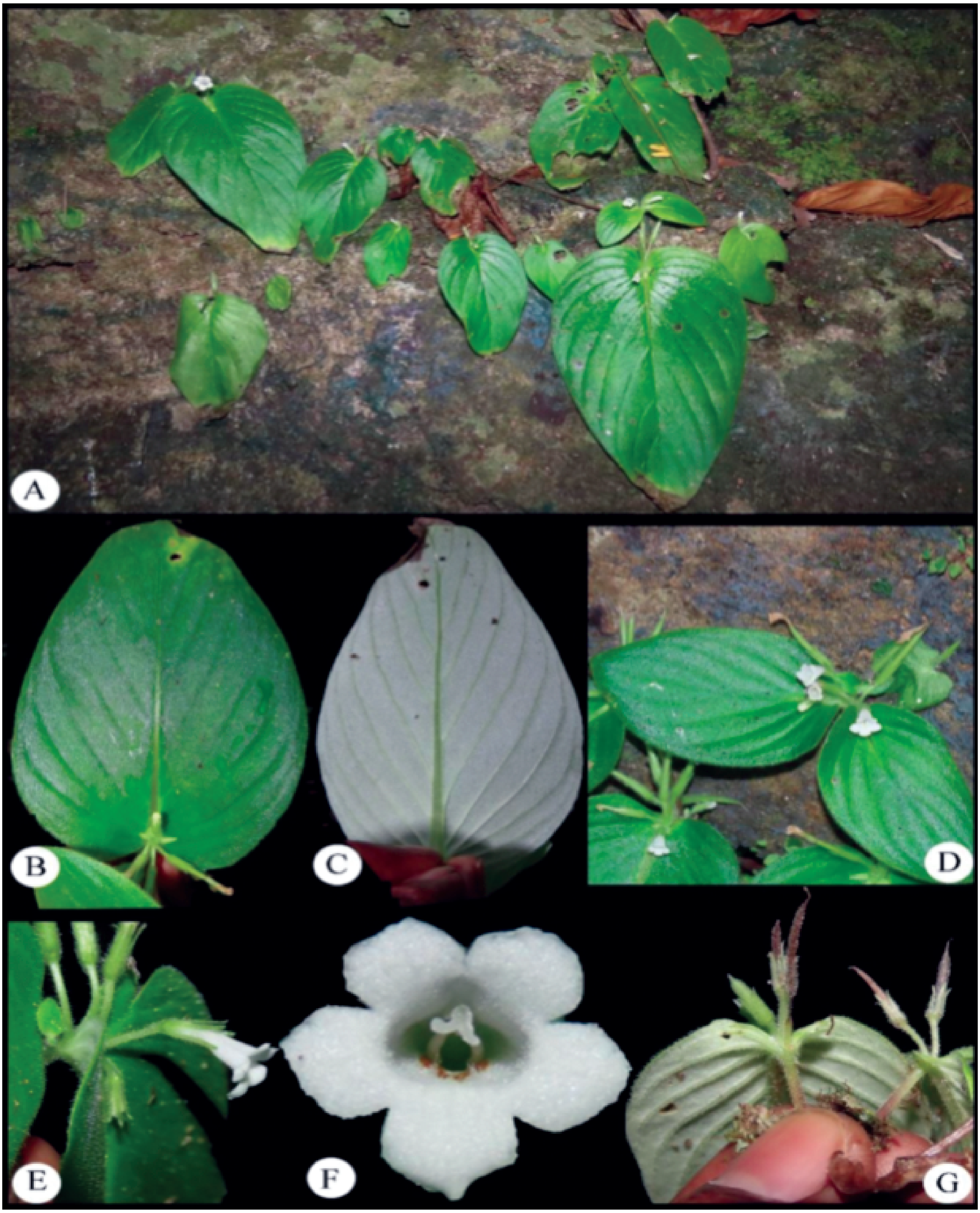

Fig. 2.1: ***Didymocarpus pygmaeus*** C. B. Clarke – A. Habitat, B & C. Leaf both surfaces, D. Inflorescence, E & F. Flower side view and close up, G. Capsules.

RESULTS AND DISCUSSION

Taxonomic treatment

Didymocarpus pygmaeus C. B. Clarke, A. L. P. P. de Candolle and A. C. P. de Candolle, Monogr. Phan. 5: 82. 1883. *Roettlera pygmaea* (C. B. Clarke) Kuntze., Revis. Gen. Pl. 2: 476. 1891.

Description: Annual, a fragile herb with a slender creeping stem, to 10 cm tall. Stem villous, pubescent, straight or curved; bearing one leaf at its apex; leaf elliptic-ovate 3–3.5 × 2–2.5 cm long, sub-opposite or alternate, subequal; cordate at base, entire to ciliate along margin, obtuse or acute at apex; pilose on both sides, midrib broad and impressed above. Pedicels few -1–8 mm, pubescent. Flowers small, 1–5, in axillary clusters; corolla white, campanulate, 5-lobed with an orange-yellow stripe ventrally in tube, tube tubular. Stamens 2 fertile, 2 linear rudimentary, glabrous, anthers 2-locular, locules ovate. Ovary with villous style; stigma small, subpeltate, bilobed. Capsules long, linear, straight or slightly curved, acuminate at apex, 2-valved, brown to black at maturity, densely hairy; seeds ellipsoid, many, smooth (Fig. 2.1).

Phenology: August – December

Habitat and Ecology: Rare on moss-clad moist rocks along streams in semi-evergreen forests in association with *Elatostema cuneatum* Wight and *Adiantum* sp.

Distribution: Native to India

Local distribution: Gujarat, Jharkhand, Karnataka, Kerala, Madhya Pradesh, Meghalaya, Odisha, Rajasthan, West Bengal, Andhra Pradesh and Telangana state (Present report).

Specimens examined (Investigator Collections): India, Telangana state, Bhadradri Kothagudem district, Kannaigudem Beat, Anantharam Section, Aswaraopet Range, Paloncha Forest Division, 21st, October, 2023, GR 0966 (HY).

Additional specimen images seen: Asia, India, Jharkhand, 28th, November, 1874, C.B. Clarke, record number (25070C), Catalogue number (BM000997735), Other catalogue numbers: NHMUK: ecatalogue:546759 (Source: NHM Data Portal).

Notes: *Didymocarpus pygmaeus* C.B. Clarke and *Microchirita hamosa* (R.Br.) Yin Z. Wang have often been compared due to their similarities, but both species are accepted and exhibit considerable variations[12-14]. *Didymocarpus pygmaeus* can be easily distinguished by its leaves have a more pronounced hairy texture, arranged in a sub-opposite or alternate (vs. smooth or slightly hairy, opposite leaves), leaf margin entire to ciliate (vs. entire or subentire), Inflorescences, bears solitary flowers or flowers in small clusters, 1-5 flowers (vs. few to many flowered).

CONCLUSIONS

The present report of *Didymocarpus pygmaeus* C. B. Clarke in the Bhadradri Kothagudem district of Telangana state is notable as it is the first documentation of the genus *Didymocarpus* in Telangana state. This finding highlights the necessity for ongoing floristic exploration endeavours to reveal and document the diverse plant life within the region.

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Research Article

STUDY OF AIRBORNE POLLEN GRAINS IN BERHAMPORE TOWN, WEST BENGAL, TO DETECT THEIR ALLERGENIC POTENTIAL

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The role of pollen in causing pollinosis is well established and documented in India. Most airborne pollen comes from anemophilous plants, with some from entomophilous and amphiphilous species. Plants pollinated by wind are the primary source of inhalant pollen allergens and are considered biological health hazards. Understanding the types of pollen present at different times of the year assists allergists in identifying the suspected pollen type responsible for allergic reactions. In light of this, an aerobiological survey was conducted in Berhampore town (approximately 200 km northeast of Kolkata, West Bengal) over two consecutive years (2001-2003). A total of 31 pollen types were identified, with the most abundant originating from Poaceae, followed by Cyperaceae, *Cassia* sp, *Acacia auriculiformis*, etc. The allergenic potential of suspected pollen types was assessed. It is known that allergic reactions depend on specific triggering antibodies, i.e., IgE-sensitized mast cells, which release mediators that induce inflammatory reactions. The concentration of total IgE was measured in selected allergic patients, revealing that individuals with atopic allergic diseases exhibit increased levels of total immunoglobulin E (IgE) in their blood.

Key Words: Airborne pollen, atopic allergy, Berhampore town, West Bengal.

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INTRODUCTION

Aeropalynology, or airborne pollen allergy study, focuses on pollen grains that carry male genetic material in plants. These grains serve as biosensors due to their easy dispersal, historically linked to conditions like "hay fever," posing a health hazard[1]. Biohazards encompass various substances like pollen, fungi, animals, bacteria, viruses, etc., with airborne pollen grains being significant organic pollutants causing respiratory allergies. The prevalence of pollen allergies is rising, affecting socio-economic aspects[2].

Allergy, termed immediate hypersensitivity, refers to an accelerated reaction upon re-exposure to a sensitizing substance. It's commonly associated with Type-I hypersensitivity[3], a global issue affecting up to 40% of industrialized populations. In Europe, chronic allergies affect over 150 million people, resembling an epidemic. In India, a significant portion, 20-30% of the population, suffers from allergic rhinitis, with 15% developing asthma[4].

Each day, adults inhale about 14-15 cubic meters of air, laden with bioparticles like pollen grains and fungal spores. Allergic reactions rely on specific triggering antibodies, particularly IgE sensitized mast cells, which release inflammatory mediators. Airborne pollen sampling aids clinicians in determining pollen season characteristics for allergen selection in treatment. Variations in allergic symptoms are observed based on location, climate change, and environmental conditions.

The present project aims to conduct an aerobiological survey focusing on pollen allergy in Berhampore, West Bengal. It seeks to understand seasonal variations in pollen concentration and their correlation with meteorological factors. Additionally, the study aims to assess the allergenicity of predominant pollen types through comprehensive patient case histories and in vivo/in vitro clinico-immunological analyses.

METHODOLOGY

Aerobiological sampling with meteorological factors

A one-day aerobiological survey was conducted for two consecutive years using a 1-day ASTIR volumetric slide sampler (Astir India Co. Ltd., New Delhi), positioned atop a domestic house in Berhampore town, approximately 200 km northeast of Calcutta, at a height of 4 meters above ground level. The sampler, with a suction rate of 10 liters of air per minute, was utilized to collect airborne particles. Pollen grains were identified and quantified in accordance with guidelines provided by The British Aerobiological Federation[5]. Meteorological data were gathered from the Pulses and Oil-seed Research Station in Berhampore. Statistical analysis of the correlation between airborne pollen content and climatic factors was conducted using the student's t-test for paired samples, following methodologies outlined by Subiza et al.[6] and Vega-Mary[7].

Clinico-immunological studies

During the pollination months, fresh pollen samples were gathered from specific plants. These pollen grains underwent purification, defatting, and extraction in a phosphate buffer with a pH of 7.2. Skin prick tests (SPT) were administered to allergic patients at the Allergy Unit of The Institute of Child Health in Kolkata, following the protocol outlined by Stytis et al.[8] Sera from both SPT positive and negative (control) patients were collected. ELISAs (Enzyme Linked Immunosorbent Assays) were conducted using the crude pollen extract and the corresponding patient sera, following the methodology detailed by Engvall and Pearlman[9]. Detailed case histories of each patient were meticulously recorded.

RESULTS

Pollen count

Thirty-one types of pollen were identified.for both years (2001-2003). The highest count belonged to Poaceae, followed by Cyperaceae, *Cassia* spp, *Acacia auriculiformis*, among others (Table 3.1).

Table 3.1: Airborne pollen types recorded at Berhampore town in two years

Taxa with Family	Annual Mean Count /Day/m³ of air			
	1st year		2nd year	
	Number	Percent	Number	Percent
Acacia auriculiformis (Fabaceae)	36.00	5.62	33.0	5.81
Areca catechu (Arecaceae)	24.0	3.75	24.0	4.22
Azadirachta indica (Meliaceae)	18.00	2.81	17.0	3.0
Barringtonia racemosa (Barringtoniaceae)	7.00	1.09	–	–
Bombax ceiba (Bombacaceae)	11.00	1.72	11.0	1.93
Borassus flabellifer (Arecaceae)	14.00	2.19	15.0	2.64
Carica papaya (Caricaceae)	17.00	2.66	16.0	2.81
Cassia spp. (Fabaceae)	52.00	8.12	32.0	5.63
Casuarina equisetifolia (Casuarinaceae)	13.00	2.03	13.0	2.28
Cheno-Amaranthaceae	34.00	5.31	34.0	5.98
Cocos nucifera (Arecaceae)	23.0	3.59	20.0	3.52
Croton bonplandianum (Euphorbiaceae)	23.00	3.59	19.0	3.43
Cyperaceae	57.66	9.01	50.33	8.85
Dillenia indica (Dilleniaceae)	5.00	0.78	6.0	1.05
Eucalyptus sp. (Myrtaceae)	14.00	2.19	14.0	2.46
Hyptis suaveolens (Lamiaceae)	7.00	1.09	–	–
Litchi chinensis (Sapindaceae)	16.00	2.5	16.0	2.81

Contd.

Contd. Table 3.1

Taxa with Family	**Annual Mean Count /Day/m³ of air**			
	1st year		2nd year	
	Number	Percent	Number	Percent
Madhuca indica (Sapotaceae)	32.00	5.0	20.0	3.52
Malvaceae	25.00	3.91	21.0	3.69
Mangifera indica (Anacardiaceae)	11.00	1.72	15.0	2.64
Mimosa pudica (Fabaceae)	18.00	2.81	17.0	3.0
Morus indica (Moraceae)	16.0	2.5	14.0	2.46
Oldenlandia sp. (Rubiaceae)	7.00	1.09	9.0	1.58
Phoenix sylvestris (Arecaceae)	14.0	2.19	11.0	1.93
Phyllanthus emblica (Euphorbiaceae)	11.00	1.72	11.0	1.93
Poaceae	70.00	11.0	71.0	12.50
Psidium guajava (Myrtaceae)	9.00	1.41	8.0	1.41
Ricinus communis (Euphorbiaceae)	16.00	2.5	13.0	2.28
Tinospora cordifolia (Menispermaceae)	4.00	0.625	6.0	1.05
Trema orientalis (Uticaceae)	28.00	4.37	25.0	4.40
Xanthium strumarium (Asteraceae)	7.00	1.09	7.0	1.23

In the present investigation, the seasonal patterns of total pollen concentration, reveal notable fluctuations throughout the year. The investigation identified two primary peak periods: the first occurring from March to May, and the second appearing between September and November (Fig. 3.1). These peaks represented times of heightened pollen activity, characterized by increased concentrations in the air. Specifically, March emerged as the month with the highest pollen count in both years under scrutiny, suggesting its significance in the overall pollen cycle. Additionally, the data depicted a smaller peak during the September to November timeframe, indicating a secondary period of elevated pollen levels. Overall, the study illuminated the dynamic nature of pollen distribution, emphasizing distinct seasonal variations and the prominence of March in driving peak concentrations.

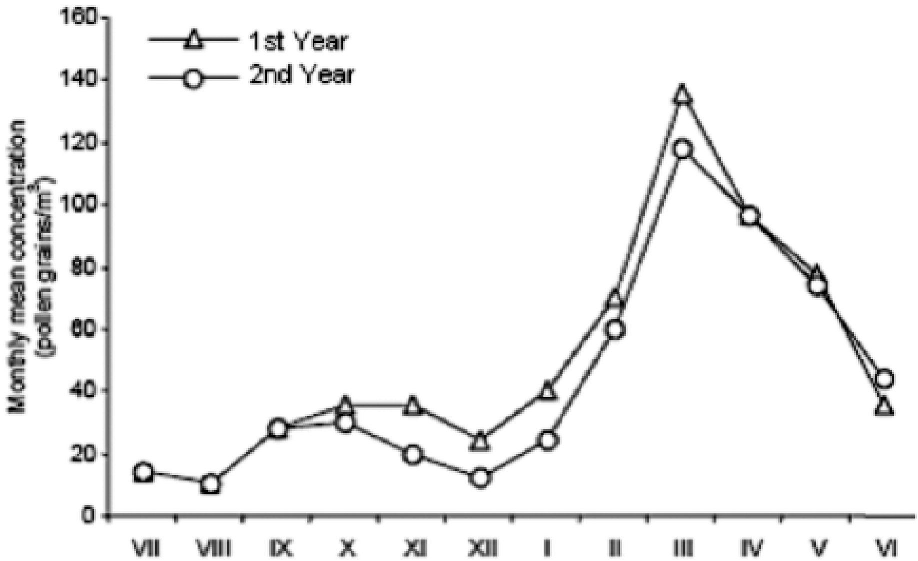

Fig. 3.1: Variations in the total pollen count in Berhampore [I = January, XII = December]

Table 3.2: Correlation Between Meteorological Parameters and Monthly Total Pollen Count

	Temperature	Rainfall	Relative humidity	Wind speed
Rainfall	0.587			
Relative humidity	0.453	0.828		
Wind speed	0.429	0.247	0.382	
Total Pollen	0.086	-0.534	-0.802	-0.120
Coefficient of Correlation (r) values				
Rainfall	0.525			
Relative humidity	0.697	0.888		
Wind speed	0.684	0.284	0.400	
Total Pollen	0.239	-0.439	-0.421	0.353

Degrees of Freedom (DF) → 12-2 = 10.

Influence of Weather on Pollination

The annual pollen load in the atmosphere experiences considerable fluctuations due to various climatic factors. Analyzing the correlation coefficients (r) reveals significant associations between pollen concentration and key weather parameters. Specifically, there is a positive correlation between pollen counts and temperature, indicating that as temperatures rise, so does the pollen count. Conversely, there exists a negative correlation between pollen concentration and rainfall, relative humidity, and wind speed (Table 3.2).

This means that when rainfall is lower, relative humidity decreases, and wind speeds are calmer, pollen concentrations tend to be higher. These relationships underscore the intricate interplay between climatic conditions and pollen dispersal. Understanding these correlations is crucial for predicting and managing pollen-related issues, such as allergies, and can inform strategies for mitigating their impact on human health and the environment.

Allergenic potentiality of the Pollen

At the Allergy Unit of the Institute of Child Health, Kolkata, in vivo skin prick tests were conducted using whole pollen extracts from eighteen common airborne pollen types on patients with relevant case histories. Each of the eighteen taxa elicited at least 1+ reaction in

Table 3.3: Results of skin-Prick test using different pollen extracts on adult respiratory allergic patients of West Bengal

Pollen allergen extract	Total No. of tested patient	No. of +Ve Patient (%)	Percent of intensity of reaction		Percent of intensity of reaction	
			+1	+2 or more	+1	+2 or more
Acacia auriculiformis	147	30 (20.41)	17.68	2.72	–	–
Amaranthus viridis	150	20 (13.33)	13.33	–	10.00	–
Areca catechu	555	222 (40.0)	34.60	5.40	9.03	4.60
Azadirachta indica	950	485 (51.05)	45.05	6.00	45.63 45.74	7.30 3.26

Contd.

Contd. Table 3.3

Pollen allergen extract	**Total No. of tested patient**	**No. of +Ve Patient (%)**	**Percent of intensity of reaction**		**Percent of intensity of reaction**	
			+1	**+2 or more**	**+1**	**+2 or more**
Bombax ceiba	450	95 (21.11)	20.00	1.11	20.33	1.20
Borassus flabellifer	455	144 (31.65)	24.45	7.20	14.89	–
Carica papaya	550	140 (25.45)	21.95	3.50	23.67 17.89	3.90 –
Cassia siamea	175	54 (30.85)	25.71	5.14	–	–
Chenopodium album	150	33 (22.00)	20.00	2.00	16.00	0.80
Cocos nucifera	945	415 (43.91)	39.41	4.50	38.74 26.66	4.90 4.70
Croton bonplandianum	200	27 (13.50)	13.50	–	–	–
Cyperus rotundas	135	52 (38.51)	33.01	5.50	31.98	5.70
Eucalyptus citriodora	260	89 (34.23)	27.69	6.53	20.33 25.18	1.20 5.61
Madhuca indica	135	33 (23.70)	20.74	2.96	–	–
Mangifera indica	180	18 (10.00)	10.00	–	10.52	–
Phoenix sylvestris	475	205 (43.16)	35.66	7.50	36.89 13.63	7.20 –
Poaceae (*Saccharum officinarum*)	350	100 (54.28)	39.28	15.00 66.02	37.86 0.64	17.00
Trema orientalis	135	12 (08.88)	8.88	–	9.16 13.95	– –

allergic patients. Among them, highly potent allergenic pollen grains, exhibiting 2+ to 3+ positivity reactions, included *Saccharum officinarum* (grass), *Azadirachta indica, Cocos nucifera*, *Phoenix sylvestris*, *Areca catechu*, *Cyperus rotundas*, and others (Table 3.3).

The concentration of total IgE was measured in case of some selected allergic patients and it was found that patients with atopic allergic diseases exhibit increased level of total immunoglobulin E (IgE) in blood (Table 3.4).

Table 3.4: Quantitative determination of IgE in human serum

Sl. No. of Patients	**Age**	**Sex**	**Skin positivity level**	**Concentration of IgE (IU/ml)**
1	35	M	–	5.5
2	25	M	++	383.3
3	34	M	–	52.2
4	37	F	+	103.2

Contd.

Contd. Table 3.4

Sl. No. of Patients	Age	Sex	Skin positivity level	Concentration of IgE (IU/ml)
5	33	M	+	399.2
6	49	M	+++	795.0
7	41	F	+++	785
8	25	M	++	775
9	52	M	+	214.1
10	33	F	+	229.6
11	24	M	+	113.9
12	64	M	+	95.9
13	16	M	++	29.1
14	25	M	++	342.6
15	23	F	+	289.1
16	44	M	++	765
17	38	F	–	38.9
18	22	M	++	113.5
19	48	M	++	78.3
20	27	M	++	599.7

DISCUSSION

Pollen allergy and Climatic factors

The research conducted in India has effectively documented the significant role of pollen allergies in triggering respiratory allergic reactions[9,10,11,12]. Our findings regarding the presence of airborne pollen in Berhampore align closely with previous studies conducted across various regions of West Bengal[9]. It's noteworthy that the frequency of pollen occurrence varies from one location to another due to factors such as geographical positioning, local flora, and other environmental conditions[6].

Interestingly, our study identified a positive and statistically significant correlation between air temperature and pollen concentration. Conversely, we observed a negative correlation between pollen concentration and factors like rainfall, relative humidity, and wind speed. These findings echo similar observations made in diverse geographical contexts and countries[6,13].

In essence, our research underscores the intricate interplay between environmental factors and pollen dispersion, shedding light on the nuanced dynamics that influence pollen concentrations in the atmosphere. By elucidating these relationships, we contribute to the broader understanding of pollen-related respiratory allergies and the factors that govern their prevalence and severity.

Impact on human health

Airborne biological pollutants pose significant health risks, with allergic reactions being among the most prevalent concerns. These reactions are frequently linked to various sources, such as molds, pollen, animal dander (especially from cats and dogs), and dust mites. These microscopic creatures inhabit common household areas like carpets, bedding, and furnishings. Of these pollutants, pollen stands out as the primary culprit behind allergic reactions.

The spectrum of allergic reactions spans from mere nuisances to life-threatening emergencies, particularly evident in severe asthma attacks. Symptoms associated with these reactions can manifest in various forms, including watery and itchy eyes, a runny nose, frequent sneezing, nasal congestion, coughing, and difficulties in breathing. Additional discomforts like headaches and fatigue may also accompany these symptoms.

Notably, asthma and allergies represent two of the most prevalent non-communicable diseases affecting children. Their impact on pediatric health is substantial, often leading to significant disruptions in daily activities and, in severe cases, necessitating urgent medical intervention.

In summary, airborne biological pollutants, notably pollen grains, pose considerable health risks through allergic reactions. Understanding these risks and implementing appropriate measures to mitigate exposure are crucial steps in safeguarding individual and public health, especially among vulnerable populations like children.

Management of the pollen allergy

Understanding the root causes of allergies and the seasonal patterns of allergen occurrence is crucial for effective management. Patients benefit from being informed about the triggers of their allergic reactions. Symptomatic relief through topical and antiallergic medications is commonly employed. However, for sustained benefits, allergen immunotherapy, commonly known as allergy shots, presents a promising long-term solution. These shots, administered subcutaneously, have demonstrated the potential to provide relief for several years, even after the treatment ceases.

By consulting a pollen calendar, individuals can locate nearby healthcare facilities, identify high-risk zones for allergen exposure, and adopt precautionary measures to mitigate allergic reactions. This proactive approach not only empowers patients to manage their allergies effectively but also reduces the socioeconomic burden associated with pollen allergies. As the incidence of pollen allergies continues to rise, initiatives integrating technology and medical insights play an increasingly pivotal role in addressing this health concern.

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Research Article

CULTURABLE AEROMYCOFLORA OF OUTDOOR MARKET AREA IN PURULIA TOWN AND STUDIES ON FUNGAL SPOILAGE OF VEGETABLES AND FRUITS

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An aeromycological study of vegetable and fruit markets provides valuable insights into the airborne fungal communities present. Post-harvest infections and rotting of vegetables and fruits result in significant economic losses for stakeholders in these market chains. Consequently, an aeromycological investigation was conducted over one year in selected markets of Purulia town to assess the overall mycoflora and its seasonal variations.

During this survey, researchers isolated 25 fungal species alongside several unidentified ones. *Aspergillus niger* (7.76%) emerged as the most dominant aeromycoflora, followed by *Aspergillus flavus* (7.15%), *Fusarium oxysporum* (6.71%), *Curvularia lunata* (6.44%), and *Penicillium chrysogenum* (5.72%). The prevalence of *Aspergillus niger* was highest during the monsoon, while *Fusarium oxysporum* dominated in the post-monsoon period. In winter, *Cladosporium cladosporioides* became the most prevalent, and during summer, *Aspergillus niger* again emerged as the most frequent airborne fungus.

Environmental factors were found to significantly influence the fungal load at the study sites, with statistical analyses confirming a notable correlation between relative humidity and fungal presence in the air. Additionally, rotted fruits and vegetables collected from these markets helped identify fungal agents responsible for spoilage, resulting in the isolation of 11 fungal genera. Tomato and banana showed the highest number of fungal isolates. Among the identified pathogens causing rot were various species of *Alternaria, Fusarium, Rhizopus, Penicillium,* and *Aspergillus*.

Overall, this study underscores the impact of airborne fungal communities on post-harvest losses in agricultural markets, highlighting seasonal variations and environmental influences on fungal diversity and prevalence.

Key Words: Aeromycology, aeromycoflora, seasonal, market, Purulia

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INTRODUCTION

Fungi are universal in distribution and their role as decomposers makes them one of the most vital parts of an ecosystem. Fungi, yeast and bacteria are responsible for causing diseases in crops and fruits. Fungi constitute a major biopollutant and contaminate the environment. Usually, they reproduce through asexual and sexual modes by producing spores as well as conidia which constitute the aerospora and may pose a risk to plants, animals and humans[1]. Fruits and vegetables are crucial in the daily diet of humans to fulfil the nutritional requirements in the form of vitamins and minerals to maintain good health[2]. India has a wide range of climatic conditions, leading to the cultivation of a wide diversity of vegetables and fruits. Despite this, India is behind other wealthy nations in the production of vegetables. The major reason is the post-harvest deterioration of vegetables with the addition of infectious diseases present in the atmosphere[3]. Post-harvest infections are those diseases manifested after the harvesting of crops. Pathogen may attack the vegetables and fruits during the time of harvesting, and packaging or at the time of transport to markets. An estimated 35-40% of fruits and vegetables are wasted due to damage from various diseases around the world, which results in significant economic losses[4]. Improper processing and unscientific storage after harvesting of vegetables and fruits, pathogens are sprouting and the main reason for the loss of nutrient value of the crops. Vegetables with high nutrient content, sugars and low pH are more prone to fungal decaying[2]. In many marketplaces, fungi grow saprophytically on dumped plant materials and diseased plant debris, where fungi produce spores in large quantities and act as pools for plant pathogens that target a wide variety of fruits, vegetables, bulbs, and seeds. Additionally, these spores are frequently disseminated in the air and can travel a distance, which is responsible for damage to fresh fruits and vegetables. Almost all fungi perform the biodegradation and biodeterioration of biological components to fulfil their prime carbon, nitrogen and other nutrient requirements[4]. Vegetable markets are thus a very common source of airborne fungi[5]. Vegetables can be generally deteriorated by physical damage, activities of their enzymes, microbial actions or in combination[6]. Aerobiological study is interdisciplinary and deals with the quality of air. Abiotic factors and environmental parameters influence the fungal load in the air of a particular location. Aeromycological study complies with the identification of fungal load, sources, release mode, dispersal, deposition and their impact on plant and animal systems. The predominance of fungal spores in the air is due to the unique structure of some fungal spores which helps them to survive in unfavorable conditions[7]. The fungal diversity of a particular area depends upon seasonal variation and atmospheric parameters like temperature, humidity, precipitation, and wind speed[5]. The airborne pathogenic fungal spores germinate under the influence of favorable climatic conditions and result in post-harvest spoilage. Airborne fungal spores present in the atmosphere can able to induce health issues among consumers and sellers in the market[8]. The most common health problems in humans are allergic asthma and rhinitis due to inhalation of airborne allergic fungal spores and mycelial fragments. This is the pioneering work on aeromycological investigation of vegetable and fruit markets of Purulia district, which will help local farmers and sellers to store the vegetables and fruits in healthy.

MATERIALS AND METHODS

Study site

Purulia is the westernmost district of the state of West Bengal. The heart of the Purulia district is Purulia town. The climate of this region is dry and sub-tropical. The temperature reaches up to 50ºC in summer and down to 5ºC in winter. The main source of water for irrigation comes from the precipitation during the monsoon. A wide variety of vegetables are cultivated in different villages of this district and sold in the vegetable markets located at different corners of Purulia town. In contrast, the majority of fruits are transported from the nearby districts or states. A total of 3 outdoor vegetable and fruit markets were selected for aeromycological surveys in different locations of Purulia town.

Aeromycological survey

Aeromycological investigation of selected vegetable markets was carried out for the duration of one year from June 2022 to May 2023. The petriplate exposure method was used to isolate the fungi from the air of the vegetable markets. Petriplates containing Potato Dextrose Agar (PDA), Corn Meal Agar (CMA), and Czapek Dox Agar (CDA) supplemented with chloramphenicol were exposed for 15 minutes at 2 m above ground level for the isolation of viable airborne fungi. The sampling was carried out between 9 am to 11 am once at 10-day intervals in a month. The exposed plates were placed in an incubator at 28ºC for 5 days. The fungal colonies were morphologically studied, stained with lactophenol cotton blue solution and observed under the microscope to identify the isolates. In order to prepare the fungal calendar, the colonies that appeared were visually counted and the concentration was calculated using the Omelyansky method[9]. The colonies were then presented as CFU per cubic meter (CFU/m^3) of air.

Monitoring of Atmospheric parameters

Different atmospheric parameters like temperature (maximum, minimum, and average) and humidity (maximum, minimum, and average) were monitored throughout the year to determination the influence of the environmental parameters on fungal concentration in the air.

Isolation of Fungi from rotted fruits and vegetables

Infected or rotted vegetables and fruits were collected from markets and transported to the laboratory in sterile containers. PDA, CMA and CDA media were prepared and sterilized by autoclaving followed by the addition of chloramphenicol (0.06 mL/L) for inhibition of bacterial contamination. The media was poured into sterile petriplates under aseptic conditions. The vegetables were surface sterilized by 1% sodium hypochlorite followed by washing with sterile distilled water. The infected part of the vegetables or fruits were sliced by sterile blades followed by placed on the culture media and incubated at 28ºC for 5 days. The identification of the isolated fungi was based on observing the micro-morphological characteristics.

Statistical analysis

The seasonal impact on the fungal density in the air of the vegetable and fruit markets was determined using a one-way ANOVA. Further post hoc analysis was performed to compare the seasonal fungal concentration and their significant difference. Spearman correlation between monthly fungal concentration and atmospheric parameters (temperature and humidity) was carried out to analyze the influence of environmental parameters on the fungal load in the air of the study sites. In all the statistical tests, the significance level was considered at 5%.

RESULTS

Aeromycological survey

The areomycological investigation was continued for one year of duration and a number of 25 fungal species of 16 genera along with some other unidentified fungi were isolated through this aeromycological sampling. The fungal concentration was found to be 95174.46 CFU/m^3 during this one-year study period. The most dominant fungal species were *Aspergillus niger* (7.76%) followed by *Aspergillus flavus* (7.15%), *Fusarium oxysporum* (6.71%), *Curvularia lunata* (6.44%), and *Penicillium chrysogenum* (5.72%). Three fungal genera – *Aspergillus, Penicillium,* and *Alternaria* had shown maximum species variation in the market atmosphere. Fungal genera like *Cladosporium, Fusarium,* and *Rhizopus* also contributed to two species. The genus *Aspergillus* was found with three species viz. *Aspergillus niger, Aspergillus flavus* and *Aspergillus fumigatus*. The genus *Aspergillus* alone contributed about 17% of total fungal concentration. Three species of *Penicillium* like *Penicillium chrysogenum, Penicillium digitatum,* and *Penicillium citrinum* were observed during this aeromycological investigation. The genus *Penicillium* contributed about 12% of the total fungal concentration. Similarly, three species of *Alternaria* like *Alternaria alternata, Alternaria brassicicola,* and *Alternaria solani* were observed during this survey and 14% of the total fungal load was found from the genus *Alternaria*. So, the genera *Aspergillus, Penicillium,* and *Alternaria* combined contributed about 43% of the total fungal concentration. Among the total isolates, 13 fungal species like *Curvularia lunata, Aspergillus niger, Aspergillus flavus, Penicillium chrysogenum, Penicillium digitatum, Fusarium oxysporum, Fusarium solani, Alternaria alternata, Alternaria brassicicola, Alternaria solani, Rhizopus nigricans, Rhizopus stolonifer, Mucor mucedo,* and *Colletotrichum gloeosporioides* were found throughout the year. The month of June was the highest in concentration, however, the month of May was the lowest (Table 4.1). The whole study duration was divided into four seasons to determine the seasonal variation of fungal spores in the air. The months from

Table 4.1: Concentration of fungal species isolated from the air of vegetable and fruit markets from June 2022 to May 2023

Fungal isolates	Month with CFU/m^3												Total (CFU/m^3)	%
	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May		
Curvularia lunata	733.32	838.08	995.22	1099.98	523.80	314.28	261.90	157.14	209.52	261.90	314.28	419.04	**6128.46**	**6.44**
Cladosporium cladosporioides	–	–	–	–	209.52	314.28	838.08	995.22	890.46	314.28	209.52	104.76	**3876.12**	**4.07**
Cladosporium herbarum	419.04	366.66	261.90	314.28	209.52	–	261.90	576.18	838.08	523.80	209.52	–	**3980.88**	**4.18**
Aspergillus niger	1466.64	733.32	628.56	523.80	471.42	419.04	419.04	314.28	419.04	628.56	785.70	576.18	**7385.58**	**7.76**
Aspergillus flavus	1152.36	680.94	576.18	733.32	628.56	523.80	419.04	366.66	419.04	419.04	471.42	419.04	**6809.4**	**7.15**
Aspergillus fumigatus	419.04	523.80	523.80	419.04	261.90	209.52	104.76	–	–	–	–	–	**2461.86**	**2.59**
Penicillium chrysogenum	838.08	261.90	261.90	366.66	471.42	523.80	523.80	733.32	576.18	471.42	314.28	104.76	**5447.52**	**5.72**
Penicillium digitatum	209.52	157.14	261.90	209.52	314.28	419.04	471.42	523.80	419.04	261.90	209.52	104.76	**3561.84**	**3.74**
Penicillium citrinum	314.28	157.14	209.52	314.28	314.28	419.04	366.66	471.42	261.90	104.76	–	–	**2933.28**	**3.08**
Fusarium oxysporum	576.18	628.56	680.94	733.32	838.08	680.94	523.80	471.42	419.04	419.04	261.90	157.14	**6390.36**	**6.71**
Fusarium solani	419.04	471.42	523.80	576.18	628.56	523.80	471.42	314.28	261.90	261.90	209.52	104.76	**4766.58**	**5.01**

Contd.

Contd. Table 4.1

Fungal isolates	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	(CFU/m³)	%
Phoma herbarum	104.76	209.52	314.28	209.52	104.76	52.380	–	–	–	–	–	–	**995.22**	**1.05**
Drechslera sp.	314.28	261.90	157.14	366.66	523.80	471.42	523.80	419.04	261.90	157.14	–	–	**3457.08**	**3.63**
Alternaria alternata	523.80	471.42	419.04	419.04	366.66	314.28	209.52	419.04	314.28	366.66	471.42	419.04	**4714.2**	**4.95**
Alternaria brassicicola	419.04	471.42	314.28	419.04	366.66	157.14	209.52	261.90	314.28	261.90	366.66	523.80	**4085.64**	**4.29**
Alternaria solani	419.04	314.28	314.28	209.52	261.90	261.90	314.28	366.66	419.04	523.80	576.18	733.32	**4714.2**	**4.95**
Rhizopus nigricans	838.08	628.56	628.56	471.42	419.04	314.28	314.28	261.90	209.52	209.52	104.76	52.380	**4452.3**	**4.68**
Rhizopus stolonifer	942.84	576.18	733.32	628.56	471.42	366.66	314.28	261.90	261.90	209.52	157.14	104.76	**5028.48**	**5.28**
Mucor mucedo	733.32	471.42	419.04	419.04	314.28	314.28	209.52	209.52	314.28	209.52	104.76	104.76	**3823.74**	**4.02**
Geotrichum candidum	–	–	–	–	–	52.38	209.52	209.52	104.76	–	–	–	**576.18**	**0.61**
Nigrospora oryzae	104.76	209.52	261.90	314.28	157.14	104.76	–	–	–	–	–	–	**1152.36**	**1.21**
Colletotrichum gloeosporioides	209.52	314.28	261.90	157.14	104.76	157.14	209.52	314.28	314.28	419.04	366.66	261.90	**3090.42**	**3.25**
Corynespora sp.	104.76	104.76	157.14	157.14	209.52	104.76	52.38	52.38	52.38	–	–	–	**995.22**	**1.05**
Cylindrocladium sp.	–	104.76	157.14	209.52	261.90	209.52	104.76	104.76	52.38	52.38	–	–	**1257.12**	**1.32**
Diplodia sp.	–	209.52	157.14	104.76	104.76	157.14	52.38	–	–	–	–	–	**785.7**	**0.83**
Other	157.14	209.52	261.90	261.90	261.90	209.52	209.52	209.52	157.14	157.14	104.76	104.76	**2304.72**	**2.42**
Total (CFU/m³)	**11418.84**	**9376.02**	**9480.78**	**9637.92**	**8799.84**	**7595.1**	**7595.1**	**8014.14**	**7490.34**	**6233.22**	**5238**	**4295.16**	**95174.46**	
%	**12.00**	**9.85**	**9.96**	**10.13**	**9.25**	**7.98**	**7.98**	**8.42**	**7.87**	**6.55**	**5.50**	**4.51**		

Table 4.2: ANOVA table for seasonal variation

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	4305732.79	3	1435244.26	3.42	0.01*	2.69
Within Groups	41864414.3	100	418644.14			
Total	46170147.09	103				

June to August were considered as monsoon, September to November as post-monsoon, December to February as winter and March to May as summer. The monsoon season exhibited the highest fungal load and the summer had the lowest. The One-way ANOVA suggested a significant variation in the concentration of airborne fungi depending upon the seasonal variation ($P<0.05$) (Table 4.2). The post hoc analysis indicated that a significant variation was observed between monsoon and summer ($P<0.05$), as well as between post-monsoon and summer ($P<0.05$) (Table 4.3). *Aspergillus niger* (9.34%) was found to be most prevalent whereas *Curvularia lunata* (8.48%) and *Aspergillus flavus* (7.96%) were the second and third most dominant culturable fungi in the air during monsoon. *Fusarium oxysporum* (8.65%) went up to the top during post-monsoon followed by *Curvularia lunata* (7.44%) and *Aspergillus flavus* (7.24%). In winter, another fungal species, *Cladosporium cladosporioides* (11.79%), became the highest occurrence,

Table 4.3: Results for Post hoc test for ANOVA

Levels	P-value
Monsoon - Post-monsoon	0.39
Monsoon - Winter	0.17
Monsoon - Summer	0.005*
Post-monsoon - Winter	0.49
Post-monsoon - Summer	0.01*
Winter - Summer	0.09

*Significance at $P<0.05$

and *Penicillium chrysogenum* (7.94%) was the second most dominant fungi in the atmosphere. Another species of *Cladosporium*, *C. herbarum* (7.26%) was the third most prevalent fungi during winter in the air. *Aspergillus niger* (12.62%) was the most dominant in the air of the summer market, whereas *Alternaria solani* (11.63%)

Table 4.4: Seasonal variation of fungi in the air of the study area

Fungal isolates	Fungal concentration (%)			
	Monsoon	Post-monsoon	Winter	Summer
Curvularia lunata	8.48	7.44	2.72	6.31
Cladosporium cladosporioides	–	2.01	11.79	3.99
Cladosporium herbarum	3.46	2.01	7.26	4.65
Aspergillus niger	9.34	5.43	4.99	12.62
Aspergillus flavus	7.96	7.24	5.22	8.31
Aspergillus fumigatus	4.84	3.42	0.45	–
Penicillium chrysogenum	4.50	5.23	7.94	5.65
Penicillium digitatum	2.08	3.62	6.12	3.65
Penicillium citrinum	2.25	4.02	4.76	0.66
Fusarium oxysporum	6.23	8.65	6.12	5.32
Fusarium solani	4.67	6.64	4.54	3.65
Phoma herbarum	2.08	1.41	–	–
Drechslera sp.	2.42	5.23	5.22	1.00
Alternaria alternata	4.67	4.23	4.08	7.97
Alternaria brassicicola	3.98	3.62	3.40	7.31
Alternaria solani	3.46	2.82	4.76	11.63
Rhizopus nigricans	6.92	4.63	3.40	2.33
Rhizopus stolonifer	7.44	5.63	3.63	2.99
Mucor mucedo	5.36	4.02	3.17	2.66
Geotrichum candidum	–	0.20	2.27	–
Nigrospora oryzae	1.90	2.21	–	–
Colletotrichum gloeosporioides	2.60	1.61	3.63	6.64
Corynespora sp.	1.21	1.81	0.68	–
Cylindrocladium sp.	0.87	2.62	1.13	0.33
Diplodia sp.	1.21	1.41	0.23	–
Others	2.08	2.82	2.49	2.33

and *Aspergillus flavus* (8.31%) were the second and third most prevalent fungi in the air of outdoor marketplaces (Table 4.4).

Monitoring of atmospheric parameters

The temperature was found to be a maximum of 48ºC during April, and the minimum temperature of 8ºC was observed in December. The average temperature was found to be in the range of 18-33ºC. The maximum relative humidity of 98% was observed from July to September, whereas the minimum relative humidity of 10% was observed during March. The average relative humidity was found to lie between 49 to 86% (Table 4.5). A significant positive correlation was observed

Table 4.5: Environmental parameters of the study sites

Months	Maximum temperature (ºC)	Minimum temperature (ºC)	Average temperature (ºC)	Maximum humidity (%)	Minimum humidity (%)	Average humidity (%)
June	42	24	29	97	30	77
July	36	25	28	98	53	84
August	35	24	29	98	50	86
September	36	24	28	98	52	82
October	35	21	26	96	31	76
November	34	15	23	92	31	74
December	30	8	19	88	23	74
January	32	10	18	84	23	74
February	34	11	21	80	18	63
March	45	22	30	88	10	51
April	48	25	33	90	12	49
May	46	24	31	90	28	64

Table 4.6: Spearman correlation between monthly fungal concentration and environmental parameters

	Maximum temperature	Minimum temperature	Average temperature	Maximum humidity	Minimum humidity	Average humidity
r	–.227	.143	–.232	.692	.717	.889
P	.478	.658	.468	.013*	.009*	.000*

between humidity (maximum, minimum, and average) and monthly fungal concentration in the air of the markets (Table 4.6). In the Spearman correlation study of the monthly fungal concentration of the five most dominant fungi with environmental parameters, it was observed that *Aspergillus niger* was positively correlated with maximum, minimum and average temperature. *Aspergillus flavus* showed a positive correlation with minimum temperature, as well as maximum, minimum and average humidity. A significant positive correlation was observed between *Fusarium oxysporum* and maximum, minimum and average humidity. Another dominant fungus *Curvularia lunata* was found positively correlated with minimum temperature and maximum, minimum as well as average humidity. In contrast one of the domin-ant fungi *Penicillium chrysogenum* exhibited a negative correlation with minimum and average temperature (Table 4.7).

Fungi isolated from decayed fruits and vegetables

A significant number of fungal species belonging to 11 genera like *Alternaria, Fusarium, Aspergillus, Rhizopus, Colletotrichum, Curvularia, Nigrospora, Penicillium, Geotrichum, Diplodia* and *Cylindrocladium* were isolated from six types of vegetables (capsicum, brinjal, onion, maize, tomato and cucumber) and six types of fruits (apple, orange, banana, pomegranate, myrobalan, and papaya). The maximum fungi were isolated from tomato and banana, followed by capsicum, brinjal and onion. Fungal genera viz. *Alternaria, Fusarium, Rhizopus, Penicillium* and *Aspergillus* were the major pathogens responsible for the rotting of fruits and vegetables with a significant economic loss (Table 4.8).

Table 4.7: Environmental parameters of the study sites

Fungal species		Maximum temperature	Minimum temperature	Average temperature	Maximum humidity	Minimum humidity	Average humidity
Aspergillus niger	r	.817	.872	.837	.540	.110	.142
	P	.001*	.000*	.001*	.070	.734	.660
Aspergillus flavus	r	.284	.583	.261	.887	.723	.685
	P	.370	.047*	.413	.000*	.008*	.014*
Fusarium oxysporum	r	-.365	-.030	-.336	.676	.776	.781
	P	.243	.925	.285	.016*	.003*	.003*
Curvularia lunata	r	.376	.696	.423	.970	.813	.753
	P	.229	.012*	.171	.000*	.001*	.005*
Penicillium chrysogenum	r	-.523	-.660	-.610	-.465	-.333	-.145
	P	.081	.020*	.035*	.127	.290	.653

* Significance at P<0.05

Table 4.8: Isolated fungal species from rotted vegetables and fruits

Vegetables	Fungal isolates
Capsicum	*Alternaria alternata, Alternaria solani, Fusarium oxysporum, Fusarium solani, Aspergillus niger, Rhizopus nigricans, Rhizopus stolonifer.*
Brinjal	*Alternaria solani, Alternaria alternata, Fusarium oxysporum, Fusarium solani, Colletotrichum gloeosporioides, Aspergillus niger.*
Onion	*Aspergillus niger, Aspergillus flavus, Penicillium* sp., *Fusarium oxysporum, Rhizopus stolonifer, Colletotrichum gloeosporioides*.
Maize	*Fusarium oxysporum, Aspergillus flavus, Penicillium* sp., *Nigrospora oryzae, Diplodia* sp.
Tomato	*Alternaria solani, Alternaria alternata, Fusarium solani, Fusarium oxysporum, Geotrichum candidum, Aspergillus flavus, Aspergillus niger, Rhizopus stolonifer, Curvularia lunata*.
Cucumber	*Aspergillus flavus, Fusarium solani, Geotrichum candidum, Rhizopus stolonifer.*
Fruits	**Fungal isolates**
Apple	*Aspergillus niger, Fusarium* sp., *Penicillium* sp., *Colletotrichum gloeosporioides*.
Orange	*Penicillium digitatum, Alternaria alternata, Aspergillus flavus, Rhizopus stolonifer.*
Banana	*Fusarium oxysporum, Rhizopus stolonifer, Rhizopus nigricans, Colletotrichum gloeosporioides, Diplodia* sp., *Aspergillus niger, Aspergillus fumigatus, Curvularia* sp., *Cylindrocladium* sp.
Pomegranate	*Aspergillus niger, Fusarium solani, Colletotrichum gloeosporioides, Alternaria solani, Curvularia* sp.
Myrobalan	*Penicillium citrinum, Colletotrichum gloeosporioides, Aspergillus niger, Fusarium* sp., *Alternaria alternata*.
Papaya	*Alternaria solani, Colletotrichum gloeosporioides, Rhizopus stolonifer, Rhizopus nigricans, Mucor mucedo*.

DISCUSSION

Aeromycological survey was conducted and 95174.46 CFU/m^3 of 25 fungal species with some sterile mycelia were detected in the air of vegetable and fruit markets in Purulia town during the study period. *Aspergillus* was found to be the dominant fungal type with three species, *Aspergillus niger, Aspergillus flavus* and *Aspergillus fumigatus* among which *Aspergillus niger* was predominant. The other prevalent fungi *viz. Aspergillus flavus, Fusarium oxysporum, Curvularia lunata,* and *Penicillium chrysogenum* in marketplaces had shown a very similar pattern of occurrence to the findings of Arya and Arya in fruit markets of Baroda where both *Aspergillus niger,* and *Aspergillus flavus*, along with *Fusarium* were the dominant fungal types[1]. A similar kind of observation with *Aspergillus niger* as a dominant fungal species was reported from Bangladesh[10]. Fungal spores are highly seasonal due to their susceptibility to variations in the atmosphere[11,12,13] and the concentrations of airborne fungal spores fluctuate according to the changes in season[14,15]. The highest fungal load in the air of marketplaces was observed in monsoon, similar to the previous study in Sudan[16] and the highest incidence of fungi was observed in June. In tropical and subtropical regions airborne viable fungi were reported to be lowest in summer[17] which strongly supports the findings of current observation where the lowest number of culturable fungi was observed in summer. The prevalence of unsanitary conditions and poor disposal systems in the market areas could be one of the main reasons for the abundance of fungal spores. Rotting vegetables and fruits, deteriorating paper bags, and packaging materials in the market serve as substrates for the luxuriant growth of fungi and are the main reasons for airborne fungal inocula. The highest occurence of fungal load in monsoon was achieved due to the enhancement of humidity and moderate temperature, which may lead to the faster growth and sporulation of fungi. Further, it is assumed that very poor ventilation of storage places and fruit containers increases the heat and humidity inside the containers, encouraging fungal growth. It was reported that during the unboxing of fruits and vegetables, the fungal spores disperse rapidly in the surrounding atmosphere[1]. Environmental variables like temperature and humidity were found to influence fungal concentration in the air, similar to the previous observation of different researchers[18,19] where temperature positively influences the spore release in the atmosphere, thus increasing the spore concentration in the air. The occurrence of *Aspergillus, Penicillium, Curvularia, Cladosporium, Rhizopus, Alternaria,* and *Fusarium* from vegetables reported by earlier workers[4,20] corroborates with the current findings where similar fungal species were isolated from rotting vegetables and fruits of Purulia markets. Among the vegetables, the maximum fungi were isolated from tomatoes, whereas bananas, among fruits, contributed the highest fungal isolates.

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Research Article

MANAGEMENT OF *POTYVIRUS* INFECTION IN CUCUMBERS IN THE TERAI REGION OF WEST BENGAL USING PLANT EXTRACTS

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Potyvirus is a well known pathogen to infect agricultural crops worldwide. Information regarding potyviral plant disease from the sub-Himalayan 'terai' region of West Bengal is meagre. Keeping this in view, the present study was conducted to survey the *Potyvirus* infection causing cucumber (*Cucumis sativus*) leaf curl disease from Terai region of West Bengal along with partial detection of the virus through reverse transcriptase polymerase chain reaction (RT-PCR). Finally, the management of its aphid vector [*Myzus persicae* (Sulzer, 1776)] was studied using plant extracts of *Clerodendrun viscosum, Azadirachta indica, Boerhavia diffusa* and *Leucas indica*.

Virus infected samples of *Cucumis sativus* was collected from March-May, 2017 from the Terai regions of West Bengal with presence of the aphid vector *Myzus persicae* on the leaves. RNA was extracted from leaves and specific genes were amplified with specific primers in RT-PCR. The amplified products were allowed to run on agarose gel. An amplicon of ~650bp was found in the extracted RNA using universal Potyvirus primer (CPuP and P9502), indicating probable infection with the RNA virus, *Potyvirus*.

Leaf extracts of the plants like *Azadirachta indica, Boerhavia diffusa, Clerodendrum viscosum* and *Leucas indica* were used for managing *Myzus persicae*, the aphid vector for *Potyvirus* and compared with healthy control set of plants. After 14 days of foliar spray and vector inoculation, the treated plants showed no sign of virus infection, whereas, the untreated control showed curling and blistering on leaves indicating presence of virus, which was confirmed by RT-PCR too. The study indicates the potential of plant extracts with antiviral compounds in the management of viral infections on agricultural crops.

Key Words: *Potyvirus* infection, *Cucumis sativus*, RT-PCR, Foliar extract, Aphid (*Myzus persicae*) vector management.

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INTRODUCTION

Viruses are submicroscopic, obligate intracellular parasites and contains either an RNA or DNA genome which remains surrounded by virus-encoded protective protein coat. Viruses depend on its host for propagation by supplying the complex metabolic and the biosynthetic machinery of the eukaryotic or the prokaryotic cells[1].

The genus Potyvirus is the largest genus of the family *Potyviridae*, with nearly 200 definite and tentative species[2]. It is named after the type species Potato virus Y. It is one of the major groups of viruses that infect agricultural, pasture, horticultural and ornamental plants[3] and cause major damage to crops worldwide, leading to huge financial loss[4].

During the late 1920s, Kenneth Smith, while studying potato viruses in the UK, named one of them *Potato virus Y*[5]. Subsequently, other viruses were shown to have similar properties, and in 1971 this group was given the acronym of "*Potyvirus*" for an experimental period of five years6. A breakthrough in potyvirus studies was achieved in 1986 when the complete genome sequences of two members of this group, *Tobacco etch virus*[7] and *Tobacco vein mottling virus*[8] were reported for the first time. Intensive research during the following years led to a greater understanding of the potyviral genome structure and expression.

Although there are many literature describing the incidence of potyviral disease all over the world, there are few or rather almost no information regarding potyviral disease profile and their management strategies available from the 'terai' region of West Bengal till now. Therefore, in the present study, the following objectives have been identified:

1. Screening of *Potyvirus* infected cucumber leaf curl from Terai region of West Bengal,
2. Partial detection of the virus through reverse transcriptase polymerase chain reaction (RT-PCR),
3. Management of insect vector [*Myzus persicae* (Sulzer, 1776)] of *Potyvirus* using four different plant extracts.

METERIALS AND METHODS

Maintenance of plants

Preparation of soil and filling of pots

For maintenance of cucumber (*Cucumis sativus* L., family: Cucurbitaceae) plants, pots were filled with sand, soil and compost in a 2:2:1 ratio (w/w). All the pots were of same size (16 cms in diameter and 12 cms in height).

Watering and Manuring

The plants in pots were grown under natural conditions of daylight and temperature (25-28°C) and maintained throughout the growing season. The plants were watered regularly with ordinary tap water to avoid the drying of soil. For maintenance of plants (both infected and healthy) in the experimental garden, each pot was supplemented with 25 mg of nitrogen, 10 mg of phosphorus and 10 mg of potash.

Preparation of sample for experiments

Leaves of the infected plants were cut with a sterile blade to avoid contamination. Cut leaves were put in zip packs and marked according to the area and date. Leaves were then washed with running distilled water to avoid any trace of dust. Similarly, healthy leaves were also collected. Washed leaves were dried with blotting paper and weighed. Dried leaves were packed in a fresh air tight plastic pack and stored at –20˚C for future experiments.

Extraction of total RNA

Total RNA was also extracted from the infected and healthy plants following the method of Ghawana *et al*.[9]. Firstly, 100 mg of tissue was ground to a fine powder in liquid nitrogen using a mortar and pestle. Then 2 ml of solution 1 was added and ground further that would help in instantaneous denaturation of protein. After that, 400 μl of diethyl pyrocarbonate (DEPC) treated RNase free water was added and mixed by grinding. Then the mixture was transferred to 2 ml micro-centrifuge tubes and left for 5 minutes at room temperature. Then 200 μl of chloroform was added to each tube, vortexed briefly (<10 s) and left for 10 minutes at room temperature. Then it was centrifuged at 13,000 rpm for 10 min at 4°C and the upper aqueous phase was transferred into fresh tubes. After that, 0.6 volumes of isopropanol was added, vortexed briefly (<10 s) and left for 10 min at room temperature. Then it was again centrifuged at 13,000 rpm for 10 min at 4°C and the supernatant was discarded. Finally, the RNA pellet was washed with 70% ethanol, air dried and dissolved in 20 to 50 μl of DEPC treated RNase free water and stored at –70°C.

Agarose Gel Electrophoresis of RNA

Agarose gel Electrophoresis was done at 5V/cm through 1.2% (w/v) agarose gels in 1X Tris Acetic acid EDTA (TAE) buffer for 60 min for visualization of RNA under UV-Transilluminator (GeNei, Bangalore).

Reverse Transcription Polymerase Chain Reaction (RT-PCR)

Reverse Transcription Polymerase Chain Reaction (RT-PCR) was done from the total RNA extracted using the One Step M-MuLV RT-PCR kit (GeNei, Bangalore) using several sets of primers following the manufacturer's protocol. After amplification, the products were electrophoresed at 5V/cm through 1.2% (w/v) agarose gels in 1X Tris Acetic acid EDTA (TAE) electrophoresis buffer, and visualized under UV transilluminator following ethidium bromide staining of the gel (60 min in 1 μg/ml ethydium bromide).

Primers used for detection of RNA virus infected samples

Primer Name	Sequence (5´-3´)
CPuP	TGAGGATCCTGGTGYATHGARAAYGG
P9502	GCGGATCCTTTTTTTTTTTTTTTTT

* N=T+C+A+G; Y=T+C; H=T+C+A; R=A+G; M=A+C; S=C+G; V=A+C+G

Mechanical sap inoculation

Mechanical sap inoculation of *Potyvirus* to healthy cucumber plants was done following the method of Reddy *et al*.[10]. Potassium phosphate buffer (0.1M, pH 7.0) was used for the mechanical inoculation of the viral disease to the test plants. Young tender leaves from the infected plants showing good symptoms were collected, washed thoroughly under tap water to remove dirt and blotted dry. The leaf samples were ground in a clean sterilized pestle and mortar by adding chilled buffer at 1 ml per gram of leaf tissue. After thorough maceration, the pulp was squeezed through sterile absorbant cotton or muslin cloth. The resultant extract was used as "standard inoculums" for mechanical sap inoculation.

Young healthy, vigorously growing test plant seedlings grown from healthy seeds and maintained in an insect-proof glass house were selected. A pinch of celite (600 mesh) was added to the standard inoculum before inoculation as an abrasive. A small piece of sterilized absorbant cotton wool soaked in the standard inoculum was gently rubbed over the upper surface of the leaves, gently and unidirectionally. The plants were labelled and kept under observation for symptom expression upto 60 days.

Extraction of plant extract and foliar spray

Collection of plants

Several plant materials (leaves) were collected from sub-Himalayan West Bengal. The plants were selected on the basis of easy availability in the growing areas of sub-Himalayan West Bengal.

Preparation of leaf extract

The leaves of *Clerodendrun viscosum, Azadirachta indica, Boerhavia diffusa* and *Leucas indica* were used for extraction. The leaf extracts were prepared following the method of Reddy *et al*.[11]. Leaf materials (500 gm each) were washed thoroughly in running tap water to remove any trace of dust particles. Then the leaf materials were pulverized in a mortar and pestle and mixed with water in a 1:1 ratio (w/v). The mixture was squeezed by using double layered muslin cloth and filtrate was diluted at 1:4 (20%) ratio and the diluted extracts were used for field spray following the method of Reddy *et al*.[11].

Field application

For spraying, the cucumber field was divided into 4 sets each contained 5 plants. In another one set sterile distilled water was sprayed and it was treated as control plot. The other 4 sets were sprayed with 4 different leaf extracts. All the spraying was done at 3 days intervals after sowing and continued up to 21 days. The concentration of the spray solute was 20%. The disease incidence was recorded.

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE)

The polyacrylamide gel was prepared according to the standard protocol of Laemmli[12]. The samples were loaded and run @100V @25 mA (two gels were run @50 mA). The gel was then placed in a plastic container, covered with a fixing solution and was incubated at room temperature for 60 minutes. Then the solution was poured off. The gel was then covered with Coomassie blue solution and was shaken at room temperature overnight. The staining solution was poured off and the gel was washed with a destainer.

RESULTS AND DISCUSSION

Field observation and virus isolation

Virus infected samples of *Cucumis sativus* was collected during the period of March-May of the year 2017 from the Sub-Himalayan Terai regions of West Bengal (Fig. 5.1). The samples were collected on the basis of the visible symptoms, such as curling of leaves, blisters

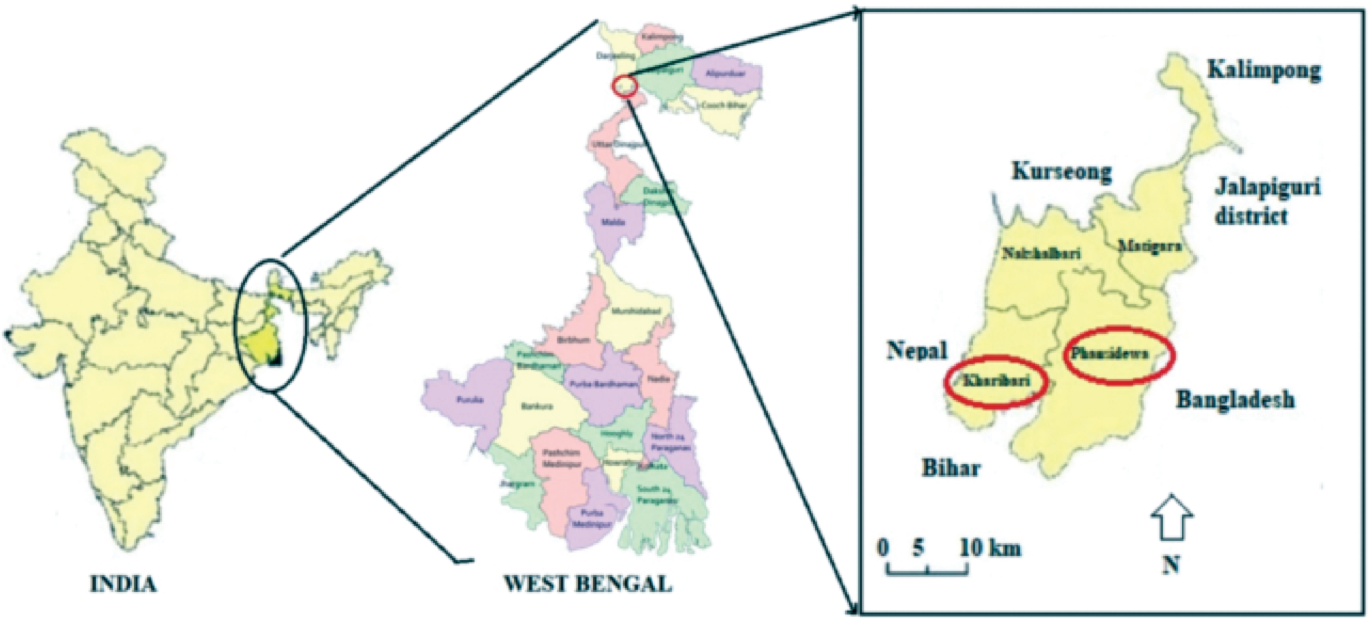

Fig. 5.1: Location map of the study area of Terai region in the Kharibari and Phansidewa of Siliguri subdivision of Darjeeling district, of West Bengal, India.

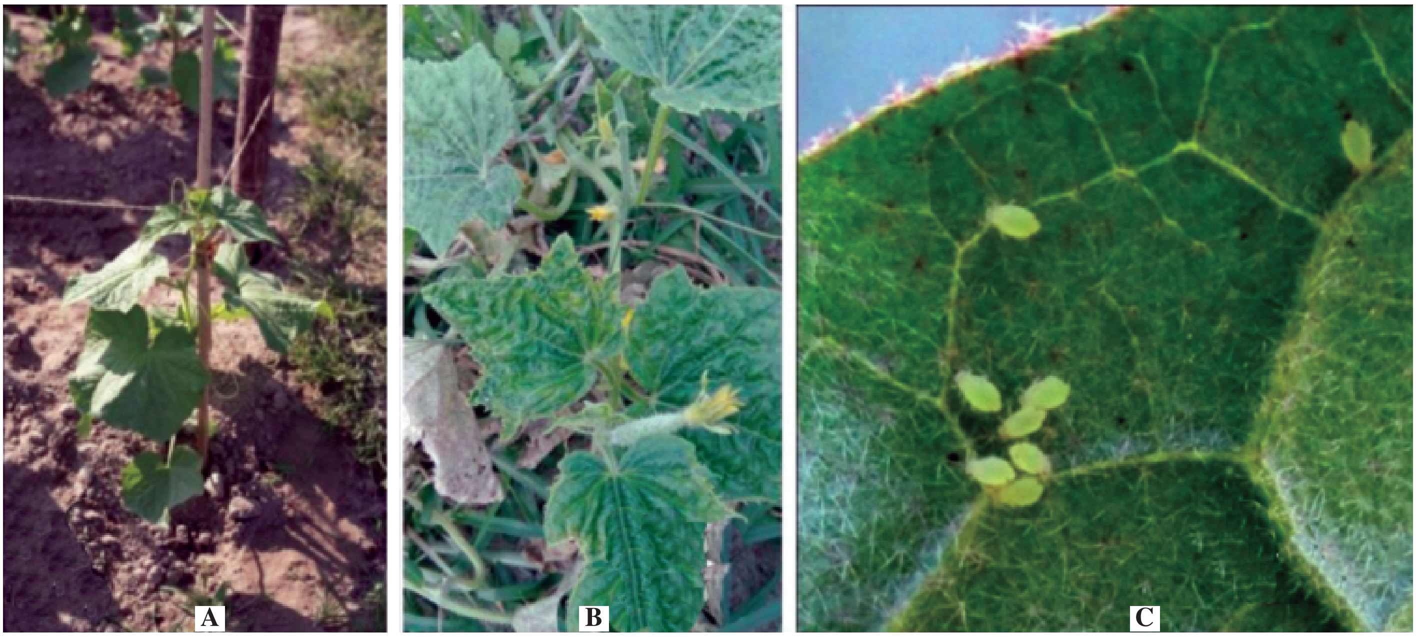

Fig. 5.2: Cucumber plants (A) Healthy, (B) Naturally infected; (C) Vector *Myzus persicae* on the lower side of the leaf

on the surface of the leaves, deformed leaves. The pre-sence of the *Potyvirus* aphid vector, *Myzus persicae* on the lower surface of the leaves helped in the identi-fication of the infected samples (Fig. 5.2).

Reverse Transcriptase Polymerase Chain Reaction (RT-PCR)

Ribinucleic acid (RNA) was extracted and specific genes were amplified in RT-PCR. The amplified pro-ducts were allowed to run on agarose gel. The RT-PCR products of the samples were amplified by specific primers and molecular weight was determined by using standard DNA-molecular weight markers. The primer was 'CPuP' and 'P9502'. The expected size of the amplified RNA by the primer as mentioned above was ~650 bp. The results of our experiments were compared with the standard results as suggested by Singh *et al*.[13] and van der Vlugt *et al*.[14]. The samples were considered to contain virus when the molecular weight of the amplified products matched with standard reported molecular weights. The results of some amplified samples in presence of DNA-molecular weight markers have been presented in Fig. 5.3.

In the present study infected cucumber leaves were collected from several places of sub-Himalayan terai region of West Bengal. RNA was extracted and cDNA was prepared from the infected leaves by RT-PCR. An amplicon of ~650bp was amplified from the extracted RNA using universal Potyvirus primer (CPuP and P9502), which indicates that the plant may be infected with the RNA virus, *Potyvirus*. On the basis of PCR amplification of viral genome, it is evident that the virus is a *Potyvirus* as reported by Singh *et al*.[13]. The isolated virus produces characteristic symptoms of vein clearing, chlorotic spots, leaf curling, leaf swelling and later varied types of symptoms including leaf reduction to shoe string, leaf distortion, puckering, mosaic patterns on leaves and stunted growth of papaya plants as repor-ted by Reddy *et al*.[10]. Subsequently, the incidence of the viral disease was also reported in Bihar[15], Madhya

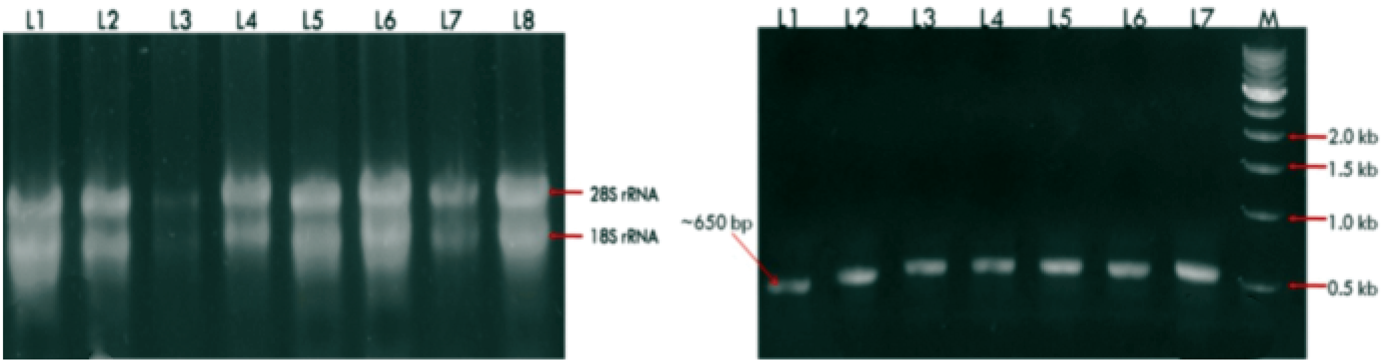

Fig. 5.3: (A) Extracted RNA product on 1% agarose gel; (B) L1-L7= RT-PCR amplicons on 1% agarose gel using CPuP/ P9502 primer pair (M= 500bp DNA ladder)

Pradesh[16], Uttar Pradesh[17], Udaipur of Rajasthan[18], Marathwada region of Maharastra[19] and Andhra Pradesh[20]. Several other reports also support the wide host range of *Potyvirus*. It is the largest and one of the most rapid growing among the 78 plant virus genera. Viruses belonging to this genus are responsible for severe disruption to many economically important crops[21,22]. Some of the hosts of the virus are papaya[23], potatoes[24], cucurbits[25], tomato[26], common bean (*Phaseolus vulgaris* L.), *Vigna unguiculata, Nicotiana benthamiana* and plants of the family Chenopodiaceae, Fabaceae and Solanaceae[27], *Cucumis metuliferus, Cucurbita pepo, Melothria pendula and Momordica charantia*[28], *Luffa acutangula, Cucumis sativus, Cucumis melo* var. *Utillissium*[29], cassava (*Manihot esculenta*), sweet potato (*Ipomoea batatas*), yams (*Dioscorea* sp.) and aroids namely taro (*Colocasia esculenta*), tannia (*Xanthosoma sagittifolium*) and elephant foot yam (*Amorphophallus paeoniifolius*)[30].

Management

Foliar extracts of *Azadirachta indica, Boerhavia diffusa, Clerodendrum viscosum* and *Leucas* indica were used for managing the vector, *Myzus persicae*. The vector was collected from the infected fields from the Sub-Himalayan Terai regions of West Bengal and it was used for artificial inoculation of the virus in samples sprayed with the mentioned extracts (Fig. 5.4).

All the plants were divided into four sets containing five plants each and each set was treated with one type of foliar extract and was artificially inoculated with the vector to check the spread of the virus (Fig. 5.5). Two sets of the plant were kept as healthy without any treatment with the extract but vector inoculation was done

Fig. 5.4: Different plants— A. *Azadirachta indica*, B. *Boerhavia diffusa*, C. *Clerodendrum viscosum* and D. *Leucas* used as source of botanical extracts for vector management

Fig. 5.5: Healthy cucumber plants before vector inoculation and treatment with A. *Azadirachta indica*, B. *Boerhavia diffusa*, C. *Clerodendrum viscosum* and D. *Leucas indica* plant extracts

Fig. 5.6: A. Healthy cucumber plants before vector inoculation (untreated control); B. Control plants after 14 days of vector inoculation showing leaf curling and blistering symptoms.

(Fig. 5.6). After 14 days of foliar spray and vector inoculation the treated plants remained healthy and there was no sign of virus infection, whereas, the untreated control showed curling and blistering on leaves indicating presence of virus (Fig. 5.7). After that, all the plants were tested for the presence of the virus using RT-PCR with the same primer set. The result of RT-PCR showed similar results as mentioned above. After that, total protein was isolated using phosphate buffer pH 7.0 from all five sets of plants and SDS-PAGE was run to see the protein band pattern. The results are shown in Fig. 5.8, where a difference in protein was obvious.

Similar types of results were obtained by several other workers[31-34]. The leaf extracts of *Boerhavia diffusa, Clerodendum viscosum, Leucas indica* and *Azadirachta indica* were used as antiviral agents in managing the vector *Myzus persicae* which causes the cucumber leaf curl. Glycoproteins obtained from *Boerhavia diffusa* incites the antiviral system in the treated plants. The glycoprotein inhibits the infection of the virus by blocking virus replication[31]. *Clerodendrum viscosum* induces antiviral state in the plants through formation of de nove synthesised protein and is active in signaling the activation of defense mechanism in susceptible hosts[32].

Presence of alkaloids, flavonoids, carbohydrates, glycosides, steroids, saponins, fixed oils, tannins, phenolic compounds, proteins and aminoacids in *Leucas indica* contributes to antimicrobial activity[33]. Azadirachtin is

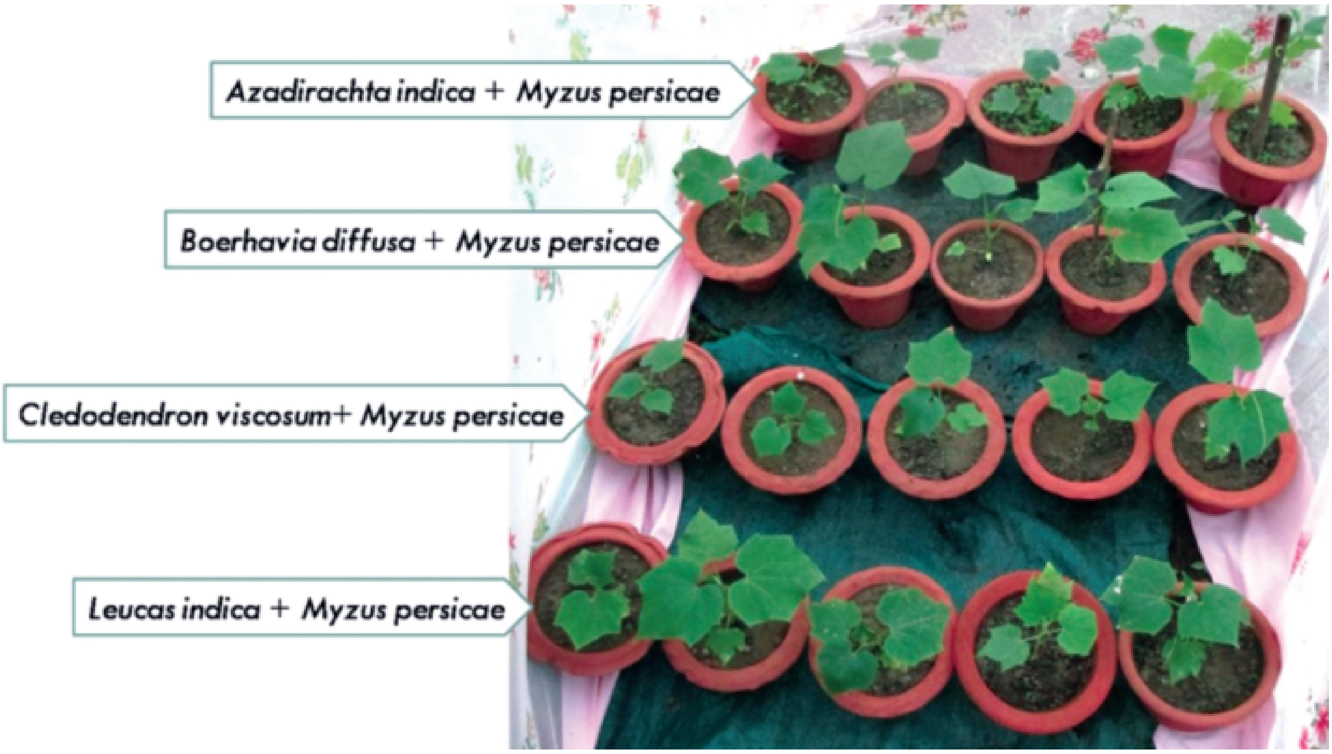

Fig. 5.7: Healthy cucumber plants after 14 days of vector inoculaton and treatment with *Azadirachta indica, Boerhavia diffusa, Clerodendrum viscosum* and *Leucas indica* plant extracts (specified in the boxes attached to the plants)

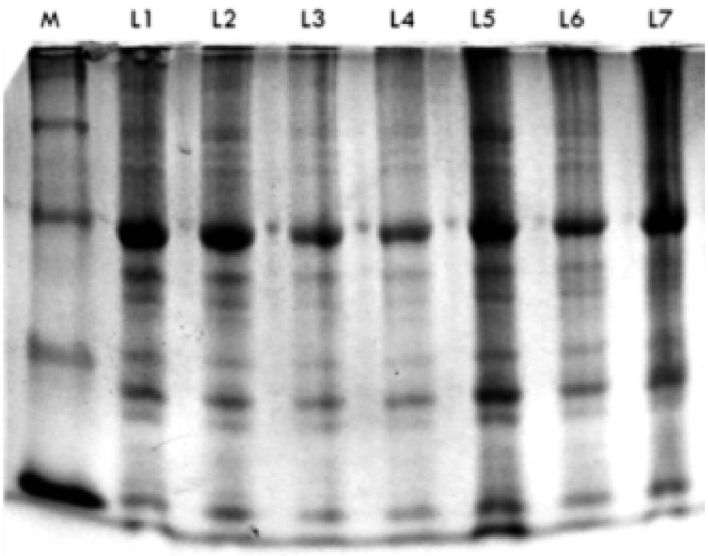

Fig. 5.8: SDS-PAGE of the extracted proteins from cucumber plants after 14 days of vector inoculaton and treatment with *Azadirachta indica* (L1), *Boerhavia diffusa* (L2), *Clerodendrum viscosum* (L3) *Leucas indica* (L4) plant extracts and untreated control (L5-L7); M= Standard protein ladder.

considered as the most active principal substance in neem which has growth regulating, fungicidal and insecticidal properties. Azadirachtin, chemically a tetran or terpenoid component of neem acts on the mitotic cells and blocks the microtubule polymerization. Certain activities of genes and proteins are also altered by azadirachtin[34].

Plant viruses are of great concern to farmers, researchers and policy makers because of the enormous loss they cause in different crops. Viral pathogens have become a major constrain on the crops[35]. Because of their peculiar nature and characteristic association with hosts and vectors, no therapeutic method to completely control them has been found successful. However, certain preventive measures, if adopted, can be of great help in avoiding viral diseases. Antiviral substances of plant origin may be used as a component for disease management. Infection of several viral diseases could be prevented by the application of extracts/antiviral compounds from various plants[36].

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Short Communication

TRADITIONAL WEATHER FORECASTING KNOWLEDGE AMONG INDIGENOUS PEOPLES OF MAHARASHTRA FOR AGRICULTURAL PLANNING AND LIVELIHOOD SUSTAINABILITY

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The study delves into the profound significance of traditional weather forecasting practices among indigenous communities in Maharashtra, particularly in the Raigad region. It highlights how these ancient methods are crucial for agricultural planning and sustaining livelihoods. By examining bioindicators, myths, and cultural practices, the research merges traditional wisdom with contemporary scientific insights. It underscores how indigenous peoples in Maharashtra predict rainfall, foresee agricultural outcomes, and adeptly navigate climate uncertainties. Their observational skills, deep ecological knowledge, and communal resilience continue to play a pivotal role in weather forecasting and agricultural adaptation, bridging ancient traditions with modern challenges.

Key Words: Bioindicators, traditional knowledge, weather forecasting, climate change, agriculture, Indigenous peoples of Maharashtra.

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INTRODUCTION

In ancient times, weather forecasting was a crucial aspect of human civilization. Numerous cultures have created advanced systems for predicting climate trends and their effects on agriculture. Weather forecasting has an extensive and distinguished history in India, having a foundation in practices and traditional knowledge systems that date back thousands of years. Accurate rainfall forecast is important for crop planning, cultivation, and overall agricultural output, especially in areas like Maharashtra where agriculture is a major source of income for farmers[1].

Weather has always captivated human interests, profoundly impacting daily life and historical events. The history of weather forecasting traces back to ancient civilizations that studied celestial patterns. Today, meteorologists utilize cutting-edge technology, but the journey began with early societies like the Vedic and Indus Valley civilizations in India. These ancient cultures demonstrated advanced knowledge of meteorology, using intricate structures and texts such as the Vedas, Puranas, and Aranyakas to predict weather patterns. The Rig Veda, for instance, venerates weather-related deities, underscoring the ancient Indian emphasis on understanding and predicting atmospheric conditions[2].

The increasing effects of climate change on global food security have prompted considerable focus on enhancing weather forecasting and agricultural technologies worldwide. Nations are investing in advanced meteorological tools, satellite imagery, and climate models to enhance agricultural adaptation and forecast accuracy. Climate-resilient agriculture is pivotal in advancing global food security in the face of climate challenges, aligning with the United Nations Sustainable Development Goals, particularly Goal 2 (Zero Hunger) and Goal 13 (Climate Action)[3].

Global initiatives such as the United Nations Framework Convention on Climate Change and the Global Framework for Climate Services are fostering collaborative efforts worldwide to enhance agricultural resilience to climate impacts. Farmers in susceptible regions are gaining from the exchange of best practices and innovations in climate-smart agriculture facilitated by multilateral partnerships, research collaborations, and knowledge-sharing platforms. The World Bank emphasizes that investments in climate-resilient agriculture are crucial for achieving sustainable development and ensuring food security for future generations[4].

In Maharashtra, India, weather forecasting relies significantly on ancient methods embedded in traditional knowledge systems. Techniques such as astronomy assessments, astrology, observations of animal behavior, plant indicators, wind patterns, and sky/cloud formations play crucial roles in predicting weather changes. For instance, heightened activity among animals like birds and frogs often precedes the onset of monsoon rains, providing valuable insights for farmers. Specific plant phenological events also serve as reliable indicators of upcoming weather conditions. Additionally, the Antariksha method, which interprets atmospheric phenomena, has historically been employed in Maharashtra to predict rainfall. By integrating insights from these ancient practices and texts, communities and farmers in Maharashtra have developed effective strategies to predict weather patterns, thereby bolstering their resilience and enhancing agricultural planning capabilities[1,5].

The Palghar district in northern Maharashtra features diverse agro-climatic zones, encompassing rugged terrain and coastal plains. Many farmers, particularly indigenous tribes, rely primarily on rain-fed agriculture. Given the region's susceptibility to unpredictable weather and climatic fluctuations, accurate weather forecasting is crucial to mitigate risks such as crop failures, water scarcity, and unstable livelihoods. To effectively implement climate adaptation strategies and enhance agricultural resilience in Palghar, understanding traditional weather forecasting techniques used by local farmers and tribes is essential.

The current study aims to investigate traditional weather forecasting methods, with a special focus on those used in Maharashtra, India. The aim is to provide insights into how ancient cultures forecasted weather patterns and their implications for the current understanding of meteorology and agricultural planning by analysing historical writings, cultural practices, and traditional systems of knowledge.

METERIALS AND METHODS

Area of the study

Raigad district, the current investigation region, is located between latitudes 18.51°N and 73.18°E. The survey was carried out in some of the selected localities for the course of the 2021-2023 season. The documentation of indigenous knowledge has benefited from this research era. The Konkani district of Raigad is well renowned for its natural resources, which include an abundance of greenery and traditional knowledge of relating to rainfall prediction. The Sahyadri hills encircle Raigad district to the east, while to the south-east lie Satara district and Pune. Alibag, Pen, Murud, Karjat, Khalapur, Panvel, Uran, Mangaon, Tala, Roha, Sudhagad, Mahad, Poladpur, Mhasala, and Shiriwardhan are the Tahsils that together constitute Raigad.

Field work

Data gathering consisted of field trips and interviews with local populations, primarily groups of tribes, to document their traditional rain-predicting procedures. Semi-structured interviews were undertaken with key informants who were knowledgeable about indigenous bioindicators and weather prediction techniques.

Documentation of Bioindicators

Several bioindicators mentioned in traditional knowledge systems were documented.

Analysis

The obtained data was analysed qualitatively to find common themes, patterns, and relationships between conventional bioindicators and meteorological occurrences. The emphasis was on understanding the cultural meaning and ecological components of these behaviours.

RESULTS AND DISCUSSION

The Thane and Palghar tribal people in Maharashtra, India, came up with transformed rain forecasting systems based on bio-indicators. These approaches are significantly based on traditional ecologic knowledge, which has been passed down through generations. These tribes can accurately anticipate weather patterns, which are critical for their agricultural and fishing operations, using a combination of observational skills, experience, and passed-down culture. Bioindicators are phenomena of nature or organisms that indicate the present condition of the environment, including meteorological conditions. In Maharashtra, indigenous people frequently use traditional knowledge and bioindicators to predict rain.

Traditional bio-indicators and practices based on myths related to rainfall prediction in Maharashtra

1. **Dragonflies' Movement**. According to regional beliefs and tradition, the sight of dragonflies migrating in swarms a few hours before rainfall is frequently regarded as a warning of predicted rain. Indigenous groups in Maharashtra have long believed that dragonflies are messengers of rain, predicting the approach of wet weather. This traditional observation, based on cultural history, enhances ecologic information passed down through generations, impacting the way communities believe in natural occurrences for weather forecasting and agricultural planning.
2. **Decreased Croaking of Frogs**. In Maharashtra folklore, an important note drop in frog cries is sometimes connected with the coming soon drought.
3. **Delay Blooming of the Kadamba Tree**. According to traditional beliefs, the late or sparse blossoming of the Kadamba tree (*Neolamarckia cadamba*) predicts less rainfall in the next season, presently it is a myth that this invariably leads to drought.
4. **Minimal Ant Activity**. In Maharashtra, the observation of fewer ants in and around anthills indicates insufficient rainfall.
5. **Absence of Fireflies**. Many believe that a decrease in the number of fireflies during summer evenings indicates less rainfall in the coming months.
6. **Normal Blooming of Champak Flower**. The predicted blossoming of the Champak tree (*Michelia champaca*) indicates appropriate rainfall for crops and vegetation.
7. **Frequent Bird Calls**. According to traditional knowledge, typical bird activity and calls, particularly the Indian cuckoo, signal moderate rainfall in Maharashtra.
8. **Increasing Earthworm Activity**. The presence of earthworms on the soil surface is believed to indicate moderate rainfall and good soil conditions for cultivation.
9. **Excessive Croaking of Frogs**. Particularly after nightfall, excessive croaking of frogs is commonly taken as an indication of heavy rainfall and flooding to come.
10. **Early and Profuse Blossoming of the Kadamba Tree**. It is regarded as a significant indication of heavy rains in Maharashtra folklore.
11. **Increasing Ant Activities**. The observation of numerous ants moving up and down trees is said to indicate significant rainfall.
12. **Dragonfly Swarming**. In Maharashtra, large numbers of dragonflies flying low are considered a warning of oncoming severe rains.
13. **Lightening of the Sky**. In Maharashtra folklore, a gentle brightening or lightening of the sky, particularly in the evening, is frequently interpreted as the arrival of distant thunderstorms.
14. **High Humidity**. The significantly increased humidity levels, combined with a moist or heavy sensation in the air, can be used to predict thunderstorms.
15. **Darker Cloud Production**. The quick production of dark, alarming clouds in the sky is sometimes regarded as a portent of nearing thunderstorms.
16. **Rapid Temperature Decrease**. A sudden decrease in temperature, typically accompanied by a cold wind, is occasionally recognized as an indication of oncoming thunderstorms.
17. **Increasing Bird Activities**. In Maharashtra, unusual bird behaviour, such as frenetic flying or seeking shelter, could be interpreted as an indicator of an oncoming rainstorm.
18. **Weather Forecast**. In Maharashtra, indigenous peoples consider the early blooming of mango trees, leafing out of neem trees, budding of tamarind trees, and flowering of palash trees as weather-predicting indications.
19. **Healthy Flowering of Mango Trees**. In Maharashtra, profuse and early blooming of mango trees is frequently seen as a good indicator of probable high harvests for the next season. Farmers look into the timing and amount of mango flowers, as they believe it coincides with ideal weather conditions and pollination success, resulting in an abundance of fruit.

20. **Limited Flowering for Vital Crops**. Traditional farmers in Maharashtra pay particular attention to the flowering patterns of key crops such as rice, wheat, and millet. Limited blooming, indicated by fewer and later blooms, is sometimes regarded as an indication of potentially poor yields.

21. **Crop Pest Prediction**. Early signals of wilting in plants are used by Maharashtra farmers to predict future disease outbreaks. Wilting, along with discoloration or stunted development, may indicate the presence of fungal, bacterial, or viral diseases.

22. **Prediction of Pollination**. Some tribes in Maharashtra predict pollination by observing floral patterns and quantity. Tribal peoples forecast the arrival of pollinators such as bees, butterflies, and birds by studying their activity patterns. They observe the frequency and behavior of pollinators as they visit flowers.

23. **Bird Migration Patterns and Airborne Diseases**. Indigenous people watch bird migration patterns, particularly for migratory species. Changes in the timing or path of bird migration are believed to indicate changes in weather patterns and the potential spread of airborne diseases.

The integration of traditional ecologic knowledge and modern-day scientific approaches improves the resilience of Maharashtra's farming communities to climate change[6]. Maharashtra can promote an integrated approach to weather forecasting and agricultural planning by collaborating with indigenous peoples, government agencies, and research institutes[7].

CONCLUSION

In conclusion, traditional weather forecasting knowledge among indigenous populations in Maharashtra is an important resource for climate adaptation and agricultural sustainability. Recognizing the significance of bioindicators, myths, and cultural practices helps stakeholders to better understand and take advantage of indigenous communities' knowledge systems to resolve climate change issues. This integration of knowledge systems is essential for developing climate-resilient populations and ensuring food security for future generations in Maharashtra.

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