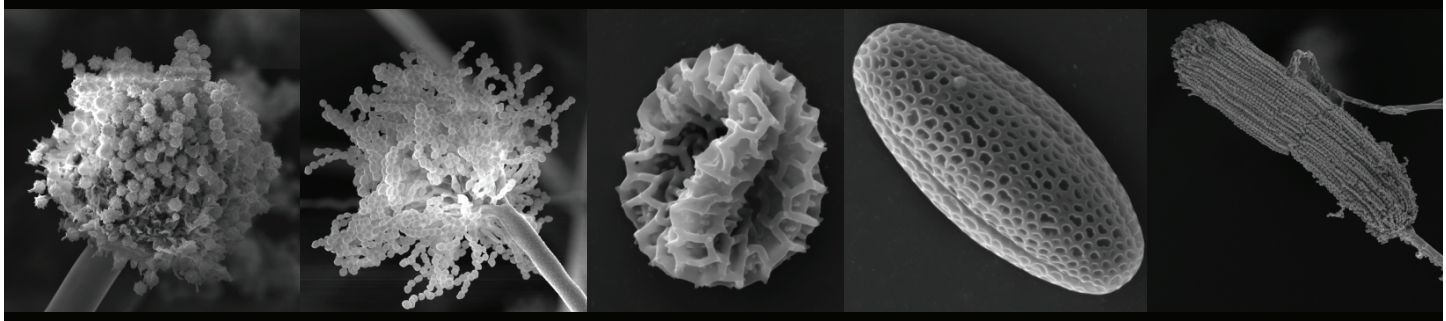
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Review Article

MICROBIAL PROTEASE – A VERSATILE ENZYME OF MODERN RESEARCH AND INDUSTRIES: A REVIEW

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Protease is one of the most prominent enzymes in use today. It catalyses the hydrolysis of peptide bonds in proteins. Owing to its catalytic activity, this enzyme is widely used across various sectors, including the pharmaceutical, detergent, food, agriculture and leather industries. At present, proteases are also extensively applied in medicine and therapeutics. Their catalytic properties make proteases emerging tools in the medical field for combating antigenic agents (microorganisms) such as viruses and bacteria. Protease enzymes help to degrade antigens and toxins by breaking down membranes and receptors. Traditionally, proteases have been sourced mainly from animals and, to a lesser extent, from plants. However, in recent times, microorganisms have emerged as the most promising source of protease enzymes. Numerous studies are currently being conducted on the production of proteases. In the future, the use and demand for this enzyme are expected to increase steadily. The production of protease enzymes using agro-waste offers a low-cost and suitable substrate that can be effectively utilised at an industrial scale.

Key Words: Enzymes; Protease; Biocatalyst; Proteins, Agro-waste.

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INTRODUCTION

Enzymes are crucial biocatalysts that are highly efficient and eco-friendly protein components synthesized by all living organisms. In living cells, enzymes are responsible for a variety of biochemical processes. The 'enzyme' word was first coined by the German biologist Wilhelm Kuhne in 1878 when he described yeast's ability of alcohol production from sugars, and it is taken from the Greek word enzume which means "within yeast"[1]. Their unique characteristics include specificity, high catalytic activity, and the ability to function at both moderate and extreme temperatures. Enzymes are required in small amounts, and they enhance the reactions without changing and involved themselves being consumed throughout the reaction. On account of their unique properties, enzymes found wide-scale applications in industrial sectors such as agriculture, food, pharmaceuticals, and biofuels. Over 90% of enzymes are simple globular proteins and the other 10% are conjugated proteins, which have a non-protein fraction referred to as the prosthetic group[2].

Classes of Enzymes

According to the international union of bio-

chemistry and molecular biology (IUBMB), enzymes are classified into six classes based on their catalytic activity[3]. The six classes of enzymes has been presented in Table 1.1. Among all the six enzyme classes, hydrolases are the main class of enzymes that are physiologically important and play a primary role in today's enzymatic industrial processes. Approximately 80% of various industrial enzymes belong to the class hydrolases[4]. Hydrolases also carry out various degradative reactions such as digestion, transport, regulation, and signaling process. Out of all the hydrolytic enzymes, proteases, amylases, and lipases have the most influence over the world enzyme market accounting for more than 70% of all enzyme sales. Proteases remain the dominant enzyme type, because of their wide applications in the detergent and dairy industries[5].

Proteases

Proteases catalyze the cleavage of peptide bonds in polypeptide chains of protein into amino acids. It possesses a varied range of applications in various manufacturing processes like detergents, leather, food, feed, and waste treatment industries. Proteases occur broadly in all living organisms including plants, animals, and microbes. They are capable of performing both degradative as well as synthetic functions[6,7,8]. These enzymes play an vital role in a variety of metabolic processes such as cell growth, death,

Table 1.1: Classes of Enzymes

Class of Enzymes	Reactions	Enzymes
EC 1 Oxidoreductases	The enzymes catalyzing oxidation/reduction reactions belong to this class	Peroxidase, dehydrogenase, oxidases, oxygenases
EC 2 Transferases	Catalyze the transfer of groups from one substrate to another, other than hydrogen	Transaminases, fructosyltransferases, acyltransferases
EC 3 Hydrolases	Hydrolases catalyze the hydrolysis of C—O, C—N, C—C, and some other bonds, including P—O bonds in phosphates	Proteases, amylases
EC 4 Lyases	This class of enzyme catalyzes the non-hydrolytic cleavage of the C—C, C—O, and C—N bond by addition or elimination reactions	Lipases, phosphatases
EC 5 Isomerases	Catalyze the structural changes by the transfer of a group from one position to another within a single molecule	Fumarase, dehydratases, decarboxylases
EC 6 Ligases	Catalyze the binding of two molecules followed by the hydrolysis of energy-rich bonds in ATP	Isomerases, epimerases, racemases

and immune responses[9]. The study of proteolytic enzymes has a renewed interest as these enzymes occupy the central position for their applications in physiological and commercial fields. Proteases are the key enzymes in regard to industries and they contribute to about 60% of the enzyme market in the world[6,10,11,12,13].

Market Potential of proteases

Advancements in various technologies to manufacture high-quality products have led to the increment of the protease market. The global protease market size costs about $3,454.3 million in 2020 and is expected to reach $5,762.7 million by 2030, with a CAGR of 5.4%, from 2021 to 2030 (*Protease Market Size, Share & Growth | Analysis Forecast,* 2030)[14]. The global protease market was dominated by microbial proteases in 2020, as the production of protease enzymes using microbial sources led to a very high yield of the enzyme meeting the demand for proteases across various industries[15]. Higher yield, cost-effective, time-saving, and easy gene manipulation are some of the important traits of microbial proteases making them perfectly suitable for commercial applications.

Classification of Proteases

The proteases are a group of very large and complex enzymes with distinct properties, including active site, substrate specificity, catalytic mechanism, pH, and temperature. Proteases can be classified in different ways by their peptide bond specificity (endopeptidases, exopeptidases, or amino acid-specific proteases). According to the pH classification, proteases may be classified by the optimal pH in which they are active like acid proteases, neutral proteases, and basic proteases (or alkaline proteases)[16,17].

Exopeptidases

The exopeptidases are active only near the end of the polypeptide chain. As per the active site at the N or C terminus, this enzyme are classified as amino and carboxypeptidases, respectively. Aminopeptidases act at the free N-terminus of the polypeptide chain and release a single amino acid residue, a dipeptide or a tripeptide. Aminopeptidases are present in many species of microorganisms, including bacteria and fungi. Generally, these are intracellular enzymes.

Carboxypeptidases act at the C-termini of the polypeptide chain and release a single amino acid or a dipeptide. Carboxypeptidases divided into three main groups, serine carboxypeptidases, metallo-carboxypeptidases, and cysteine carboxypeptidases, based on the nature of the amino acid residues at the active site of the enzymes. These enzymes can also hydrolyze the peptides with acyl groups.

Endopeptidases

These are identified by their special ability to hydrolyze the inner region of the peptide bonds in a polypeptide chain away from the N and C termini. The endopeptidases are further classified into four subgroups based on their catalytic activities including serine proteases, cysteine proteases, aspartic proteases, and metalloproteases.

Serine proteases

Serine proteases are characterized based on the

presence of a serine group (e.g. subtilisins) in their active site. They are numerous and common among viruses, bacteria, and eukaryotes, suggesting that they are important to organisms. Serine proteases generally function at neutral and alkaline pH, within an optimum range of pH 7 to 11. The molecular mass of these enzymes lies between 18-35 kDa.

Cysteine proteases

These enzymes occur in prokaryotes and eukaryotes and are functionally active at neutral pH. Their enzymatic activity depends on a catalytic dyad containing cysteine or histidine. Commonly, cysteine proteases function actively in the presence of reducing agents such as HCN. Streptopain is a type of cysteine protease produced by *Streptococcus* species and is found to have wide specificities, including oxidized B insulin chain and other synthetic substrates[6].

Aspartic proteases

These are commonly known as acidic proteases as they show maximum activity at low pH ranging from 3–4. These are the type of endopeptidases that rely on aspartic acid residues for their catalytic reactions. Their molecular weight falls within the range of 30-40 kDa.

Metalloproteases

These are the most distinct type of proteases, requiring a divalent metal ion for their action. Metalloproteases can be bifurcated into neutral and alkaline proteases based on their functional specificity. The neutral proteases are specific for hydrophobic amino acids, whereas the alkaline proteases show a very wide specificity. Many of the metalloproteases contain catalytically active atom zinc (Zn^{2+}) in their site of action. The molecular mass of metalloproteases lies in the range of 48 to 60 kDa.

Acid proteases

This type of protease functions optimally within a pH range of 2–4. It is commonly used in medicine, in industry for the breakdown of soy proteins during soya sauce production, and for the digestion of wheat gluten in the baking industry. Rennin-like proteases are examples of acid proteases derived from fungi and are chiefly used in cheese manufacture.

Neutral proteases

These are secreted by both bacteria and fungus. The neutral proteases are comparatively unstable due to which ions such as Ca^{++}, Na^{+}, and Cl^{-} are added to increase their stability. The pH range of activity is near about 6.5–7.0 and are unstable at high temperature.

Alkaline proteases

These proteases are functionally active at optimum pH greater than or equal to 9. These are commonly used as additives in detergents. They attain their stability in high temperatures or when associated with chelating agents[6].

Sources of protease enzyme

Since proteases are ubiquitous—being found in plants, animals, and microorganisms—their demand has increased significantly in response to growing requirements in the global market.

Plant proteases

Proteases can be produced from plants through several processes including cultivation and climatic conditions that are suited to growth. Papain, bromelain, and keratin are examples of proteases produced by plants[18].

Animal proteases

The most common type of animal proteases are trypsin, and chymotrypsin, pepsin is produced in bulk quantities in pure form[19]. Due to the insufficient production of proteases from animal sources, the researchers have extended their work to produce a sufficient amount of proteolytic enzymes for industrial applications through microbial sources[11].

Microbial proteases

Microbial proteases – mainly occurring in bacteria and fungi – are among the most important hydrolytic enzymes, and they have been extensively studied since the development of enzymology. Microorganisms are the excellent sources for the production of enzymes knowing to their wide biochemical diversity. Microbial proteases account for about 40% of total global enzymatic sales[13]. The suitable microbes for production of enzymes include *Bacillus subtilis, B. licheniformis*[20,21,22], *Aspergillus niger, A. oryzae, A. flavus, A. fumigatus, Cheotomium globusum*[23,24,25,26], thermophilic bacterium like *Halobacillus karajensis*[27], *Bacillus laterosporus, B. altitudinis, B. cereus, B. halodurans, Pseudomonas gessardii*[13,25,28,29]. Proteases from microbial sources are preferred over enzymes from plant and animal sources as microbial proteases can be cultured in huge quantities in comparatively less time by established fermentation methods and they also possess several characteristics required for various biotechnological applications.

Substrate for protease production

Various synthetic sources of proteins, carbohydrates, oils, and fats are used as substrates for the industrial production of enzymes. The use of all the synthetic sources for the production of a sufficient quantity of enzymes for industrial applications is an expensive deal. Furthermore, these synthetic substrates are the reason for increased industrial waste and are a major cause of pollution. Considering the scenario, the scientific community has started to make use of cost-effective alternative sources that requires less energy, and also reduce the number of pollutants in industrial effluents. The most preferred alternative is the use of agricultural residues. The agro-industrial residues are the most useful sources of lignocellulosic material, an important structural component in plants, found in cell walls, and composed of cellulose, hemicellulose, lignin, and various organic salts and minerals[7,8].

A variety of agricultural residues such as sugarcane bagasse, wheat bran, rice bran, potato peel, rice husk, green gram husk, oil cakes, etc. is helpful for purpose of growth and production of various industrial enzymes including proteases[30,31]. The main advantage of using these agricultural wastes as a substrate for the production of enzymes is that they are easily available, are cheap or cost-effective, and are non-toxic[12].

Agricultural by-products are utilized for protease production viz. gram husk and rice husk. The whole gram is rich in protein, fiber, vitamins, and essential minerals. The husk of a gram is the outer coat obtained after the milling process. The gram husk is composed of 24-27% cellulose, 30-33% hemicellulose, 22-25% lignin, 15-17% crude protein, and 5-7% ash[13,32]. A hard shell-like covering of paddy rice seed is the rice husk, which provides nutrients and protection

from pathogens and various factors during grain development. Rice husk consists of about 80% of organic substance. The rice husk contains 2.0-2.8% crude protein, 0.3-0.8% fat, 34.5-45.9% crude fiber including 28-41.5% cellulose, 14-28.6% hemicellulose, and 20.4-33.7 % lignin[33]. Protease enzyme production is done by *Bacillus* spp. from agricultural waste (Soy cake) as a substrate[20,34]. Paddy straw, wheat bran, sawdust, black gram husk, green gram husk[13,29] and other agro-industrial wastes like, coffee pulp and corncob are also used for protease production[35].

Protease production process

The process of fermentation has been widely used for the easy production of various useful industrial substances. The fermentation technique is a process of conversion of complex substances into simple ones by different bacteria and fungi[36]. The course of rapid development in industries and the increased demands have led to the emergence of two broad fermentation techniques viz. solid-state fermentation technique (SSF) and submerged fermentation (SmF)/liquid fermentation (LF).

SSF is applicable for solid substrates, such as bagasse, husk, bran, etc. This technique is helpful in the easy recycling of nutrient-rich substrates. It supports the controlled release of nutrients by slow utilization of the substrates thus the substrates are useful for a long fermentation period. SSF is mostly preferred for microorganisms like fungi that require very low moisture content and thus cannot be used for the processes involving organisms with high water content such as bacteria[37].

SmF utilizes liquid broth and molasses for the fermentation process. Various metal-containing compounds are secreted into the fermentation broth. Quick utilization of substrate takes place thus the broth is continuously supplemented with various required nutrients. It is the best-suited technique for bacteria as it requires high moisture content. An extra benefit of using SmF is that it provides easier purification of the products. This technique is most commonly used in the extraction of various secondary metabolites that are to be used in the liquid state[26,38].

Applications of protease enzyme

Microbial proteases are known to be the most remarkable hydrolytic enzymes and are of great importance to various industries possessing innumerable applications[17].

Detergent industry

The protease enzyme is broadly used at a commercial scale in detergent industries. They are the key ingredient of various detergents that are used for cleaning household laundry, and as a reagent for cleaning contact lenses. The use of protease enzyme in laundry detergents accounts for approximately 20-25% of overall world enzyme sales[6,17]. Serine proteases produced by Bacillus strains are also of great use in detergent industries[6].

Leather industry

The use of protease in leather industries has been proven to be the best alternative for various pollution-causing chemical methods. These enzymes are primarily used in leather processing which involves steps like soaking, bating, dehairing, and tanning[17]. Using enzymes

instead of chemicals is an eco-friendly alternative as well as it has increased the quality of produced leather. Alkaline proteases, neutral proteases, and lipases are most commonly used in leather industries. Alkaline proteases are used to separate the non-fibrillar protein components during the soaking step, also to make the leather soft in bating, and along with neutral proteases, they reduce the wastage of water in the dehairing step of leather processing[3].

Dairy industry

Proteases are useful for the industrial production of cheese, yogurt, and various other milk products[3]. It is majorly used in the production of cheese in large quantities and its function is to hydrolyze specific peptide bonds to make para-casein and macro peptides[3,17].

Pharmaceutical industry

On account of their diversity and specificity, protease enzymes play a vital role in the field of medicine and diagnosis. Proteases possess the ability to be useful in the production of various drugs against life-threatening diseases like anticancer, antimicrobial, and anti-inflammatory[15]. Recently, proteases are being used for diseases like cystic fibrosis, digestive disorders, retinal disorders, etc. Asparaginase produced by *Escherichia coli* is utilized to separate asparagine from blood as lymphocytic leukemia[15,19]. Alkaline proteases occupy the major application part in the pharmaceutical industries. Gauze bandages and various ointment compositions are some of the widely applied medical formulas using protease enzymes[15,17,19].

Food industry

The application of enzymes in the food industry has proven to enhance the quality and other components such as flavor, aroma, texture, colour, self life, and nutritional value of the produced food item[3]. The utilization of proteases in food industries has been distributed in various sectors such as baking, dairy, and breweries.

Baking industries utilize protease enzymes for the enhancement of flour quality, and to improve the texture, volume, and colour of the dough for the prolonged freshness of the baked bread[6]. Dairy industries use proteases as a coagulant to increase the self life and also for the higher yield of various milk products like cheese and yogurt[19]. Breweries use enzymes as a processing aid and for the consistent production of good quality products. Enzymes are used as an integrating ingredient in canned juices to increase their self life and also to maintain their taste, smell, and colour. To be specific, brewing industries use proteases by adding these enzymes in beers to avoid the collision of particles when it is chilled thus preventing the beer to look cloudy or hazy[3].

Management of waste

The most beneficial factor of using enzymes in cleaning methods is that they are biodegradable and hence will not be harmful in anyways to the environment even after they are no longer needed. Protease enzymes degrade the complex waste substances into small simple molecules that are consumed by various microbes for their metabolic activity, thus reducing the waste and making the environment clean[11]. Proteases produced by *Bacillus subtilis* along with a reducing agent ‘thioglycolate’ help in cleaning clogged pipelines[39]. Microbial proteases such as keratinase significantly degrade keratin-containing

wastes from domestic poultry and leather industries without causing pollution[40]. Protease enzymes can efficiently degrade various halogenated organic compounds containing harmful pollutants like herbicides, insecticides, plasticizers, and various intermediates used for chemical synthesis and make the environment pollution-free[17].

CONCLUSION AND FUTURE PROSPECTS

Proteases are the unique class of world's most demanding enzyme. As mentioned above, proteases account for nearly 60-70% of total commercial enzyme market. Proteases being ubiquitous in nature have a great influence in every commercial sector (food, pharmaceutical, feed, etc.). For ages, proteases produced by microbes are in biotechnological trend to meet proliferating demands of the consumers. Microbes are considered as a goldmine of proteases and are immensely preferred over other enzyme sources because of their easy and rapid growth, requirement of limited resources, and easy accessibility of gene manipulation[6]. Proteases possessing numerous characteristic features and their increasing commercial market have influenced researchers to discover and generate novel proteases that are more stable with regard to their pH, temperature and genetic engineering techniques.

The emerging branch "recombinant DNA technology" of biotechnology has made it possible for researchers to easily manipulate the microbial isolates for the desired amount of production of various enzymes. Gene cloning by site-directed mutagenesis has provided new possibilities for the introduction of advantageous changes, resulting in the production of desirable quality and quantity of the enzyme[15]. Protease enzymes having potential to be applicable in pharmaceutical industries are being tested to overcome the threat caused by diseases like AIDS, cancer, etc. Also, many researchers have illustrated the use of waste generated from agricultural industries, as a cost-effective substrate for the production of enzymes at the commercial level by various bioprocessing techniques. The use of agro-industrial waste in microbial fermentation has upgraded the economy along with reducing the disposal issues, making the environment eco-friendly[41].

In the future, protein engineering techniques will possibly provide the facilities for the discovery of newly functioning proteases by advanced approaches as *in vitro* change in the primary structure of protein by computational biology and techniques[42]. Thus, having a part in green and clean technology desirable eco-friendly applications of protease are very much diversified, promising a potential future for the enzyme.

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Research Article

AERIAL POLLEN LOAD OF PURULIA TOWN WITH ITS ALLERGENIC POTENTIAL

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Airborne pollen is a major environmental concern in relation to respiratory allergies, yet region-specific aerobiological surveys from eastern India remain limited. Purulia, the westernmost district of West Bengal, is situated on the eastern slope of the Chotanagpur Plateau. This study investigated airborne pollen diversity in Purulia town from January to December 2024 using a Burkard volumetric air sampler. A total of 60 pollen types belonging to 32 families were identified, with the peak pollen season occurring from late winter to early summer, particularly during March and April. A monthly pollen calendar was developed to illustrate seasonal variations in the aerial pollen load. Skin prick tests (SPT) conducted on 150 allergic patients revealed that *Argemone mexicana, Typha angustifolia*, and *Tridax procumbens* elicited the highest sensitisation rates. A strong correlation between total IgE and pollen-specific IgE levels confirmed IgE-mediated hypersensitivity to these pollens. Patients sensitised to *Typha angustifolia* pollen exhibited a wide range of total IgE levels (approximately 250–522 IU/mL), while ELISA-determined specific IgE values (P/N ratios) ranged from about 3.11 to 5.49. Similarly, patients sensitised to *Tridax procumbens* showed total IgE levels of approximately 280–523 IU/mL, with specific IgE (P/N ratios) ranging from about 3.05 to 5.89. For patients sensitised to *Argemone mexicana*, total IgE levels ranged from approximately 255–522 IU/mL, and pollen-specific IgE values (P/N ratios) varied from about 3.01 to 6.23. The pollen calendar and allergen profiles established in this study will enable medical practitioners to predict high-risk periods more accurately and to improve the management and treatment of pollen-allergic patients.

Key Words: Airborne pollen, Purulia, West Bengal, Skin prick test, Total IgE, Specific IgE

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INTRODUCTION

One of the most prevalent chronic diseases in the world is allergic disease[1]. An allergy is an immune system disorder in which foreign substances, most often called as allergens, trigger high production of immunoglobulin E (IgE) antibodies. In general, allergens are protein molecules found in various allergy-causing substances, including dust mites, fungi, pollen grains, and certain food items. Allergy diseases can range in severity from minor ailments like rhinitis and skin irritation to severe, sometimes

fatal problems like anaphylaxis and allergic asthma. Over a quarter of the global population suffers from various allergic diseases. About 20 to 30 percent of Indians suffer from at least one allergic illness, according to the country's National Health Portal[2]. As a result, it is now a significant global health issue affecting millions of people's quality of life[3]. Pollen from the Poaceae family, *Azadirachta indica, Alstonia scholaris, Areca catechu, Cocos nucifera, Carica papaya, Delonix regia, Peltophorum pterocarpum*, and several others have been found to cause pollinosis in a number of aerobiological studies conducted in West Bengal[4,5,6,7,8].

Airborne pollen grain concentrations vary not only between locations but also within nearby areas; this variability may be attributed to pollen-pollutant interactions as well as meteorological conditions[9]. To accurately define the timing and intensity of the pollen season, and to support the prevention and diagnosis of allergic diseases, pollen calendars are essential. Pollen calendars for a specific area are particularly valuable, as they provide clear and easily interpretable visual information on the different types of airborne pollen present throughout the year[10,11].

The present study aims to quantify the aeropollen load in the study area, prepare a pollen calendar for Purulia town, and identify allergenic pollen grains. The study also emphasises the distribution patterns of pollen grains, their seasonal variations, and the influence of meteorological factors on airborne pollen concentrations. Air sampling was conducted over a one-year period (January to December 2024) at different locations within Purulia town.

MATERIALS AND METHODOLOGY

Study area

Purulia is the westernmost district of West Bengal. Situated between latitudes 22.40° and 23.50° north and longitudes 85.75° and 86.65° east, it lies on the eastern margin of the Chota Nagpur Plateau. Purulia town, the district headquarters, supports a wide variety of indigenous and exotic trees, herbs and shrubs. The region is characterised by a lateritic landscape, moderately high temperatures and low humidity. May is the hottest month, with maximum temperatures reaching 42-48 °C. The monsoon season begins in June and continues until October, during which daily mean temperatures range between 32 and 37 °C. In winter, temperatures drop considerably, reaching as low as 6°C. All 22 wards of the Purulia municipality were surveyed for aeropalynological investigation.

Aerobiological monitoring

Using the Burkard portable volumetric sampler, biomonitoring of airborne pollen flora was carried out in the different municipal wards of Purulia town. The sampling was conducted from January to December, 2024. The sampler was positioned between two meters above the ground level in the selected locations.

Sampling Methods

To monitor and evaluate the airborne pollen spectra at those experimental sites, a Burkard personal air sampler with an air suction rate of 10 liters per minute was operated for 30 minutes. Air sampling was carried out at three distinct times: morning (09:30-10:30 hr.), afternoon

(12:30-13:30 hr), and evening (19:30-20:30 hr) in every week round the year.

To capture the pollen grains, a 1 cm thick microslide coated with a tiny layer of glycerine jelly was inserted through the slide slit of the sampler. At the end of the sampling, the slide was removed by rotating the upper ring using a forcep. The sampler pulled air through the top slit, depositing a 2 × 14 mm sample on the glass slide. The particles formed a thin band on the microslide covered with glycerine jelly. The cover slip was placed over the exposed slides, which were then mounted using DPX and left to dry for two to three days. A high-resolution light microscope was used to scan the slides.

The flowering periods of both anemophilous and entomophilous plants were observed and recorded. Identification of airborne pollen was carried out primarily using reference slides prepared in our laboratory, together with information from published literature[12, 13,14,15].

Collection of pollen sample

During the peak flowering time of the relevant trees, pollen samples were taken from mature anthers of their fresh flowers. They were dried at 37°C, mildly crushed, and passed through different sieves (50, 105, 150, 250, and 300 mesh/cm^2) successively to remove the other floral impurities. The purity of the isolated pollen material was checked under the microscope. Pollen grains were stored at -20°C for further use.

Preparation of pollen extract

The pollen grains were defatted with diethyl ether, and then one gram of the pollen was extracted in phosphate buffer saline (PBS: 0.1 M Na-phosphate 150 mM, pH 7.2) by continuous stirring at 4°C for 16 h in a 1:10 (w/v) ratio. After centrifugation at 14,000 g for 20 min, the clear supernatant was dialyzed and passed through a 0.22 μm Millipore filter. The filtrate was then lyophilized and stored at -35°C in sterile vials containing aliquots of known volume.

Clinical study and collection of patient sera

Skin tests were performed with some dominant pollen allergen extracts such as *Acacia auriculiformis, Delonix regia, Cassia* sp., *Tridax procumbens, Argemone mexicana, Alangium salvifolium, Typha angustifolia, Azadirachta indica, Chromoleana odorata, Borassus flabellifer* on 150 adult respiratory allergic patients (Age range: 18-55 years), attending the Shakti Sangha Allergy Unit, Purulia, during 2024. The study excluded patients with chronic illnesses, smokers, and pregnant women. Clinical and demographic information for these patients was gathered and examined. 20 µL of the diluted antigen solution was applied to the ventral side of the forearm and picked with a sterile lancet without causing bleeding after the antigenic extract was diluted in PBS (1:10 w/v). The positive and negative controls were histamine diphosphate (1 mg/mL) and PBS (0.01 M, pH 7.2) respectively and the wheal reaction was assessed after 15 minutes[16]. 3mm of wheal diameter was considered a favourable response. SPT was performed by the clinic. Twenty of these patients was agreed to have their blood for further study. For additional immunological research, the sera fractions of the collected blood samples were kept at -20°C for further study. Nine non-atopic people's sera were also collected as negative control. The entire study

was approved by the ethical committee of the institute.

Total IgE and Specific IgE estimation

Indirect ELISA was used to measure the specific IgE level in patients' serum against three pollen extracts. Crude antigenic extracts (10 ng/µL) of selected pollens (50 µL/well) were applied to wells of microtiter ELISA plates (AccuBind ELISA Micro wells) and incubated at 4°C overnight. Following two hours of blocking with 3% Bovine Serum Albumin (BSA) and repeated washing with 0.1 M PBS buffer (pH 7.2) with 0.05% Tween-20 (PBS-T) at 37°C, wells were incubated with 50 µL of 1:10 diluted individual patient sera at 4°C for 16 hours. The secondary antibody was a monoclonal anti-human IgE-alkaline phosphatase conjugate produced in mouse (Sigma-Aldrich), whereas the substrate was para-nitro phenylphosphate (pNPP) (Sigma-Aldrich). After adding 3 N NaOH to halt the reaction, the absorbance was measured at 405 nm using an ELISA reader (company name with model). The average OD 405 value of the three replicas for each patient's serum was marked as "P", while the mean OD 405 value of all the healthy patients were labelled as "N". According to Sircar *et al*.[17], the ratio of P/N was used to determine the amount of serum-specific IgE. A P/N ratio (patient vs. non-allergic participants) of more than 3.5 for a given serum sample was considered as in vitro "positive" with noticeably higher IgE levels against the study antigen.

Statistical analysis

A linear regression analysis was performed to evaluate the relationship between Specific IgE (P/N value) and Total IgE levels. The coefficient of determination (R^2) indicated the proportion of total IgE variability explained by Specific IgE. A higher R^2 suggests a stronger linear relationship. The p-value <0.05 indicates that the relationship is statistically significant. Pollen calendar was conducted using Python (v3.x) within the Jupyter Notebook environment. The libraries used included pandas, numpy, seaborn, matplotlib, and statsmodels.

RESULTS

Airborne Pollen Composition and Relative Abundance

A wide range of pollen spectra were found in the atmosphere of Purulia town over the one year, according to the aero palynological study. Over the course of the one-year study period, 60 pollen types from 32 families of angiosperms families from Purulia were identified; however, a few remained unidentified. Poaceae and *Acacia* sp. alone provided the most pollen, whereas *Morus* sp. contributed the least.

Pollen Calendar and Seasonal Variation

Most trees had a flowering season that started in late winter and lasted until the early monsoon season. During this study, the maximum monthly pollen concentration was observed in March (9280/m^3), followed by April (8097/m^3), February (6363/m^3), and May(4746/m^3). Poaceae were the most abundant (7.10 %) of the total pollen load, followed by *Acacia* sp. (4.07%). The next in the order of abundance were *Eucalyptus* sp. (3.92%), *Cyanthillium cinereum* (3.83%), *Amaranthus spinosus* (3.56%), *Tridax procumbens* (3.48%), Malvaceae (3.37%),

Alangium salviifolium (3.37%), *Lantana camara* (3.36%), *Chromoleana odorata* (2.22%), *Argemone mexicana* (2.17%), *Ricinus communis* (2.04%), *Alstonia scholaris* (2.01%), *Cocos*

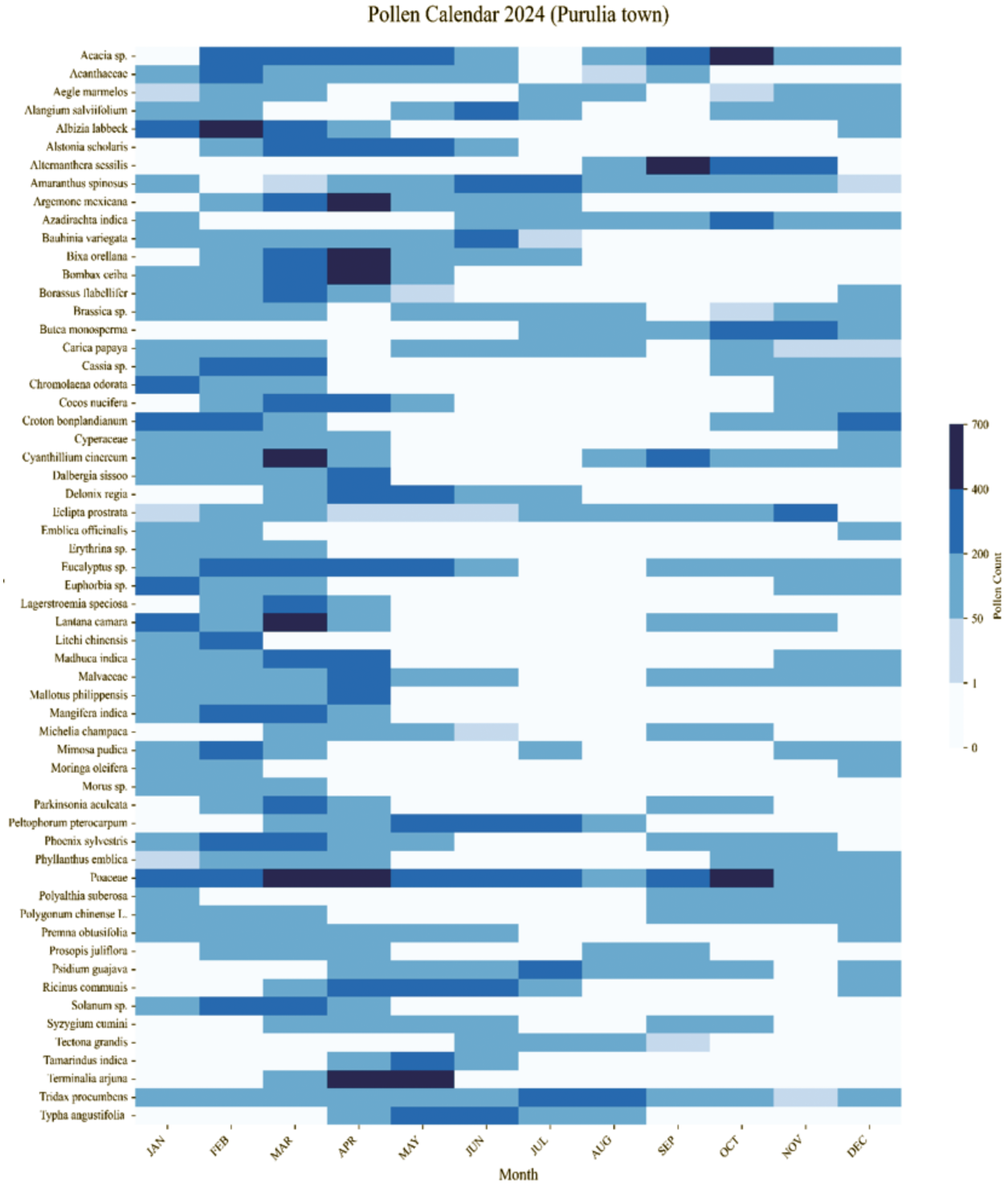


Fig. 2.1: Monthly pollen calendar of Purulia town in the year 2024

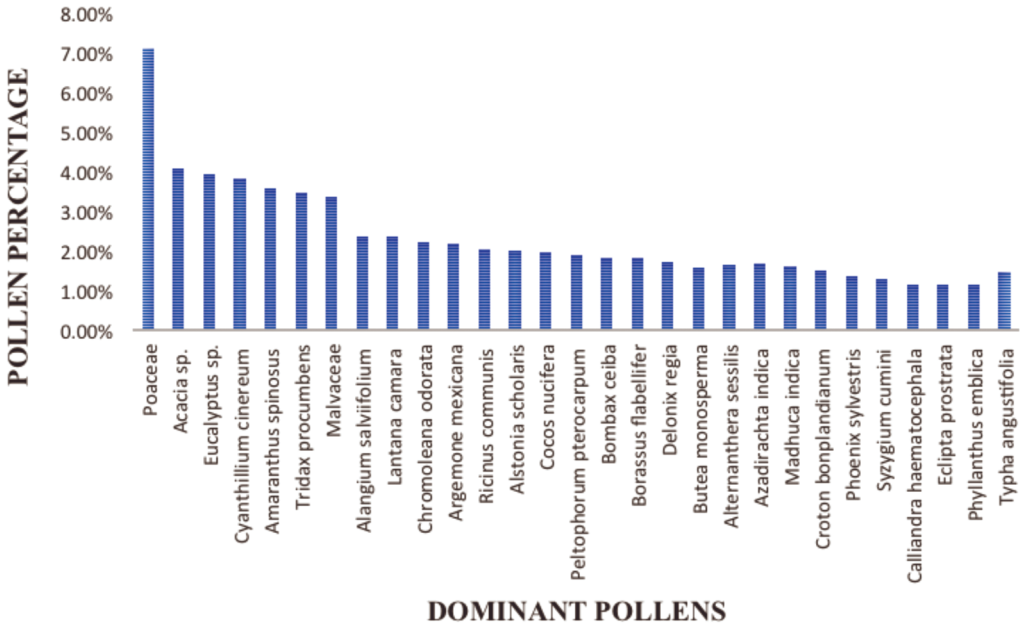


Fig. 2.2: Air borne pollen spectra of dominant pollens in Purulia town

nucifera (1.97%), *Peltophorum pterocarpum* (1.90%), *Bombax ceiba* (1.83%), *Borassus flabellifer* (1.83%), *Delonix regia* (1.71%), *Butea monosperma* (1.58%), *Alternanthera sessilis* (1.64%), *Azadirachta indica* (1.68%), *Madhuca indica* (1.62%), *Croton bonplandianum* (1.50%), *Typha angustifolia* (1.48%), *Phoenix sylvestris* (1.36%), *Syzygium cumini* (1.28%), *Calliandra haematocephala* (1.16%), *Eclipta prostrata* (1.15%), Phyllanthus emblica (1.14%). The remaining types contributed less than 1.0% (Fig. 2.1 & 2.2).

Allergenicity assessment by skin prick test (SPT)

Data from skin prick testing showed that out of

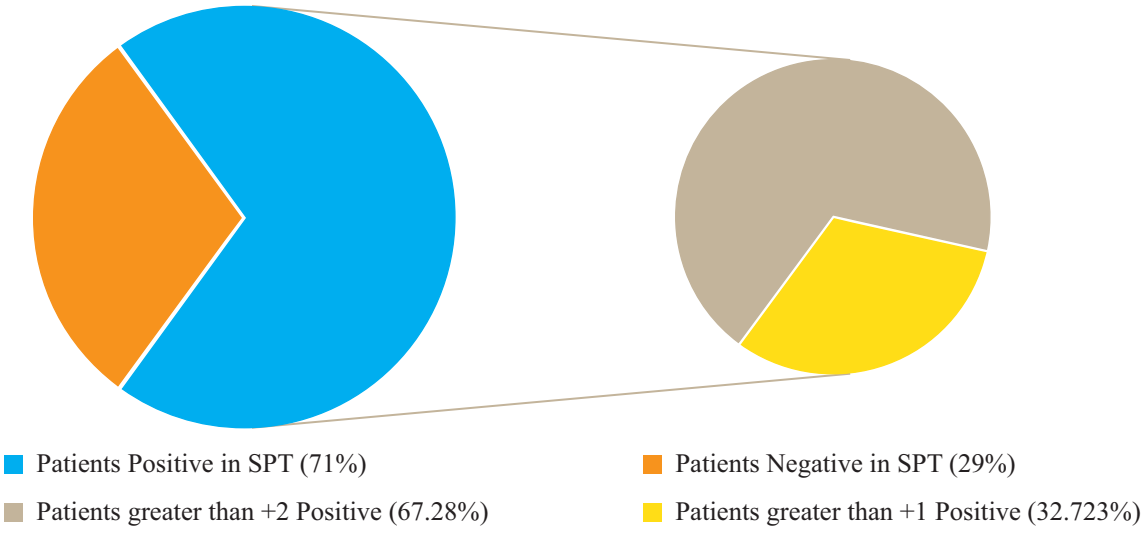


Fig. 2.3: Results of skin prick (SPT) with *Argemone mexicana* antigenic extract at diagnostic clinic on 150 patients.

10, three pollens have a high potential to induce allergies. Of the 150 patients (age range: 18-55), 60 showed a positive skin reaction (SPT) to three pollen allergens which namely *Argemone mexicana, Typha angustifolia, and Tridax procumbens*. Most of the sensitive patients showed a +3 grade of positivity, followed by +2 and +1.

SPT report of Argemone mexicana antigen

Out the 150 patients, 71% patients showed a positive reaction to *Argemone mexicana* antigen (Fig. 2.3). Among them, 29% of patients showed ≥2+ grade in SPT and were considered clinically significant. A $\frac{+3}{+4}$ grade of positivity was found to be most predominant, followed by +2. Only 20 patients with marked positive reactions in SPT agreed to donate blood, and the collected serum samples were analysed for specific IgE by indirect ELISA.

SPT report of Typha angustifolia antigen

Out of 150 patients 67% patients showed a positive reaction to *Typha angustifolia* antigen (Fig. 2.4). Among them, 33% patients showed ≥2+ grade in SPT and were considered to be clinically significant. A $\frac{+3}{+4}$ grade of positivity was found to be predominant, followed by +2. Only 20 patients with marked positive reactions in SPT agreed to donate blood, and the serum samples collected were analysed for specific IgE estimation by indirect ELISA.

SPT report of Tridax procumbens antigen

Out of 150 patients, 70% patients showed a positive reaction to *Tridax procumbens* antigen (Fig. 2.5). Among them, 30% patients showed ≥2+ grade in SPT and were considered to be clinically significant. A $\frac{+3}{+4}$ grade of positivity was found to be most predominant followed by +2. Only 20 patients with marked positive reactions in SPT agreed to donate blood, and the serum samples collected were subjected to specific IgE estimation by indirect ELISA.

Estimation of total and specific IgE

Twenty clinically symptomatic patients and three healthy persons as controls were included in the study for testing sensitivity to each of the 3 major pollen allergens. For each patient,

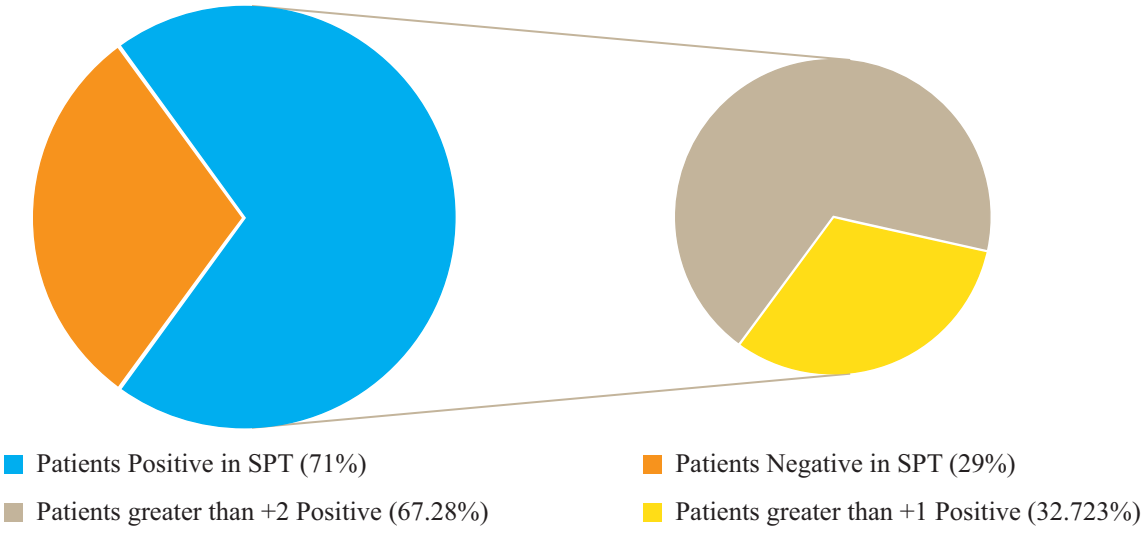


Fig. 2.4: Results of skin prick (SPT) with *Typha angustifolia* antigenic extract at diagnostic clinic on 150 patients.

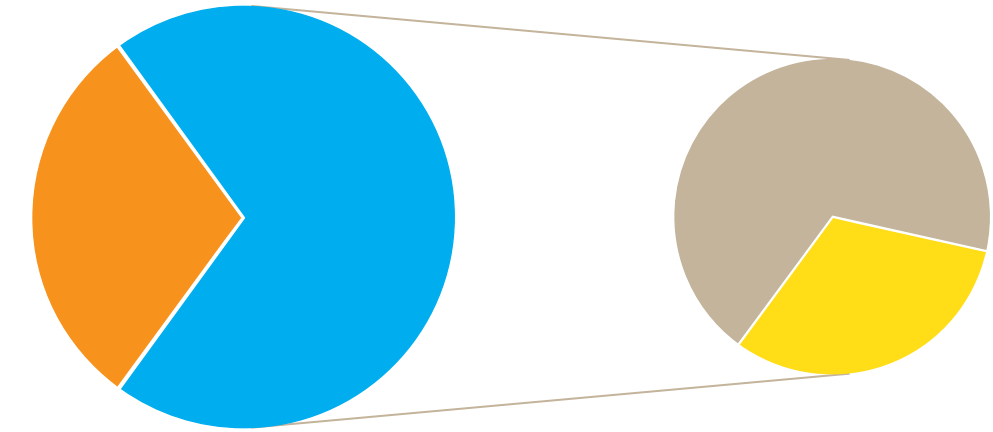


Fig. 2.5: Results of skin prick (SPT) with *Tridax procumbens* antigenic extract at diagnostic clinic on 150 patients.

demographic details (age and sex), clinical symptoms, skin prick test (SPT) grades, Total IgE levels, Specific IgE (P/N values), and established co-sensitizations were recorded. The symptomatic patients (P1-P20) presented with various allergic manifestations including cough (C), breathlessness (B), sneezing (S), nasal blockage (NB), allergic conjunctivitis (AC), and skin reactions (SR). SPT grades ranged from +2 to +4, indicating moderate to extreme reactivity.

In *Typha angustifolia*, pollen-sensitized patients total IgE levels showed (Table 2.1) wide variation (range approx. 250-522 IU/mL), reflecting

Table 2.1: The total Clinical characteristics of the 20 *Typha angustifolia* pollen sensitive patients

Sl. No.	Age/ Sex	Symptoms/ Clinical history	Grades of SPT	Total IgE	Specific IgE-Titer by ELISA (P/N Value)	Established Co-sensitization
P1	45/M	B, S	++++	344.34	5.49	AI, PS, CF, AA,
P2	32/M	C, B, S	++	388.98	3.32	SC, TA
P3	55/M	B, NB, S, NB, SR	+++	345.66	3.23	CN, RN, AF, PP
P4	38/F	B, SR	+++	456.89	4.54	PP, CD, AAl, SC
P5	28/F	C, B, SR	++	326.66	3.11	CD, RN, CN, TA
P6	22/M	C, S, SR	++++	443.87	4.73	AI, AN, CO
P7	34/F	NB, AC, SR	++	377.67	3.23	AA, TA, CD
P8	43/M	NB, C, B	+++	456.32	3.66	BF, AI, CS, CD
P9	45/F	B, AC, SR	+++	389.66	4.61	BF, AAI, RN, AN, AF

Contd.

Contd. Table 2.1

Sl. No.	Age/ Sex	Symptoms/ Clinical history	Grades of SPT	Total IgE	Specific IgE-Titer by ELISA (P/N Value)	Established Co-sensitization
P10	25/F	C, B, AC, S, NB, SR	+++	422.12	3.88	AI, TA, AF
P11	38/M	AC, NB, SR	++	250.89	3.21	PP, AI, AN, AP
P12	44/M	AC, NB	+++	301.89	3.96	SC, AN, RN, TA, CP
P13	36/M	B, S, NB	+++	499.12	4.78	AN, CN, SC
P14	33/F	C, B, NB, SR	++	279.45	3.22	AAl, AA, SC
P15	52/M	B, AC, SR	++	425.67	4.19	PE, CS, SC
P16	54/M	C, AC, NB, SR	++++	488.34	5.62	CS, CD, TA
P17	49/F	B, AC, SR	+++	489.22	4.52	PP, CD, RN, AM
P18	29/M	AC, C, B	+++	265.38	4.56	BF, CP, TA
P19	31/F	AC, C, B, SR	++	318.56	3.22	TA, BF
P20	28/M	B, C, AC, S	++++	522.34	5.34	AI, CD, TA
Healthy Control Patients						
C1					1.05	No
C2					0.88	No
C3					0.67	No

Note: Symptoms like Cough (C), Breathlessness (B), Allergic Conjunctivitis (AC), Sneezing (S), Nasal Blockage (NB), and Skin Rash (SR).

The grading scale of skin prick test like negative if wheal diameter is <3mm, +1 if wheal diameter is 3–5mm, + 2 if wheal diameter is >6mm, +3 if wheal diameter is >6mm, with one or two small pseudo pods, and +4 is any reaction that is more pronounced than +3.

Aeroallergen abbreviations like *Acacia auriculiformis* (AA), *Chromolaena odorata* (CO), *Azadirachta indica* (AI), *Borassus flabellifer* (BF), *Carica papaya* (CP), *Typha angustifolia* (TA), *Syzigium cumini* (SC), *Cocos nucifera* (CN), *Peltophorum pterocarpum* (PP), *Phoenix sylvestris* (PS), *Argemone mexicana* (AM), *Cassia sophera* (CS), *Solanum sisymbriifolium* (SS), *Cynodon dactylon* (CD), *Tridax procumbens* (TP), *Delonix regia* (DR), *Alangium salvifolium* (AS), *Alternaria alternata* (AAI), *Aspergillus flavus* (AF), *Aspergillus niger* (AN), *Rhizopus nigricans* (RN).

varying degrees of systemic allergic response. Specific IgE values (P/N ratios) measured by ELISA ranged from approximately 3.11 to 5.49, suggesting measurable allergen-specific sensitivity. In contrast, the healthy controls (C1-C3) showed very low Specific IgE values (0.67-1.05) and no co-sensitization, confirming their non-allergic status.

In case of *Tridax procumbens* pollen-sensitized patients, total IgE levels showed (Table 2.2) wide variation among patients (range approx. 280-523 IU/mL), reflecting different levels of

Table 2.2: Clinical characteristics of the 20 Tridax procumbens pollen sensitive patients

Sl. No.	Age/ Sex	Symptoms/ Clinical history	Grades of SPT	Total IgE	Specific IgE-Titer by ELISA (P/N Value)	Established Co-sensitization
P1	42/F	C, B, S	+++	314.03	3.49	SC, AI, PS, SS, TP
P2	30/M	B, NB, SR	++	280.67	3.22	CF, AA
P3	25/M	C, B, AC, NB, SR	+++	491.57	4.55	CD, CN, PP, PS, TP
P4	39/M	C, SR, AC	+++	423.16	4.39	Al, SC, RN, AF, DR
P5	38/F	NB, SR, B	++++	523.56	5.21	AN, RN, CN
P6	42/F	C, NB, SR	++++	454.67	4.78	PE, AC, TP
P7	36/M	C, B, AC, NB	++	387.23	3.22	AA, TP, CD, AI, RN
P8	40/M	NB, SR, C	++	412.11	4.32	BF, AI, CS, AN, AF
P9	33/F	CB, AC, NB, SR	+++	329.49	3.55	RN, BF, AI, CS
P10	28/F	C, B, AC, S, NB, SR	+++	394.76	3.95	AA, PE, RN, AF, TP
P11	39/M	B, S, NB, SR	+++	387.23	3.23	AA, AI, AN
P12	45/F	B, S, AC, SR	++	248.44	3.05	SC, AN, RN, TP
P13	36/F	B, NB, SR	+++	480.55	4.76	CP, SC, AN, TP
P14	38/F	AC, NB, SR	++	311.59	3.59	SC, Al, AAI, CO
P15	42/M	B, C, SR, NB	+++	491.57	4.89	AA, AAI, CC, SC
P16	52/F	B, AC, NB, SR	++++	525.45	5.89	AA, CD, AA, TP
P17	46/M	B, AC, NB, SR	+++	412.11	4.32	SC, CD, TP
P18	30/M	B, C, SR	++	311.59	3.54	AI, BF, CP
P19	21/M	C, S, NB, SR	++	309.22	3.42	AA, CP, CF, SC
P20	26/F	B, NB, AC, SR	+++	454.67	4.34	AN, TP, AS
Healthy Control Patients						
C1					0.88	No
C2					0.34	No
C3					1.09	No

Note: Abbreviations same as Table 2.1

systemic allergic response. Specific IgE values (P/N ratios) measured by ELISA ranged from approximately 3.05 to 5.89, suggesting measu-rable allergen-specific sensitivity. In contrast, the healthy controls (C1-C3) showed very low Specific IgE values (0.88-1.09) and no co-sensitization, confirming their non-allergic status.

Table 2.3: Clinical characteristics of the 20 Argemone mexicana pollen sensitive patients

Sl. No.	Age/ Sex	Symptoms/ Clinical history	Grades of SPT	Total IgE	Specific IgE-Titer by ELISA (P/N Value)	Established Co-sensitization
P1	45/M	C, S	++	318.23	3.01	AM, SC, AI, PS
P2	32/M	C, B, NB	+++	279.17	4.27	CF, AA, AS
P3	55/M	B, AC, CS, NB, SR	++	488.47	6.23	CD, CN, RN, AM
P4	38/F	C, B, SR	++++	412.01	3.45	PP, PS, Al, SC
P5	28/F	C, B, NB, SR	++	517.45	3.56	CD, AN, RN, CN
P6	22/M	C, S, NB,	+++	452.37	4.12	AI, AN, RN
P7	34/F	C, AC, NB	+++	379.35	4.34	AA, CO, AM
P8	43/M	B, S, SR	+++	409.78	5.43	BF, AI, CS, AM
P9	45/F	C, B, AC, S, NB, SR	++++	334.56	3.78	RN, AN, AF
P10	25/F	B, S, SR	++	390.45	4.45	AA, PE, TA, AM
P11	38/M	C, B, NB, SR	++++	369.55	3.78	CD, AI, AN
P12	44/M	B, S, NB	++	255.12	3.52	SC, AN, RN, TX
P13	36/M	C, S, NB, SR	++++	455.12	4.23	CP, CF, CN, RN, AM
P14	33/F	AC, NB, SR	+++	376.33	4.56	AAI, TP, CO
P15	52/M	B, AC	++	471.66	4.33	CS, TX, SC
P16	54/M	C, AC, SR	+++	522.33	3.78	CS, TX, CD, AA
P17	49/F	B, AC, NB, SR	++++	410.59	3.27	SC, RN, AM, DR
P18	29/M	AC, NB, SR	++	318.12	3.89	AI, BF, CP
P19	31/F	C, S, SR	+++	312.44	3.19	BF, PP, CF, AM
P20	28/M	B, AC, SR	++++	476.37	3.90	AN, RN, AM
Healthy Control Patients						
C1					1.23	No
C2					0.58	No
C3					0.78	No

Note: Abbreviations same as Table 2.1

In patients with *Argemone mexicana* pollen sensitivity total, IgE levels showed (Table 2.3) wide variation (range approx. 255-522 IU/mL), reflecting varying levels of systemic allergic response. Specific IgE values (P/N ratios) measured by ELISA ranged from approximately 3.01 to 6.23, suggesting measurable allergen-specific sensitivity.

The healthy controls (C1-C3) showed very low Specific IgE values (0.78-1.23) and no co-sensitization, confirming their non-allergic status.

DISCUSSION

This aerobiological survey provides a comprehensive understanding of airborne pollen diversity, seasonal patterns, and allergenic potential in Purulia town. During the one-year monitoring period, a total of 60 pollen types belonging to 32 angiosperm families were observed, indicating that the area retains a rich pollen habitat that can affect respiratory health. The predominance of Poaceae, *Acacia* sp., *Tridax procumbens*, *Eucalyptus* sp., *Typha angustifolia*, *Cyanthillium cinereum*, *Amaranthus spinosus*, *Argemone mexicana*, and other weedy taxa reflects the vegetation profile of Purulia, where open fields, roadside flora, and unmanaged weeds contribute substantially to atmospheric pollen.

Clear seasonal trends were observed, with the highest pollen concentrations recorded during late winter to early summer. March had a particularly high pollen load, which was followed by April and February, when many wind-pollinated species were in flower. These results correlate with aerobiological evidence from other tropical and subtropical regions where lower humidity and pre-monsoon temperature facilitate pollen release and spread. Therefore, the monthly pollen calendar derived from these data has substantial clinical significance, serving as a useful tool for predicting high-risk periods for allergy patients and assisting doctors in scheduling preventive treatments.

Three pollen species - *Argemone mexicana*, *Typha angustifolia*, and *Tridax procumbens* - elicited the highest rates of sensitivity in allergic individuals, according to skin prick tests. These species are confirmed to be the most common aeroallergens in Purulia due to their great abundance and continuously high SPT reactivity. Notably, the majority of individuals exhibited moderate to intense $\left(\frac{+3}{+4}\right)$ allergic responses, indicating severe IgE-mediated hypersensitivity.

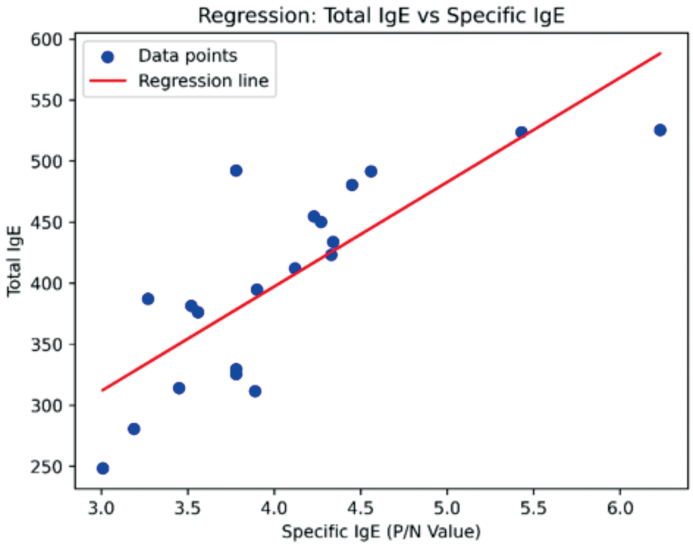


Fig. 2.6: The regression graph of T*ypha angustifolia* shows fitted line plots (straight line) displaying the results from simple regression along with observed values (dots), which is one predictor variable (specific IgE) and the total IgE.

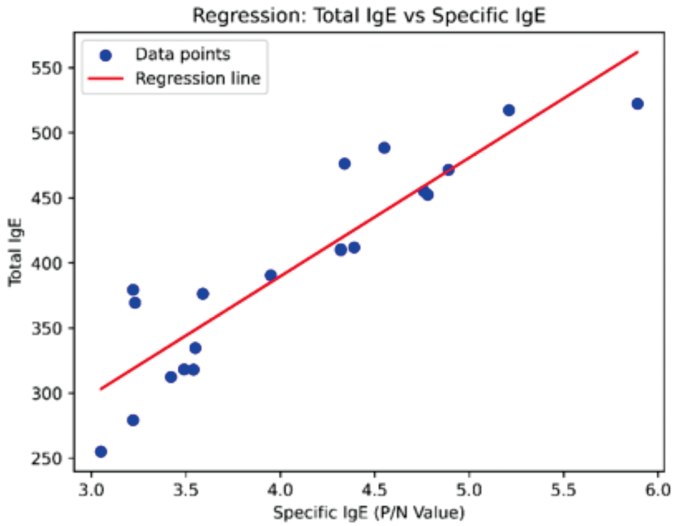


Fig. 2.7: The regression graph of *Argemone mexicana* shows that fitted line plots (straight line) displaying the results from simple regression along with observed values (dots), which is one predictor variable (specific IgE) and the total IgE.

The immunological findings further confirm these observations. Systemic atopy was indicated by significantly higher total IgE and histamine levels in patients with allergy symptoms as compared to healthy controls. The immunological mechanisms are further clarified by the regression graphs, which show correlation between total serum IgE and pollen-specific IgE for each of the three major allergens.

For ***Typha angustifolia***, the regression graph shows a strong positive linear correlation between total IgE and specific IgE (Fig. 2.6). This suggests that Typha's sensitivity is closely related to total IgE-mediated atopic status, emphasizing its significance as a prominent allergen in the study region.

In the case of ***Argemone mexicana***, the correlation was moderately strong, with a clearly positive slope but a slightly wider data spread. This trend indicates that although *Argemone*-specific IgE often rises with total IgE, sensitization can happen even in those with merely borderline or moderately raised total IgE, suggesting its potency as an allergen (Fig. 2.7).

The correlation for ***Tridax procumbens*** demonstrated a distinctly linear positive trend, with tight clustering of values around the regression line (Fig. 2.8). According to the regression plot, patients with higher allergen-specific IgE typically have higher overall IgE levels, clearly demonstrating a positive association between specific and total IgE. Despite some dispersion, the rising trend indicates a continuous IgE-mediated allergic response to pollen. This suggests that *T. procumbens* is a significant cause of pollen allergy in Purulia and that there is a very consistent IgE-mediated response among allergic individuals.

These three regression graphs show that IgE-dependent immune processes play a key role in pollen allergy in this group, and that the detected pollens are immunologically and clinically relevant allergens. The positive correlation between SPT, total IgE, and specific IgE values

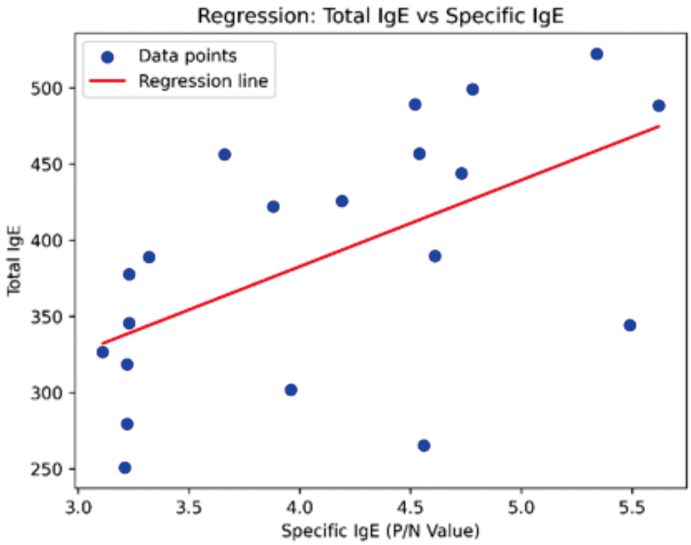


Fig. 2.8: The regression graph of *Tridax procumbens* shows that fitted line plots (straight line) displaying the results from simple regression along with observed values (dots), which is one predictor variable (specific IgE) and the total IgE.

presents strong validation of the allergenic potential of these species. Overall, this work highlights the need for region-specific diagnostic assessments and immunotherapeutic approaches, and provides a comprehensive aerobiological and clinical profile of Purulia. Incorporating antigens from *Argemone mexicana, Typha angustifolia* and *Tridax procumbens* into routine allergy testing could substantially improve patient care and diagnostic accuracy in this region.

CONCLUSION

It is known that pollen grains can induce respiratory allergies[18]. Meteorological factors significantly impact air bone pollen concentration as temperature (minimum and maximum), rainfall, relative humidity, and wind speed[19,20,21]. This study shows distinct seasonal peaks in the airborne pollen variety of rural Purulia, with the largest pollen load occurring from late winter to early summer. *Typha angustifolia, Tridax procumbens*, and *Argemone mexicana* are the main allergenic pollens in the area, according to clinical evaluation. Pollen-specific IgE and total IgE have strong relationships, indicating their important involvement in IgE-mediated hypersensitivity. These findings emphasize the need for region-specific pollen calendars and allergen assessments to improve the diagnosis and treatment of allergic conditions.

ACKNOWLEDGEMENT

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Research Article

IPOMOEA SINDICA STAPF (CONVOLVULACEAE) — AN ADDITION TO FLORA OF TELANGANA, INDIA

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Ipomoea sindica Stapf was collected from black soil habitats occurring along roadsides and at forest margins in Tandur mandal of Vikarabad district, as well as from the Mudimiyal Reserve Forest in Ranga Reddy district, Telangana State, India. Based on critical examination and comparison with available taxonomic literature, the identity of the species was confirmed. This collection represents a new distributional record and is reported here as an addition to the flora of Telangana State.

To support accurate recognition and future documentation of this species in the region, a comprehensive taxonomic account has been prepared. This includes a detailed morphological description covering diagnostic vegetative and reproductive characters, along with photographs illustrating key identifying features. These details are intended to facilitate easy identification of Ipomoea sindica in the field and to aid further floristic and taxonomic studies.

Key Words: Addition, Convolvulaceae, *Ipomoea*, Telangana, Vikarabad district.

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INTRODUCTION

Members of the family Convolvulaceae are predominantly twiners and trailing herbs, while shrubs and trees are rare. Most species are cultivated as ornamental plants or used for food, medicinal purposes, and religious rituals[1]. The genus Ipomoea L. is a large and complex group comprising more than 800 species[2], distributed across tropical and warm temperate regions of the world. In India, the genus is represented by 90 species, excluding cultivated and taxonomically uncertain species[3].

While conducting random floristic documentation in Telangana, the authors collected an interesting *Ipomoea* species closely resembling *Ipomoea eriocarpa* R. Br. The specimens were collected from black soils along roadsides and forest margins in Tandur Mandal of Vikarabad District and the Mudimiyal Reserve Forest of Ranga Reddy District during July and September 2024.

Following critical morphological and microscopic examination of the specimens, along with a review of relevant literature, the collected

specimen was identified as *Ipomoea sindica* Stapf. This species was first described by Stapf[4], who noted its close affinity to *I. eriocarpa*, from which it can be distinguished by its glabrous capsules and velvety seeds. Jafri[5] and Bhandari[6] accepted *I. sindica* as a distinct species. However, Austin and Ghazanfar[7] confused *IIpomoea sindica* with *Convolvulus scindicus* Stocks and treated it as a synonym under the name *Ipomoea sindica* (Stocks) Stapf in the *Flora of Pakistan*.

Subsequently, Khatoon and Husain[8], based on chemotaxonomical and micromorphological evidence, clarified that *I. sindica* is distinct from *I. eriocarpa* and that it is not based on *Convolvulus scindicus* Stocks. The collected specimens were prepared as herbarium sheets following standard methodology[9] and deposited in the Herbarium of Osmania University, Hyderabad (HY). The genus *Ipomoea* L. is represented by 26 species in Telangana[10,11]; however, *I. sindica* has not been reported previously from the state. Therefore, the present collection constitutes a new addition to the flora of Telangana state. A detailed description, distribution, and photographic plates are provided herein.

RESULTS AND DISCUSSIONS

Taxonomic treatment

Ipomoea sindica Stapf, Kew Bull. 93: 346. 1894; Bhandari, Fl. Ind. Desert 231. 1990; Cooke, Fl. Pres. Bombay 2: 252. 1905; Chavan and Oza, Fl. Pavagadh, 153. 1966; Mudgal *et al*., Fl. Madhya Pradesh 2: 164. 1997; Singh *et al*., Fl. Maharashtra Dicot. 2: 470. 2001.

Morphological description: Annual, slender, laticiferous creeper, up to 3 m long. Stem slender, hairy, terete. Leaves 5 - 10 × 4 - 5 cm, dimorphic, basal leaves ovate, cordate at base; upper leaves linear lanceolate, longer than broader, hairy, entire, with rounded leaf base, acuminate apex, margin entire. Petioles 2 - 4 cm long, densely hairy. Inflorescence axillary, 1 - 3 flowered cymes. Flowers bracteate, pedicels 3 - 5 mm; bracts leafy, caducous, densely hairy. Sepals 5, sub-equal, linear, faintly veined, densely hairy, white ciliated at margin, faintly veined, hairy. Corolla 5-lobed, infundibuliform, 0.8 - 1 cm long, tube 0.3 - 0.5 cm long, pure white; lobes 0.5 cm long, white, 1.0 - 1.5 cm wide at mouth, apiculate, with hairy white tuft at the base of corolla lobes, mid-petaline bands white, shallow apiculate, hairy at outside. Stamens 5; filaments sub-equal, 0.5 - 0.8 cm long; attached at 0.2 cm above the tube, included, hairy at base; anthers 0.3 cm long. Ovary ovoid, light green, hairy, 4-locular, ovule 1 in each locule; style glabrous; stigma bilobed, capitate, white, sometime pink in color. Fruits 0.5 - 0.8 cm, globose, 4-valved, glabrous and brown, dehisces from top into 4 parts. Seeds 4 per capsules, 0.3 × 0.4 cm, brownish black, pubescent.

Pollen Description: Polyporate (pantoporate), isopolar and radially symmetrical, with spheroidal to suboblate-oblate grains and circular outline. The exine is tectate and typically echinate, with circular pores between echinae.

Distribution: India - Delhi, Gujarat, Haryana, Himachal Pradesh, Madhya Pradesh, Maharashtra, Rajasthan, Telangana (present study) and Uttar Pradesh.

Flowering and Fruiting time: July - October.

Specimens examined: India, Telangana state, Vikarabad district, Tandur mandal, L. Paramesh

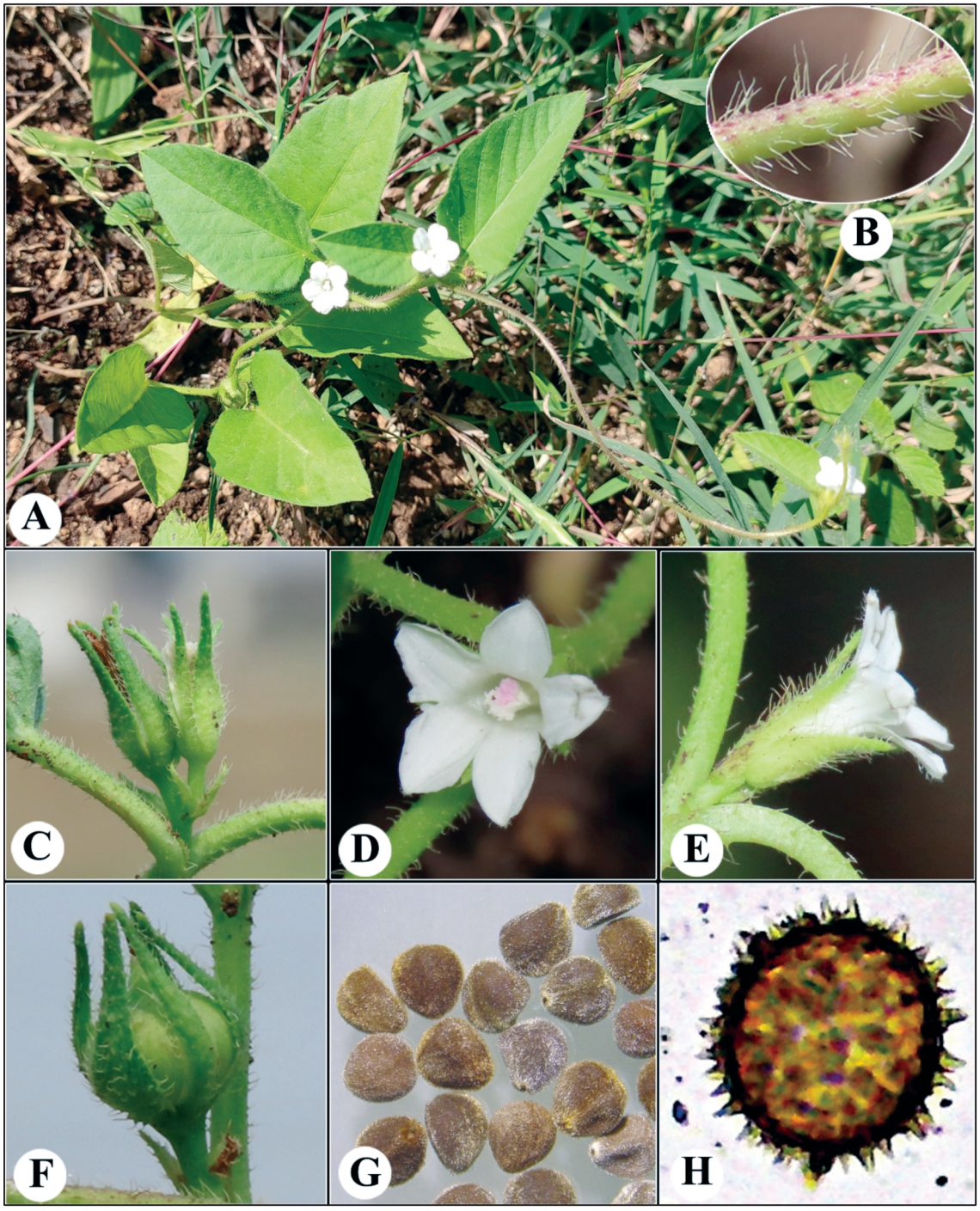


Fig. 3.1: *Ipomoea sindica* Stapf: A. Habit; B. Stem; C. Flower buds; D. Flower top view; E. Flower (side views); F. Fruit; G. Seeds; H. Pollen grain.

1295 and Ranga Reddy district, Mudimiyal R. F, L. Paramesh and B. Suresh Kumar 1310.

Habitat and Ecology: It was growing on black cotton soils in association with *Acalypha*

malabarica Müll. Arg., *Iseilema prostratum* (L.) Andersson, *Merremia emarginata* (Burm.f.) Hallier f., *Orthosiphon pallidus* Royle ex Benth., Rungia repens (L.) Nees, *Senna uniflora* (Mill.) H. S. Irwin & Barneby, *Urochloa reptans* (L.) Stapf.

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Research Article

AN APPRAISAL OF AEROPALYNOLOGICAL RESEARCH ON ALLERGY IN EASTERN INDIA OVER THE PAST DECADE

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A substantial number of airborne pollen studies have been carried out in eastern India over the past decade, with a primary focus on West Bengal, Bihar, Jharkhand and Odisha. These investigations have identified predominant pollen types, their seasonal patterns and allergenic potential, with particular reference to meteorological conditions and respiratory disorders. Aerobiological surveys from various parts of West Bengal reveal distinct pollen seasons, although the precise timing varies according to local environmental factors. Research conducted between 2015 and 2025 in eastern India demonstrates a consistent pattern of elevated pollen concentrations during specific seasons, especially spring and autumn. Studies on pollen allergy in eastern India, particularly in West Bengal, have concentrated on identifying locally important allergenic pollen types, their seasonal fluctuations and the increasing prevalence of associated allergic conditions such as allergic rhinitis. The most prevalent aeroallergens identified include grasses (Poaceae), *Parthenium hysterophorus, Alstonia scholaris, Cassia* species, and pollen from common avenue trees such as *Lagerstroemia speciosa, Spathodea campanulata, Eucalyptus citriodora* and *Cassia siamea*. A review published in 2023 reported that 20-30% of the Indian population suffers from allergic rhinitis each year. Recent studies from Bihar have highlighted the influence of specific invasive plant species and urban air pollution on airborne pollen composition. One study involving schoolchildren in Bihar reported a combined prevalence of 35% for at least one allergic disorder, with marginally higher asthma rates in urban areas. In Odisha and Jharkhand, several of the same avenue tree species identified in West Bengal have also been associated with allergic reactions. However, very limited documented data are available in the literature for this particular decade. The aim of the present study is to provide detailed information on the seasonal periodicity of allergenic pollen grains, thereby enabling susceptible individuals, healthcare professionals, policymakers and scientists to better understand the local environment and to develop effective mitigation strategies.

Key Words: Aeropalynology, meteorology, seasonal periodicities, aeroallergen, allergy.

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INTRODUCTION

Aeropalynology deals with airborne pollen grains, which carry the male genetic material of higher plants. Among plant species, pollen grains are important biosensors because of their characteristic patterns of production and their efficient mechanisms of dispersal. The first report of comprehensive aerobiological work in India was published in 1873 by D. D. Cunningham[1], which examined the atmosphere of Calcutta, then the capital of India. The significance of pollen as a cause of pollinosis is well established and well documented in India. An

aeropalynological survey was initiated in Calcutta by Chanda and Sarkar in 1972, partly using a gravitational sampler[2].

The eastern part of India includes the states of Bihar, Jharkhand, Odisha and West Bengal. West Bengal lies between 21°45′ and 27°16′ N latitude and 85°55′ and 89°56′ E longitude. Over the last decade (2015-2025), aeropalynological research in West Bengal has focused extensively on the study of airborne pollen and its impact on human health, particularly respiratory allergies. Major studies have been carried out in key population centres and biogeographical zones, including Santiniketan, Chandannagar, Berhampore, Burdwan, Habra and Kolkata.

Bihar is situated in the eastern region of India, between latitudes 24°20′ and 27°31′ N and longitudes 83°19′ and 88°17′ E. Over the past decade, aeropalynological research in Bihar has primarily focused on the identification of allergenic pollen present in the atmosphere. These studies have documented airborne pollen types, prepared pollination calendars, and examined the association between atmospheric particulates and allergic reactions among the local population. Research activities have been concentrated in specific locations, including Chapra, Doriganj and Patna.

Odisha is located in the eastern part of the Indian peninsula, with the Bay of Bengal to its east. The state lies between latitudes 17°78′ and 22°73′ N and longitudes 81°37′ and 87°53′ E. During the past ten years, aeropalynological research in Odisha has comprised a preliminary study in the Rourkela region, along with ongoing, broader investigations in aerobiology and environmental monitoring in Rourkela and the Sundargarh district. A key study conducted in the Rourkela region focused on the relationship between modern pollen assemblages and local vegetation.

Jharkhand is a state in eastern India, located between latitudes 21°57′ and 25°14′ North and longitudes 83°20′ and 87°58′ East. No documented aeropalynological studies focusing specifically on Jharkhand have been identified over the past ten years (2015-2025). Although broader research on aerosols and air quality has been carried out in the state, studies specifically addressing airborne pollen appear to have been conducted mainly in neighbouring regions or other parts of India.

India is the most populous country in the world, with approximately 1.428 billion people, accounting for about 17.74-17.99% of the global population. An estimated 35 million individuals in India suffer from asthma, and recent studies have reported an increasing prevalence of allergic rhinitis and asthma[22].

Among the various aerobiological components, pollen constitutes one of the most significant bioparticles in the air. Atmospheric pollen grains are a major cause of allergies, historically referred to as hay fever[3], and they affect human health in the form of respiratory allergies[4,5]. Knowledge of the temporal and spatial distribution of allergenic pollen taxa is therefore of utmost importance for the effective management of pollen-related allergic diseases. Pollen calendars graphically represent the temporal dynamics of airborne pollen taxa within a particular geographical area[6]. With this in mind, the present paper compiles data from aeropalynological

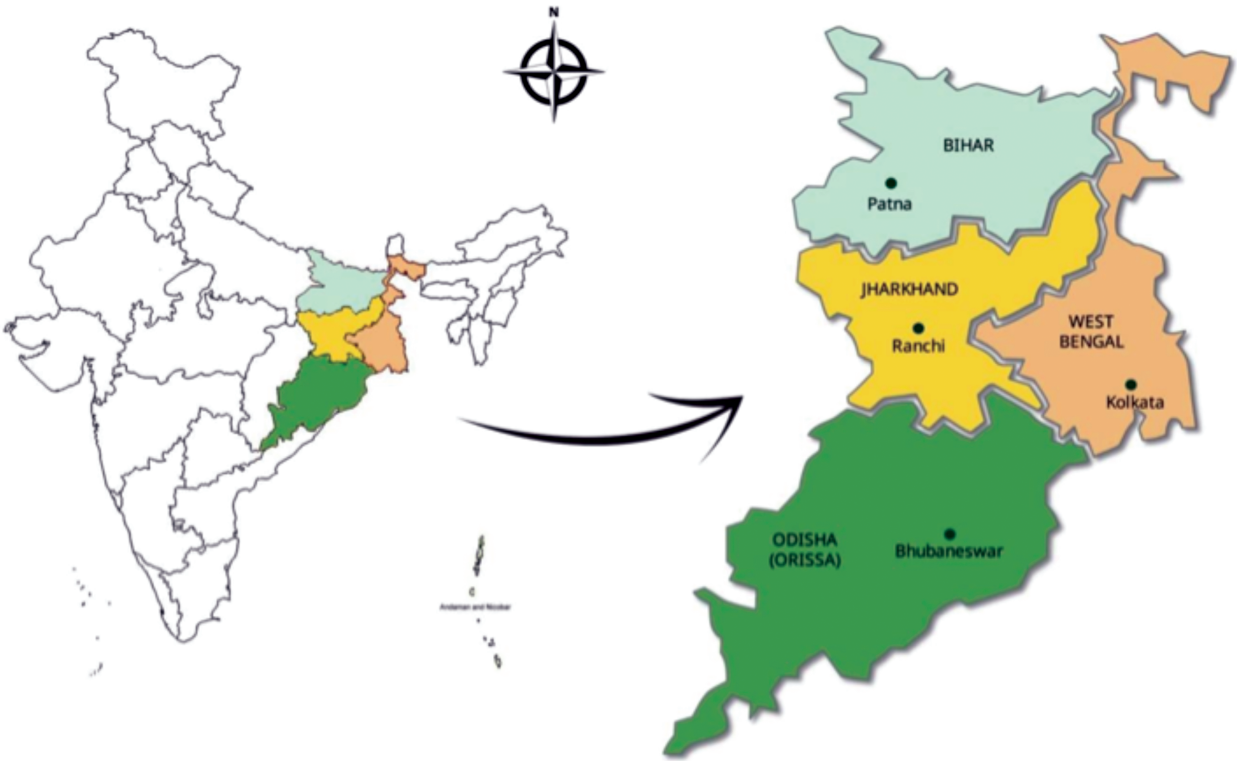


Fig. 4.1: Map showing aeropalynological study area performed in four different states in India.

surveys related to allergy conducted over the past decade in different parts of eastern India, with the aim of identifying periods of hypersensitivity in susceptible individuals so that appropriate precautions may be taken by patients and timely treatment provided by clinicians.

MATERIALS AND METHODS

The present review is based on an analysis of papers published in various journals over the last ten years, focusing on aeropalynological surveys conducted in eastern India. In these surveys, airborne pollen samples were collected, and several methods were employed to prepare pollen calendars, along with other studies such as statistical analyses of the relationship between pollen concentration and meteorological parameters, and tests of allergenicity. Fig. 4.1 shows a map of the aeropalynological study areas across four Indian states: West Bengal, Bihar, Odisha, and Jharkhand.

Clinico-Immunological studies

In general, clinico-immunological studies are conducted using purified pollen grains, which are subsequently defatted and extracted, most often in a phosphate buffer. Skin prick tests (SPT) are carried out on allergic patients in clinical settings according to the method described by Stytis *et al*.[7]. In some cases, ELISA is also performed following the method of Engvall and Perlmann[8].

RESULTS AND DISCUSSIONS

In West Bengal

Numerous studies on aerobiology and allergies in eastern India, particularly in West Bengal, have identified the principal allergens, their

seasonal prevalence, and their correlation with respiratory conditions such as allergic rhinitis and asthma. The accumulated research provides a detailed overview of the region's airborne pollen types and overall aeroallergen profile.

During 2014–2015, an airborne pollen survey conducted in Santiniketan and Chandannagar, West Bengal, identified a total of 64 pollen types belonging to 38 angiosperm families and one gymnosperm species in Santiniketan, and 56 pollen types belonging to 37 families in Chandannagar. A small proportion of the total pollen grains (approximately 2.26%) remained unidentified[9]. The study reported high pollen diversity, attributed to the region's rich vegetation, with peak pollen concentrations occurring in April. Grass pollen (Poaceae) was found to be the most dominant type at both sites, accounting for 6.64% and 9.01% of the total pollen load, respectively.

Approximately 157,400 pollen grains per cubic metre of air were collected over a two-year period. The annual pollen index (API) for Santiniketan was 77,272 pollen grains per cubic metre of air. The most prevalent pollen types were grasses (Poaceae), *Cassia* species, *Acacia* species, the Solanaceae family, the Asteraceae family, the Cyperaceae family, *Cycas* species and *Lagerstroemia* species. Based on monthly pollen diversity, two main pollen seasons were identified: one from mid-February to May and another from mid-September to the end of November. The study showed that airborne pollen spectra vary from place to place in both qualitative and quantitative assemblages, owing to differences in geology, vegetation and other climatic factors.

Clinical skin prick tests revealed that, out of 1,687 patients, 70.92% showed a positive reaction to at least one antigenic extract. The most sensitive allergens were *Saccharum spontaneum* (58.91%), *Alstonia scholaris* (54.30%) and *Cassia sophera* (54.30%). This research contributes to the development of a pollen calendar and helps to forecast allergy-related illnesses for local residents. A hospital-based study from Kolkata[10] described the prevalence of environmental allergens in children with asthma and allergic rhinitis. In Kolkata, among asthmatic children aged 4-12 years, the most common inhalant allergens were house dust mite and cockroach, while the commonly identified food allergens were milk and milk products, egg and egg products, and mustard.

The aeropalynological and meteorological investigation concerning respiratory problem – related hospital admissions among local inhabitants in and around Santiniketan and Sriniketan, West Bengal, was conducted by S. Ghosh *et al*.[11]. The study was carried out over a one-year period (July 2019–June 2020) using a seven-day Burkard volumetric air sampler. A total of 38 pollen types were recorded from the ambient air, with the highest pollen concentration observed in November and the lowest in July.

Meteorological factors such as average temperature, rainfall, relative humidity and wind speed, along with environmental pollutants including NO_2, SO_2 and PM_{10}, were analysed simultaneously. All these environmental factors were found to be positively and/or negatively correlated with the dispersion of pollen grains. The air quality index showed a positive correlation with monthly pollen counts. These findings

indicate that meteorological parameters have a significant impact on the concentration of airborne pollen, which in turn influences respiratory problem-related hospital admissions.

The effect of meteorological parameters on airborne Cyperaceae pollen in West Bengal over a two-decade period (1997–2017) at Madhyamgram was recorded[12]. Perennially airborne Cyperaceae pollen exhibited two peaks, in March and September, throughout the twenty-year period. The concentration showed a gradual increase from 1997 to 2017. Regarding meteorological parameters, the mean daily temperature exhibited an upward trend over the two decades, whereas no significant changes were observed in relative humidity or wind speed, with only slight variations in precipitation. In Murshidabad, an aeropalynological study examined the impact of airborne pollen on the environment and on human respiratory allergies, highlighting the importance of developing pollen calendars for local clinical applications[13].

An aeropalynological survey conducted in Berhampore town, Murshidabad district, West Bengal, recorded a total of 31 pollen types, with the most abundant originating from Poaceae, followed by Cyperaceae, *Cassia* spp., *Acacia auriculiformis*, and others[14]. The allergenic potential of the suspected pollen types was assessed. In Berhampore, skin prick tests on patients revealed strong allergic reactions to pollen from *Eucalyptus citriodora*, *Madhuca indica*, and *Acacia auriculiformis*. Aeropalynological surveys have thus identified the key aeroallergens in Berhampore town, and a pollen calendar has been prepared to assist residents and healthcare professionals.

In Bihar

Recent aeropalynological research in Bihar has concentrated on identifying airborne pollen and spores associated with respiratory allergies, developing pollen calendars, and evaluating the effects of environmental pollution. Studies conducted over the past decade (2015–2025) have built upon earlier work, pinpointing key allergenic plant species across different regions of the state. Researchers have mapped the predominant aeroallergens, including both pollen and fungal spores, for public health purposes. The main airborne pollen identified include *Ambrosia artemisiifolia, Parthenium hysterophorus, Holoptelea integrifolia, Acacia auriculiformis, Cynodon dactylon*, and various species from the family Asteraceae[15,16].

A study conducted in Chapra city between 2018 and 2020 revealed high concentrations of *Parthenium hysterophorus* pollen, with peak levels observed during the spring[15]. Research indicates that pollen seasons in Bihar vary depending on the plant species. A study examining pollen in honeybee forage plants in Muzaffarpur reported peak pollen availability from January to May and again in November. The seasons for allergenic pollen often peak in spring, coinciding with increased reports of allergic conditions. Similarly, a 2022 study in Saran district established a link between environmental pollution, pollen, and allergic reactions. An aerobiological study conducted in Doriganj, Saran, specifically focused on identifying the airspora, or airborne pollen and spores, responsible for allergic reactions[16]. Another study highlights the considerable prevalence of asthma and allergic disorders

among schoolchildren in the Bihar region, with 12% of children diagnosed with asthma and 35% affected by at least one allergic condition, linking higher asthma rates in urban areas to urban air pollution compared with rural areas[17]. Recent studies in Bihar have also emphasised the impact of specific invasive species and urban pollution on airborne pollen.

In Odisha

Over the past ten years, aeropalynological research in Odisha has encompassed a preliminary study in the Rourkela region as well as ongoing broader aerobiology and environmental monitoring. A notable study was carried out in Rourkela, Sundargarh district, focusing on the relationship between modern pollen and the local vegetation[18]. The study found that non-arboreal pollen (originating from herbs) predominated in the airborne taxa over arboreal pollen (from trees and shrubs). Airborne pollen was dominated by herbaceous taxa, particularly grasses from the family Poaceae. This research provides a refe-rence for the modern pollen-vegetation relationship in the region, aiding the interpretation of older, fossilised pollen records. Aeropalynological studies remain limited in Odisha, although broader aerobiological and air quality research has been ongoing, particularly in urban centres such as Bhubaneswar[19]. These studies often examine a wider range of airborne pollutants, including fungal spores alongside pollen. Despite the study in Rourkela, comprehensive, state-wide aeropalynological research in Odisha over the past decade appears limited or is not widely accessible in easily searchable databases.

In Jharkhand

In the past ten years (2015–2025), no dedicated aeropalynological studies specifically focusing on Jharkhand have been identified. While broader investigations into aerosols and air quality in Jharkhand are currently ongoing[20], the rapid pace of industrialisation has significantly worsened air pollution, creating serious public health concerns globally. Mohammad *et al*.[21] examined spatio-temporal changes in air pollution and their impact on public health in Jharkhand. Their study revealed that urban populations are 49.75% more affected than rural populations, with urban males being more affected by 23.60% and urban females by 58.26%. The average growth rate of patients diagnosed with asthma was 92.84% and 90.13% from 2010 to 2013, respectively. Research specifically targeting airborne pollen and spores appears to have been conducted in neighbouring or other regions of India.

Pollen seasons in Eastern India: The aeropalynological surveys conducted indicate the presence of two distinct pollen seasons, although the precise timing may vary according to local conditions. The first occurs from February to April (spring) and is characterised by a peak in tree pollen. The second occurs from September to November (autumn) and is dominated by high concentrations of weed and grass pollen.

Impact of meteorological factors on airborne pollen load: Analysis of the correlation between pollen concentrations and meteorological parameters reveals that pollen levels are positively correlated with temperature and wind speed, and negatively correlated with rainfall and relative humidity. Recent studies suggest that

climate change may be influencing both the timing and duration of pollen seasons, as well as increasing airborne pollen counts and their allergenicity.

Common allergenic pollens in Eastern India: Research has identified several key plant species whose pollen triggers allergies in Eastern India, including *Azadirachta indica, Lantana camara, Cocos nucifera, Cassia fistula, Phoenix sylvestris, Eucalyptus citriodora, Madhuca indica, Parthenium hysterophorus, Ricinus communis, Aegle marmelos, Saccharum officinarum, Alstonia scholaris, Cassia sophera, Peltophorum pterocarpum*, members of the family Cyperaceae, and grasses, specifically those of the Poaceae family. The principal airborne pollen grains responsible for allergies in Eastern India, along with their peak pollination seasons and pollination patterns, are summarised in Table 4.1.

Prevalence of allergic conditions

Allergic rhinitis, often triggered by pollen, has been on the rise in India, including in the eastern regions. A 2023 review reported that

Table 4.1: Important aeroallergenic pollen grains of Eastern India

Sl. No.	Pollen grains	Peak pollination season	Pollination pattern
1.	*Azadirachta indica*	February-May	Seasonal
2.	*Aegle mermelos*	March-June	Seasonal
3.	*Alstonia scholaris*	September-November	Seasonal
4.	*Acacia auriculiformis*	September-January	Seasonal
5.	*Ambrosia artemisiifolia*	August-September	Seasonal
6.	*Cassia sophera*	May-July	Seasonal
7.	*Cocos nucifera*	Present throughout the year, Peak -August	Perennial
8.	*Cyperaceae*	Round the year	Perennial
9.	*Cynodon dactylon*	Early summer through mid-autumn	Perennial
10.	*Delonix regia*	April- July	Seasonal
11.	*Eucalyptus citriodora*	February-April	Seasonal
12.	*Madhuca indica*	January-April	Seasonal
13.	*Parthenium hysterophorus*	March-August	Seasonal
14.	*Peltophorum pterocarpum*	March-June & August-September	Seasonal
15.	*Poaceae*	Round the year	Perennial
16.	*Ricinus communis*	August-November	Seasonal
17.	*Saccharum officinarum*	September-November	Seasonal

20–30% of the Indian population experiences allergic rhinitis annually. A 2024 study summarising common allergens in Eastern India found that, while dust mites and fungi were predominant, weed pollen was also a significant allergen.

Factors contributing to rising allergy rates: Several factors are thought to contribute to the increasing prevalence of pollen allergies and related conditions:

Environmental pollution: Air pollution and elevated levels of particulate matter can increase the allergenic potency of pollens and exacerbate allergic symptoms.

Urbanisation: Rapid urbanisation and associated environmental changes, including shifts in local flora, affect the types and concentrations of airborne pollens. A 2023 review suggests that urban environmental factors may play a significant role.

Lack of awareness and misdiagnosis: Allergic rhinitis is frequently underdiagnosed or mistaken for the common cold, resulting in suboptimal management.

Lifestyle changes: Changes in diet and exposure to various environmental factors, such as mosquito repellents, can influence susceptibility to allergies.

CONCLUSION

It can be concluded from various research articles that aeroallergen profiles vary depending on location, season, and environmental conditions. While studies have been conducted in Kolkata and certain parts of West Bengal, data from other eastern Indian states remain limited. A wide range of airborne pollen types have been identified to develop a pollen calendar and assess seasonal variations. Climate change has been suggested to cause shifts in pollination timing and potential lengthening of pollen seasons, although local evidence is still emerging.

Such information is crucial for health officials, as it helps identify high-risk zones for susceptible individuals and facilitates the implementation of precautionary measures to reduce allergic reactions. A major challenge in good clinical practice for allergists in India is the lack of local aerobiological data on pollens and fungal spores. A large proportion of the Indian population resides in areas where no aerobiological studies have ever been conducted[22]. This makes it difficult for allergists to select an aeroallergen panel that is regionally relevant.

Despite progress, significant research gaps persist in eastern India, particularly over the past decade. There is a need to focus on systematic aeropalynological surveys to produce local pollen calendars and establish ongoing monitoring, thereby supporting effective allergy management.

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Research Article

FICUS LACOR BUCH.-HAM. (MORACEAE): AN ADDITION TO THE FIG FLORA OF TELANGANA STATE

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Ficus lacor Buch.-Ham. (family Moraceae), an ecologically and ethnobotanically significant fig species, is reported here for the first time from the state of Telangana. The species was recorded from the Amrabad Tiger Reserve, thereby constituting a new distributional record for the region. A comprehensive taxonomic description of the species is provided to facilitate accurate identification, along with details of its known geographical distribution. In addition, a photographic plate has been included to support field recognition and to assist researchers, forest managers, and conservation practitioners in distinguishing this species from allied taxa. This new record highlights the floristic importance of the Amrabad Tiger Reserve and underscores the need for continued botanical exploration in the region.

Key Words: Amrabad Tiger Reserve, Eastern Ghats, *Ficus*, New record, Telangana.

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INTRODUCTION

Ficus L., commonly known as fig, belongs to the family Moraceae and represents an abundant and diverse genus characterised by a wide range of life forms, including free-standing trees, epiphytes, semi-epiphytes, rheophytes, and lithophytes. *Ficus* species can grow as deciduous and evergreen trees, shrubs, herbs, climbers, stranglers and creepers. The genus has a worldwide distribution, mainly in tropical and subtropical areas[1]. Ficus species support a wide range of animals owing to their nutrient-rich fruits, which are available throughout the year as a food source, and are therefore considered keystone species[2,3].

Global figure diversity comprises approximately 850 species[4], including 96 species and 12 infraspecific taxa reported from India[5]. The genus Ficus is divided into six sub-genera, viz., *Ficus, Pharmacosycea, Sycidium, Sycomorus, Synoecia,* and *Urostigma*[6], among which *Urostigma* is dominant, comprising about 280 species[7]. The present paper deals with a new distributional record of *Ficus lacor* Buch.-Ham. to fig diversity of the Telangana state.

MATERIALS AND METHODS

Study Area

Amrabad Tiger Reserve is one of the Tiger Reserves of India, covering an area of 2611.39 km^2. It comprises a group of moderately steep hills located in the Central Eastern Ghats between 79.403094°E, 16.703926°N and 78.48951°E, 16.215721°N. The reserve is located in Nagarkurnool and Nalgonda districts of Telangana and was named in 2014 following the bifurcation of the former state of Andhra Pradesh. The elevation in the Tiger Reserve ranges from 150 m to 900 m MSL. The climate is moderate, with average temperatures ranging from 24o to 30oC and during the summer temperatures ranges between 35oC to 45oC. The average annual rainfall is around 900-1000 mm, due to the south-west monsoon. The Krishna River, after flowing through flat plains, enters the mountainous region and follows the southern boundary of the Tiger Reserve. Moist deciduous, dry deciduous, and scrub forests are the major forests types in the study area.

During recent floristic explorations in the Amrabad Tiger Reserve, Telangana, carried out during the Saleswaram Jathara in April within the core area of the Nallamala forests, the authors observed a large evergreen *Ficus* tree providing shade to numerous pilgrims from the intense heat, despite its occurrence within a dry deciduous forest. The authors collected specimens, prepared herbarium specimens using standard methodology8, and deposited at Telangana State Herbarium (TBGH), Dr. BRR Government Degree College (A), Jadcherala, Mahabubnagar District, Telangana. Complete details of the plant, habitat, geographical coordinates, and elevation were recorded in the field. The detailed description, distribution, field and microscopic photographs are provided to facilitate easy identification of the species.

RESULTS AND DISCUSSIONS

After a critical study of the specimen and relevant literatures[9,10], the collected specimens were identified as *Ficus lacor* Buch.-Ham., belonging to the subgenus *Urostigma*. Several authors have reported various number of *Ficus* from the Eastern Ghats, such as 37 taxa including 10 cultivars[11] and 30 taxa[9]. Pullaiah and Sadasivaiah[12] reported 22 taxa of *Ficus* from Telangana State. The present study records *Ficus lacor* Buch.-Ham. as a new addition to the figure diversity of Telangana state. The species may be confused with *F. virens*, but it differs in having larger elliptic leaves and smaller, globose, silky pubescent figs. Earlier it was collected from Semi evergreen forests of Maredumilly, Andhra Pradesh and the present collection is far away from the first location and it is the second collection of the species from dry deciduous forests of Eastern Ghats.

Taxonomic Treatment

Ficus lacor Buch.-Ham., Trans. Linn. Soc. London 15: 150. 1827; Corner, Gard. Bull. Singapore 21 (1): 8. 1965; Chaudhary & al., Taiwania 57 (2): 208. 2012. *Ficus avium* Gagnep., Notul. Syst. (Paris) 4: 85. 1927.

Large tree, up to 30 m tall; aerial roots absent; young branches cylindrical, whitish puberulous to tomentose, glabrous at maturity; bark whitish-grey. Leaves alternate, subcoriaceous-coriaceous,

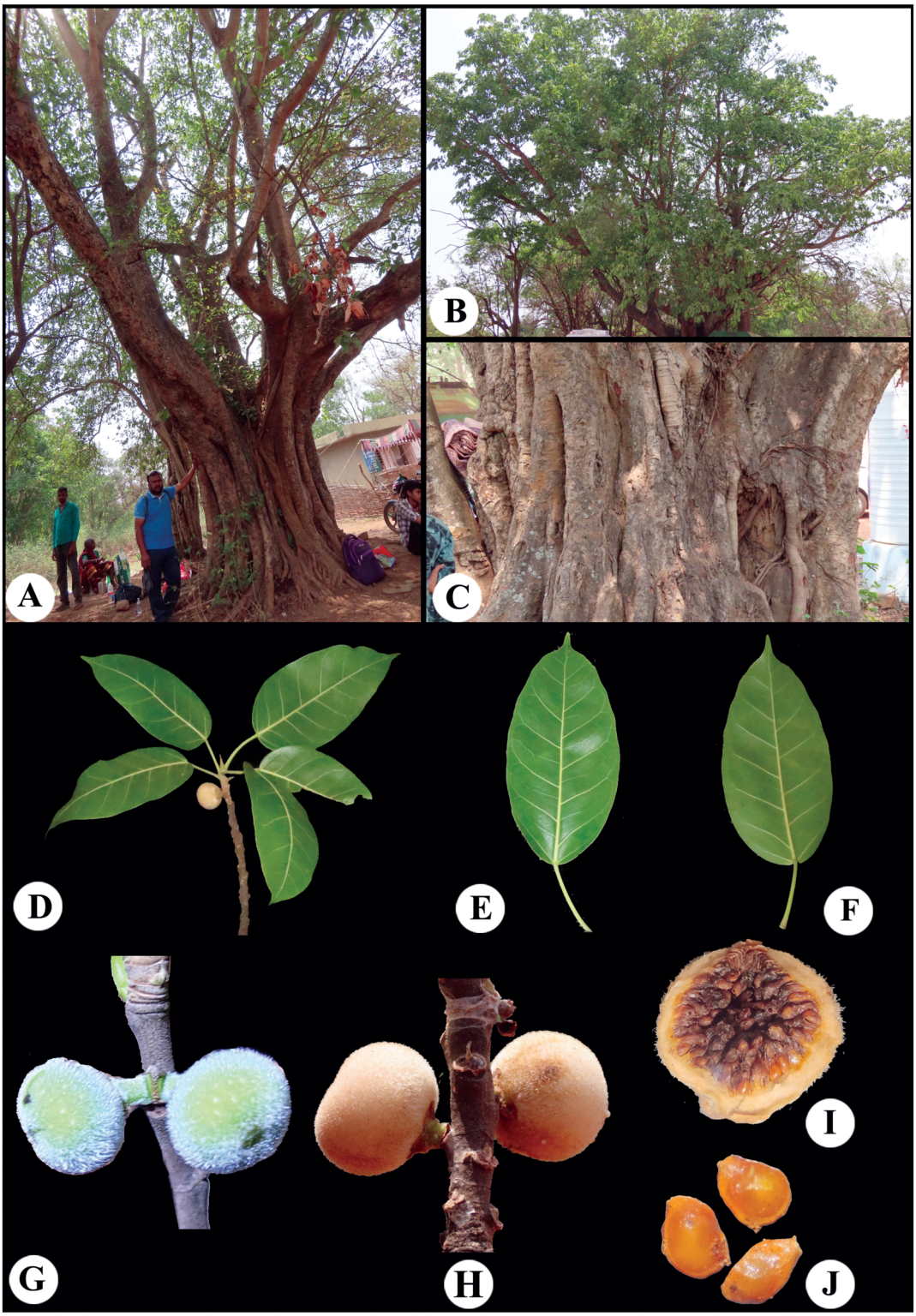


Fig. 5.1: *Ficus lacor* Buch.-Ham.: A & B. Habit; C. Stem; D. Twig; E & F. Adaxial and Abaxial surfaces of Leaves; G & H. Young and Mature Figures; I. Left side of Figure; J. Achenes.

glabrous, ovate-lanceolate or ovate to elliptic to oblong, 5–11 × 4.5–6 cm, apex acute to acuminate, acumen 0.5–1 cm, base obtuse to cuneate, margin entire or sub-undulate, light to dark green above, pale beneath, younger leaves pinkish-reddish; midrib flat above, prominent beneath, lateral veins 5–9 pairs, looped at margin, prominent beneath; petiole 3–6 cm, grooved at upper side. Stipules ovate-acute or broadly ovate, 0.3–1 cm (2–5 cm long in opening shoots), pubescent to tomentose, rarely glabrous, caducous or sub-persistent. Figures in pairs, rarely solitary, usually crowded at the end of the branchlets, shortly peduncled or subsessile; peduncle 0.5–1 cm, woolly tomentose; basal bracts 3, free, ovate-acute or obtuse, triangular, 1.5–3 mm long, brownish, tomentose or pubescent, persistent; receptacle globose-subglobose, 1–1.8 cm in diam., white or greenish at maturity, woolly tomentose or densely pubescent at maturity; ostiole slightly raised or depressed, 2–3 mm in diam., opened; ostiolar bracts 2–3, ovate-obtuse, brownish, densely pubescent; inter floral bracts many, chaffy, linear-lanceolate, 1–1.5 × 0.2–0.3 mm, hyaline, glabrous. Male flowers near ostiole, 1-3 rows, subsessile or pedicellate, 1–2 mm; tepals 5–6, free, linear-lanceolate, 1.2–1.5 mm, white with brownish tinge, glabrous; stamen 1, 0.7–1 mm; filament 0.2–0.5 mm, anther 0.5 mm, rounded, depressed at apex. Gall flowers throughout, sessile or pedicellate, 1.7–3 mm; tepals 3–5, free, spathulate, 1–1.2 mm, white with brownish tinge, hyaline, glabrous. Ovary obovoid, ca. 1 mm long, stipitate, style ca. 0.3 mm long, stigma short. Female flowers many, throughout the receptacle, sessile, 1.6–2.6 mm; tepals 3–5, free, spathulate or ovate-lanceolate, 0.8–1.6 mm, white with reddish or brownish tinge, hyaline, glabrous. Ovary ovoid, 0.5–1 mm, white with brownish tinge, smooth; style lateral, 1–1.5 mm, white with brownish tinge, glabrous; stigma clavate. Achenes ovoid, oblong-ovoid or obovoid, 0.7–1.6 mm, whitish, reddish or brownish, glabrous, smooth.

Flowering and Fruiting time: March – June

Habitat: The species occurs in dry deciduous forest areas at elevations up to 876 m MSL.

Distribution: ***World*** – Bhutan, Cambodia, Myanmar and Nepal. ***India*** – Andhra Pradesh, Bihar, Jharkhand, Madhya Pradesh, Maharashtra, Telangana (present report), Uttarakhand and Uttar Pradesh.

Specimens examined: Pullayapalli Base camp, Nagarkurnool district, Telangana, India, 20.04.2024, (16.202593° N and 78.658868°E), B. Sadasivaiah, L. Paramesh & P. Ranjith Kumar 4556 (TBGH).

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Research Article

CELLULASE-PRODUCING MICROORGANISMS AND AGRI-FOOD WASTE SUBSTRATES: A MICROBIAL APPROACH TO LIGNOCELLULOSE BIOCONVERSION

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As global sustainability concerns continue to intensify, there is growing worldwide interest in transitioning from the current fossil fuel-based economy to a more sustainable, bio-based economy. Lignocellulosic by-products, predominantly generated from agri-food wastes, are abundantly available and possess a high concentration of sugar- and nutrient-rich components, making them promising and low-cost feedstocks for bioprocesses. Current circumstances surrounding global climate change, together with increasing interest in lignocellulose conversion, have further drawn global attention to the development of improved industrial cellulase production. However, traditional approaches have not met industrial demands in an economically viable manner. The valorisation of agri-food wastes through microbial cellulase production offers a sustainable and cost-effective alternative. This paper provides an updated overview of the different types of agri-food wastes utilised as substrates for cellulase production, including field residues, process residues and food processing wastes. It examines the roles of bacterial and fungal strains, highlights recent advancements in fermentation technologies and optimisation strategies, and discusses applications within the food-related industries. In addition, key challenges and future perspectives for enhancing cellulase production are critically addressed.

Key Words: Agri-food waste, biomass, cellulase, enzyme, lignocellulose, food industry

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INTRODUCTION

The United Nations estimated that by 2030, the world population will surpass 8.5 billion, 9.7 billion by 2050 and 10.9 billion by 2100[1]. It has been widely reported that the agricultural yield nearly quadrupled between 1960 and 2015 as Food and Agriculture Organization (FAO, 2020) claims that the production of just fruits and vegetables scaled by 115% between 2000 and 20181. However, despite the developed innovation and technology, significant rise in the consumption and demand in the fresh food does not lead to decrease in the global food waste. The Food waste have garnered worldwide attention due to their detrimental impact on the planet, economy and nutrition[2]. The yearly global production of food waste is estimated to be 931 million tons, among which approximately 53 to 61 percent is domestic trash[3]. From agricultural production to post-harvest handling, processing, distribution, and retail,

food waste happens at every stage of the supply chain, culminating at the consumer stage[4]. Aslam *et al*.[5] specified that the implications of this waste are widespread and include environmental damage, ineffective resource utilization, and considerable economic losses in addition to global food insecurity.

Effective ways of curbing food waste include promoting responsible consumption, enhancing processing efficiency, avoiding preventable losses and adopting sustainable valorization of food residues[6]. Valorization is considered as one of the most sustainable methods which converts waste into value-added products, reducing the landfill accumulation and greenhouse gas emission while simultaneously offering economic advantages through the recovery of valuable materials and expanding up new market opportunities[7]. Over the past few years, the traditional methods for food waste management such as anaerobic digestion and composting, has been gradually replaced by biotechnological approaches that offer higher specificity, efficiency and product value[8]. Among these, enzymatic bioconversion has received a significant attention as a sustainable strategy for converting food waste into different types of value-added products.

Aligned with principles of zero waste economy, agri-food waste possesses considerable potential as a renewable feedstock which can be hydrolyzed into fermentable sugars using enzymes for producing innovative and value-added products across various sectors. Agri-food waste have been found to be high in nutrients including many essential macromolecules such as minerals, proteins, lignin and carbohydrates[9]. Owing to their high nutritional value, they can be utilized primarily to cultivate variety of microbial commodities[10].

Additionally, a wide variety of microorganisms were examined to produce economically valuable cellulase enzymes when grown in agri-food waste. These microbial cellulase enzyme holds enormous potential in food sector such as in juice clarification, olive oil extraction, carotenoid extraction, baking, wine industry and extraction of phenolic compounds. A wide range of microbial strains have been used to produce cellulase enzyme from different types of agri-food waste. While most of the existing literature primarily focuses either on combined enzyme system production or extensively studied enzyme-mediated valorization of agro-industrial residues without addressing cellulase or food sector relevance.

RESULTS AND DISCUSSIONS

Although several reviews have examined cellulase production from agro-industrial residues, most are largely descriptive and primarily emphasize applications in the biofuel, textile, and paper industries. In contrast, critical aspects such as techno-economic feasibility, the use of machine learning and artificial intelligence for process optimization, and food-industry-specific applications are rarely discussed in depth. Furthermore, comparable assessment of cost, scalability and substrates remain unexplored. The present paper delivers an entirely novel perspective by (1) comparing substrates obtained from food waste not simply by enzyme yield but also by pretreatment specifications and scalability, (2) describing breakthroughs in AI/ML-based optimization beyond traditional

tools like RSM, (3) mapping cellulase applications significant to food industry that have not been sufficiently addressed in previous reviews, (4) illustrating substantial research gaps in techno-economic, regulatory and industrial translation. Through the intersection of these dimensions, this paper frames cellulase as a strategic enabler of the circular bio economy

Cellulase Enzyme

The most meticulously investigated multi-enzyme complex, cellulase, hydrolyze the glycosidic bond of cellulose microfibrils comprise of endoglucanase, exoglucanase and β-glycosidase. A variety of products are liberated as a result of this enzymatic action, notably, oligosaccharides (short sequences of sugar molecules), cellobiose (a disaccharide consists of two glucose molecules) and at last glucose (a monosaccharide)[11]. Despite its abundance in nature, cellulose is a polymer offering challenges to breakdown. Only a confined number of species have evolved elaborate enzyme systems to degrade cellulose entirely due to the recalcitrant identity of its glycosidic bonds, necessitating a cooperative action of the distinct components of the enzyme complex to depolymerize cellulose into simple sugars. Microorganisms, plants and some animals serve as the source of the cellulase enzyme[12]. However, the process of production of most industrial and commercial enzymes is accomplished by the microorganisms due to their simplicity, reliability, stability, ease of cultivation, catalytic efficiency, consistent enzyme production, cost-effective, high yield within a short time frame thus maximizing productivity, ease of scalability i.e. from lab-scale to industrial production, vast diversity of enzymes production, ease in genetic manipulation, secretion capability and environment friendly[13]. Mostly, bacterial and fungal species are extensively studied and optimized for enzyme production. As the field of biotechnology advances and microbial hosts are tailored for elevated enzyme yield, versatility and stability, their usage in enzyme manufacturing continues to expand to meet the industrial demand proficiently and economically.

Despite its numerous potential applications, cellulase production confronts obstacles that hinder large scale commercial adoption. One of the most enduring challenges is the high cost of production, which is mostly determined by substrate selection, fermentation condition and downstream processing demands. To enhance enzyme accessibility, various lignocellulosic waste need energy-intensive pretreatment processes, which reduces their overall economic feasibility. Moreover, the production of cellulase may differ significantly based on microbial strain efficiency, type of substrate, production inhibition, substrate specificity, stability, enzyme composition, enzyme activity and synergy, physical characteristics, strain selection, purification and genetic and protein engineering[14]. These drawbacks emphasize the ongoing call for improved strains, optimized fermentation techniques and sustainable substrate replacements such as agri-food waste.

Agri-food waste as substrates for cellulase production

Every step of the food supply chain produces these wastes, that is during food production, processing, transport, storage, packaging, spoilage as well as discarding. However, many of

these wastes can be used as cost-effective substrates for cellulase production due to their richness in plant-derived structural polysaccharides especially when combined with fermentation techniques and optimized microbial system. Agri-food waste can be classified based on their source of generation. There are two categories of agri-food waste such as residues produced from agriculture and food processing. Agricultural residues are further classified into field residues and process residues. The residues that are left in the field following crop harvesting such as leaves, stalks, straw, seed pods, and stems, are referred to as field residues. On the other hand, trash produced when crops are processed to produce any valued product may be considered process residues. Depending on the crop variety and processing method, different amounts of agri-food waste may be produced[8,15].

Micro-organism used in cellulase production

Owing to its broad spectrum of industrial applications and complex structure, microbial cellulase has been recognized as an essential biocatalyst. Many microbes, including bacteria and filamentous fungi, synthesize cellulases (Table 6.1). The genera of Clostridium, Cellulomonas, Thermomonospora, Trichoderma, and Aspergillus are often identified as the producers of cellulase among micro-organisms[16].

Cellulase producing fungi

Fungal cellulases are considered as effective cellulases due to their capacity to generate wide

Table 6.1: Representation of different cellulase producing microorganism using different agri-food waste substrates

Name of microbe	Source of isolated microbes	Lignocellulosic substrate	Type of fermentation	References cite
Trichoderma asperellum	Wheat Straw	Wheat Straw	Submerged	[19]
Aspergillus flavus, *Bipolaris* sp. and *Fusarium* sp.	Decayed irish potato tubers	Potato peel and tuber tissue	Submerged fermentation	[20]
Rhizopus microspores and *Trichoderma viride*	Coconut meat waste	Cassava peel powder	Submerged fermentation	[21]
Aspergillus oryzae OY-3		Onion peel	Submerged fermentation	[22] [23]
Aspergillus terreus NIH2624	Sugarcane bagasse, sugarcane straw and rice bran	Sugarcane bagasse, sugarcane straw and rice bran	Solid state fermentation and Submerged fermentation	

Contd.

Contd. 6.1

Name of microbe	Source of isolated microbes	Lignocellulosic substrate	Type of fermentation	References cite
Cladosporium cladosporiodes	Wheat spikes	Wheat spikes tissue		[24]
Fusarium graminearum	Corn cob	Corn cob residue	Submerged	[25]
Penicillium subrubescens STDF-EG	Corn cob waste	Corn cob powder	Solid state fermentation	[26]
Aspergillus niger spp (HT3, Aa20)	Pineapple peel waste	Pineapple peel	Solid state fermentation	[27]
Aspergillus protuberus	Spoiled rice husk	Rice husk	Solid state fermentation	[28]
Bacillus subtilis QY4		Potato Peel waste	Submerged fermentation	[29]
Acromobacter S3	Strawberry	Mango peel waste	Submerged fermentation	[30]
Trichoderma spp. and *Aspergillus* spp.	Banana pseudostem	Banana pseudostem	Solid state fermentation	[31]
Penicillium rolfsii CCMB714	Jackfruit seed	Jackfruit seed	Solid state fermentation	[32]
Penicillium crustosum	Fruit peels	Water hyacinth (agro-waste)	Solid state fermentation	[33]
Penicillium miczynskii	Pineapple peel	Pineapple peel	Submerged fermentation	[34]
Trichoderma viride	Barley straw and grains	Barley straw and grains	Separate Hydrolysis & Fermentation (SHF)	[35]

variety of enzymes such as endoglucanases, exoglucanases, β-glucosidases which synergistically work to enhance the degradation of cellulose, high substrate specificity, vigorous growth on variety of substrates making them excellent option for the large scale production at lower cost than alternative sources, stability and activity throughout a broad range of pH and temperature contributing to the versatility for different industrial applications.

There are a number of fungi that have been shown to be capable of producing cellulase. Some of the common cellulase producing fungi are *Trichoderma reesei, Aspergillus niger, Peni-*

cillium species, Fusarium species. Because of their high rates of enzyme production, fungal cellulases, such as *Aspergillus* sp., are beneficial for producing cellulases from waste residues and play a significant role in industries[17].

Cellulase producing bacteria

Bacterial sources have been the focus of efforts to discover and understand new lignocellulolytic enzymes because of the high specific activity and efficient in the synthesis of these enzymes heterologously[18]. Studies indicate that the bacterial cellulases are suitable for variety of industrial applications as these offer advantages such as high growth rates, genetic versatility and resistance to alkaline and thermophilic conditions. Majority of identified bacterial strains that are able to break down crystalline cellulose are limited to particular lineages within the four main phyla Actinobacteria, Firmicutes, Proteobacteria, and Bacteroidetes[18].

Preference of bacterial cellulases over fungal cellulases

Bacterial cellulases are becoming more and more favored over fungal cellulases because of their resilience, effectiveness, and versatility in a range of industrial conditions. Bacterial cellulases are appropriate for a variety of situations and processes due to their exceptional capacity to operate in harsh situations, including high temperatures and fluctuating pH levels. For sectors like detergents, where enzyme stability is critical to performance, this flexibility is vital. Numerous bacterial genera, such as *Acetivibrio, Alteromonas, Bacillus, Clostridium, Cellulomonas,* and *Ruminococcus*, have been described for producing extracellular cellulase[36].

Fermentation strategies

To meet the industrial need for microbial cellulase production, two techniques are now used in production methods such as Solid-state fermentation (SSF) and submerged fermentation (SMF). The SSF and SMF are the two methods that they differ in terms of environmental factors and modes of conduction. One of the most critical elements in differentiating different kinds of cycles is the examination of the amount of water involved in the reaction[37].

Submerged Fermentation

In the past, submerged fermentation (SMF) has been used to create industrially significant enzymes because of the need for precise management and control of environmental elements such as pH and temperature. The effectiveness of SSF is relies upon multiple elements, as well as the interplay among them. The outcome of the bioprocess under study depends on selecting the right microorganism in relation to the substrate, the establishing ideal process parameters, and the downstream processing of the final product or products. All of these factors are intricately linked to the technological hurdles encountered when one shifts the paradigm from laboratory shake flasks to enormous bioreactors. The SSF provides various benefits, including reduced catabolite repression, increased yield, increased productivity, and minimal capital investment; however, the process lacks enough technology for estimating biomass, purifying final products, and maximizing productivity at the industrial level[38]. Recent advancements have been made in scaling up technology and addressing the issue of sufficient heat transfer which was

previously considered to be the main bottleneck have been accomplished recently.

Solid-state fermentation

Solid-state fermentation is cheaper since it uses less expensive substrates for the production of cellulase[39]. The technique holds potential because of its low infrastructure and skill requirements, low priced dewatering costs and high product concentration. With the correct technology, enhanced bioreactor design, and a competitive cellulase production method, solid-state fermentation can achieve higher cellulase yields than submerged fermentation while significantly cutting down the production costs[40].

The components of the medium also plays a role in affecting how specific bacteria synthesize enzymes. Complex substrates including wheat flour, steamed rice, sugarcane bagasse, rice bran, sayo humps, banana waste, cotton oil cake, cassava flour, rice straw, cornflour, apple pomace, sesame oil cake, mustard oil cake, sayo hulls, wheat bran, rice bran, rice straw and corncobs were utilized in solid-state fermentation to generate microbial metabolites[41]. For example, wheat bran was employed as a substrate and the filamentous fungus *Trichoderma reesei RUT C30* was used to produce cellulase under SSF. Another example was using wheat bran as a feedstock and the filamentous fungus *Trichoderma reesei RUT C30* under SSF substrate[42].

Enzyme production is a common application for SSF methods because to its cost-effectiveness and simplicity. Inhomogeneous mass transfer, uneven temperature maintenance, pH maintenance, unequal fungal growth in the SSF media and absence of a technique to predict the precise moisture maintenance, expanding SSF for an industrial application poses difficulties associated with fungal biomass concentration[42].

Applications of cellulase in food industry

Cellulases have been utilized in commercial areas for more than 30 years, yet despite this, industry and academic research continue to be fascinated in this enzyme. Depending upon the

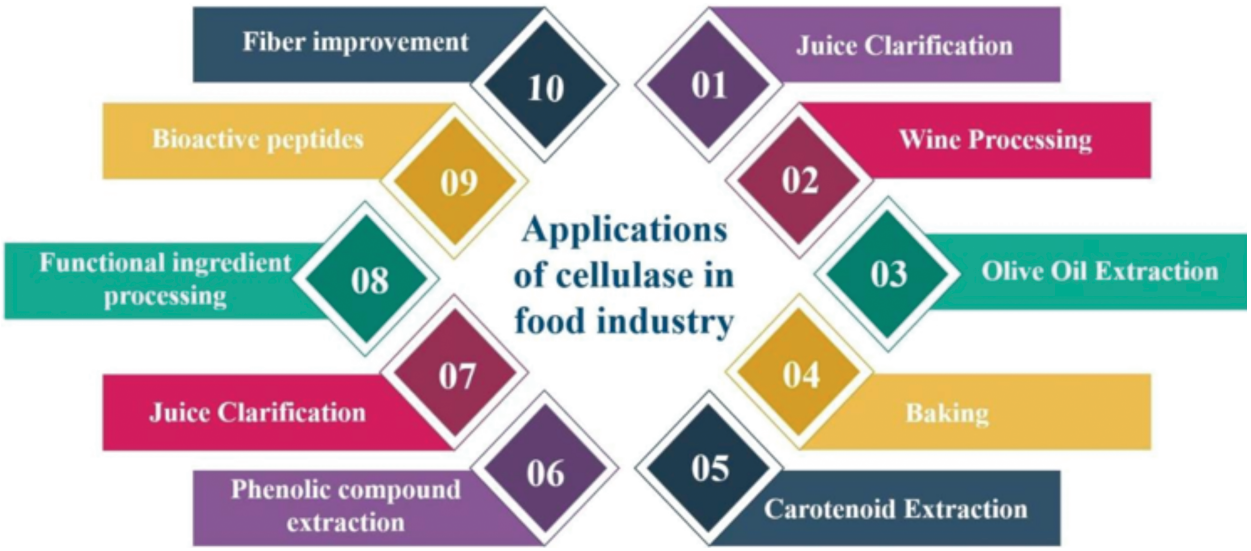


Fig. 6.1: Schematic representation of applications of cellulase in food industry.

business volume, their considerable uses in the different industries positioned cellulase enzyme at the topmost of the worldwide industrial enzyme market. The food sector may make use of cellulase derived from fungus (*Trichoderma* and *Aspergillus*) and bacteria (Paenibacillus and Bacillus)[12]. Cellulase is used in the food industry for a variety of purposes (Fig. 6.1), such as softening fruits, clarifying fruit juices, extracting flavorings and essential oils, and enhancing the filterability of vanilla extracts, to enhance sensory quality of mass, used in the clarification and extraction of beverages such as wine and juices, preserving the rheological stability of the end products[43].

CONCLUSION

One potential approach to solving industrial and environmental issues is the commercialization of agri-food waste for the manufacturing of cellulase. Recent developments in microbial strain production, fermentation and optimization strategies are highlighted in this study, illustrating the emerging trend of producing high-value biocatalysts from inexpensive, renewable substrates. Process efficiency and predictability can be further be improved by the use of intelligent technologies like genetic editing platforms and AI-driven modeling. Because of their adaptability and practical advantages, cellulases are becoming increasingly popular in the food and bioprocess industries for anything from fruit juice clarifying to wine processing and bioactive extraction. However, factors including feedstock unpredictability, downstream processing, and economic viability must be taken into consideration on the way to widespread industrial use. The production of microbial consortia, integrated biorefinery models, and techno-economic analyses that support the objectives of the circular economy should be given top priority in future research. To fully utilize cellulases made from agri-food waste, it will be crucial to prioritize sustainability and application relevance

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Research Article

SEASONAL VARIATION OF INDOOR AIRBORNE FUNGAL DIVERSITY AND ITS ASSOCIATION WITH PATIENT LOAD IN A MEDICAL COLLEGE HOSPITAL IN AMRAVATI, INDIA

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The present study investigated seasonal variations in indoor airborne fungal diversity and their association with hospital medical workload across selected functional areas of Dr Panjabrao Deshmukh Memorial Medical College and Hospital, Amravati, during the period from July 2024 to June 2025. Air sampling was carried out in the OPDs, Gynae-ICU, NICU, OT-I and OT-II using standard aeromycological techniques. The predominant airborne fungal genera identified were *Aspergillus, Penicillium, Cladosporium, Alternaria, Rhizopus* and *Mucor*. A clear seasonal pattern in fungal concentration was observed, with the highest mean CFU/m^3 recorded during the monsoon, followed by winter, and the lowest levels during summer. The OPDs consistently showed the highest fungal burden, largely dominated by *Aspergillus*. In contrast, OT-II recorded the lowest fungal counts, with *Penicillium* as the dominant genus, reflecting the effectiveness of strict aseptic practices.

Analysis of monthly hospital records indicated that OPD attendance, ICU and NICU admissions, and surgical procedures peaked during the monsoon months. Statistical correlation analysis demonstrated a strong and significant positive relationship between airborne fungal load and OPD attendance, ICU/NICU admissions, and surgical activity ($p < 0.01$). These findings confirm that both seasonal climatic conditions and hospital workload jointly influence indoor fungal dynamics, highlighting the need for regular aeromycological surveillance and enhanced infection-control measures, particularly during the monsoon season.

Key Words: Aeromycology, Patient's load, Medical College, Amravati, Maharashtra

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INTRODUCTION

Indoor air quality is especially important in hospitals, where patients, staff, and visitors are continuously exposed to biological pollutants. Airborne fungal bioaerosols are of particular concern because they can cause allergies, respiratory diseases, and severe infections, especially in immunocompromised individuals[1,2]. Common indoor fungal genera such as *Aspergillus, Penicillium, Cladosporium, Alternaria, Rhizopus*, and *Mucor* are major allergens and opportunistic pathogens. Species like *Aspergillus fumigatus, A. flavus,* and *A. niger* frequently lead to hospital-acquired infections, including invasive aspergillosis in high-risk patients[3,4].

Exposure to these fungi is linked to allergic rhinitis, asthma, hypersensitivity pneumonitis, and related disorders, making continuous aeromycological monitoring essential for hospital safety and public health.

Hospitals are highly dynamic built environments characterized by heavy human movement, high patient turnover, repeated ventilation disturbances, increased moisture levels, and accumulation of organic matter, all of which favor fungal growth and dispersal[5,6]. Different hospital sections, such as outpatient departments (OPDs), general wards, intensive care units (ICUs), neonatal ICUs (NICUs), and operating theatres (OTs), exhibit substantial variation in fungal concentration due to differences in patient density, ventilation, sanitation, and clinical activity. High OPD footfall, increased inpatient occupancy, and frequent surgical procedures lead to continuous resuspension of settled dust and spores, resulting in elevated airborne fungal loads[7].

In addition to hospital workload, seasonal climatic factors play a decisive role in determining the concentration and composition of airborne fungi. Temperature, relative humidity, rainfall, and wind speed collectively influence fungal sporulation, survival, and dissemination[8]. In tropical and subtropical regions of India, fungal bioaerosol concentrations usually show distinct seasonal fluctuations, with the highest levels during the monsoon, followed by winter, and the lowest during summer[9]. High humidity during the rainy season creates ideal conditions for fungal growth, while dry summer conditions suppress fungal survival in indoor air.

Several aeromycological studies conducted across different parts of India have documented seasonal variation in airborne fungal diversity in residential, educational, and hospital environments[10]. Most of these studies highlight the predominance of allergenic fungi during the monsoon and winter seasons. However, very few investigations have attempted to correlate airborne fungal concentrations with hospital medical activity, such as OPD attendance, ward-wise patient occupancy, ICU admissions, and the number of surgical procedures. These operational parameters play a crucial role in influencing indoor microbial air quality but are often ignored in conventional aerobiological studies.

In the Vidarbha region of Maharashtra, including Amravati district, limited indoor aeromycological studies have been carried out in schools, libraries, and rural healthcare centers, reporting the presence of allergenic fungal spores in indoor air[11]. However, systematic, year-round seasonal studies from medical college hospitals in this region remain scarce, and quantitative assessment of fungal bioaerosols in relation to hospital medical statistics is largely unexplored. The integration of aerobiological data with hospital workload indicators provides a more realistic assessment of the risk of nosocomial fungal exposure. Increased patient density and clinical activity elevate indoor fungal aerosols through frequent disturbance of contaminated surfaces, increased moisture from respiratory droplets, and continuous movement of staff and equipment[12]. Correlating fungal load with medical data can help to identify high-risk hospital zones and seasons associated with maximum exposure.

The present study aims to evaluate the seasonal

dynamics of indoor airborne fungal diversity and to determine its association with hospital medical workload, including OPD visits, ward-wise patient occupancy, ICU admissions, and surgical activity, in a tertiary care medical college hospital at Amravati.

MATERIALS AND METHODS

Selection of Sampling Sites

The study was conducted across five selected indoor locations within the Medical College Hospital, Amravati, Maharashtra state, India. The hospital receives patients from both urban and rural areas of the Vidarbha region and comprises multiple high-occupancy zones, including outpatient departments (OPDs), surgical wards, gynecological intensive care unit (ICU), neonatal ICUs (NICUs), and operation theatres (OT-I & OT-II). Due to continuous movement of patients, diagnostic procedures, and surgical interventions, the hospital provides an ideal setting for aeromycological monitoring.

Study Duration and Seasonal Classification

The study was conducted for a period of twelve consecutive months, from July 2024 to June 2025, to assess seasonal variation in indoor airborne fungal diversity. Based on regional climatic conditions, the sampling period was divided into three major seasons:

- ➢ Summer: March–June
- ➢ Monsoon: July–September
- ➢ Winter: October–February

Collection of Airborne Fungal Samples

Airborne fungal spores were sampled using the settle plate technique[13,14]. Petri plates containing Potato Dextrose Agar (PDA) were exposed to the indoor environment for 10–15 minutes at a height of about one meter, approximating the breathing level of individuals in the area. Concurrently, environmental parameters such as temperature, relative humidity, and rainfall were documented to evaluate their impact on spore presence. After exposure, the plates were closed, properly labeled, and transported to the laboratory under sterile conditions.

Growth, Observation and Isolation

PDA plates were incubated at 28 ± 1°C and examined on the 3rd, 5th, and 7th days to record CFUs. Selected colonies were transferred aseptically to fresh PDA to obtain and maintain pure cultures through regular sub-culturing.

Microscopic and Macroscopic Identification

Lactophenol Cotton Blue staining was used to study spore and hyphal structures under the microscope, while colony color, texture, pigmentation, and sporulation were documented. Fungal isolates were identified to genus or species level by comparing these traits with standard taxonomic keys[15].

Collection of Hospital Medical Workload Data

To establish a correlation between airborne fungal load and hospital activity, monthly hospital medical statistics were collected from the Medical Records Department (MRD) and hospital administration with prior official permission.

The following parameters were recorded:

- Monthly OPD attendance
- Ward-wise inpatient admissions
- ICU and NICU admissions

- Number of surgical procedures performed per month
- Average bed occupancy rate

All medical data were collected in aggregate form only, without access to individual patient identities, to ensure confidentiality.

Statistical Analysis

The data obtained from aeromycological sampling and hospital medical records were statistically analyzed using Microsoft Excel.

- Mean and standard deviation (Mean ± SD) were calculated for seasonal and ward-wise fungal counts.
- One-way Analysis of Variance (ANOVA) was applied to determine significant seasonal variation.
- Pearson's correlation coefficient (r) was used to evaluate the association between:
  - Fungal load and OPD attendance
  - Fungal load and inpatient admissions
  - Fungal load and ICU occupancy
  - Fungal load and number of surgeries

A p-value < 0.05 was considered statistically significant.

Ethical Considerations

Prior approval for the study was obtained from the Dean of Dr. Panjabrao Deshmukh Memorial Medical College, Amravati. All hospital data were collected in compliance with ethical guidelines, and patient confidentiality was strictly maintained.

RESULTS

The present study provides a detailed assessment of the seasonal variation of indoor airborne fungal diversity and its relationship with hospital medical workload across different functional units of a hospital (Tables 7.1-7.6).

The indoor hospital environment was characterised by a clear predominance of fungal genera, notably *Aspergillus, Penicillium, Cladosporium, Alternaria, Rhizopus,* and *Mucor* (Table 7.1 & Table 7.3). Among these, *Aspergillus* species were particularly widespread, indicating their strong adaptability to the hospital microclimate (Table 7.2). The highest concentrations of air-

Table 7.1: Seasonal Variation of Airborne Fungal Load and Dominant Aerofungi (CFU/m³)

Fungal Taxa	Summer (CFU/m³ ± SD)	Monsoon (CFU/m³ ± SD)	Winter (CFU/m³ ± SD)
Total Fungal Load	180 ± 25 (Min. 140 – Max. 220)	420 ± 40 (Min. 360 – Max. 480)	290 ± 30 (Min. 240 – Max. 340)
Aspergillus	120 ± 15	210 ± 25	170 ± 20
Penicillium	60 ± 10	95 ± 15	75 ± 12
Cladosporium	38 ± 8	68 ± 12	50 ± 10
Alternaria	32 ± 7	42 ± 9	55 ± 10
Rhizopus	22 ± 6	39 ± 8	28 ± 6

Table 7.2: Site-wise Distribution of Airborne Fungal Load in Hospital Environment

Sl. No.	Hospital Site	Mean CFU/m³ ± SD	Dominant Aerofungus
1.	OPDs	410 ± 35	*Aspergillus*
2.	Gyn-ICU	250 ± 22	*Aspergillus*
3.	NICU	190 ± 18	*Penicillium*
4.	OT-I	125 ± 12	*Penicillium*
5.	OT-II	110 ± 10	*Penicillium*

Table 7.3: Dominant Aerofungi: Overall Load, Abundance, and Clinical Significance

Fungal Genus	Mean CFU/m³ ± SD	Relative Abundance (%)	Clinical Significance
Aspergillus	165 ± 22	42.3	Allergic & invasive infections
Penicillium	76 ± 14	19.5	Allergic reactions
Cladosporium	52 ± 11	13.3	Respiratory allergy
Alternaria	41 ± 9	10.5	Severe asthma, rhinitis
Rhizopus	30 ± 7	7.7	Mucormycosis
Mucor	26 ± 6	6.7	Opportunistic infections

Table 7.4: Monthly (2024-2025) medical workload for selected sites

Month	OPD Attendance	Gyn-ICU Admissions	NICU Admissions	OT-I Surgeries	OT-II Surgeries
July 2024	13,600	160	130	190	175
August 2024	14,200	170	140	200	185
September 2024	13,850	165	135	195	180
October 2024	12,900	155	125	185	170
November 2024	12,650	150	122	180	165
December 2024	13,100	158	128	188	172
January 2025	12,480	145	120	180	160
February 2025	11,950	138	115	175	155
March 2025	10,880	122	105	160	145
April 2025	9,940	110	98	150	130
May 2025	9,210	105	90	140	120
June 2025	10,450	118	102	155	135

Table 7.5: Relationship between Dominant Aerofungal Load and Hospital Medical Workload (Correlation Analysis)

Parameter Compared	Correlation Coefficient (r)	p-value
Total Fungal Load vs OPD Attendance	+0.85	< 0.01
Total Fungal Load vs Gyn-ICU Admissions	+0.77	< 0.01
Total Fungal Load vs NICU Admissions	+0.73	< 0.01
Total Fungal Load vs OT-I Surgeries	+0.76	< 0.01
Total Fungal Load vs OT-II Surgeries	+0.74	< 0.01

Table 7.6: Integrated Relationship Between Dominant Aerofungi, Season, Site, and Hospital Workload

Dominant Aerofungus	Peak Season	High-Risk Site	Mean CFU/m³	Associated Hospital Activity
Aspergillus	Monsoon	OPDs, Gyn-ICU	210	High patient crowd & ICU admissions
Penicillium	Monsoon	NICU, OT-I, OT-II	95	Neonatal care & surgeries
Cladosporium	Monsoon	OPDs	68	Heavy human movement
Alternaria	Winter	OPDs	55	Seasonal respiratory cases
Rhizopus	Monsoon	OPDs	39	High humidity & organic moisture
Mucor	Monsoon	OPDs, ICU	34	High moisture & immuno-compromised exposure

borne fungi, expressed as colony-forming units (CFU), were consistently recorded during the monsoon season (Table 7.6). This period coincided with peak hospital activity, including increased outpatient department (OPD) attendance, higher rates of intensive care unit (ICU) admissions, and a greater volume of surgical procedures, suggesting a seasonal amplification of fungal burden linked to both environmental and human factors.

Spatial analysis within the hospital revealed notable variation in fungal contamination across different functional areas (Table 7.2 & 7.4). The OPDs emerged as the most heavily contaminated locations, with *Aspergillus* species dominating the aeromycological profile, likely reflecting high patient turnover, f requent movement, and repeated door opening. In contrast, Operation Theatre II (OT-II) exhibited the lowest CFU counts, indicative of more stringent environmen-

tal control measures, and was predominantly colonised by *Penicillium* species.

Statistical analysis demonstrated a strong and highly significant positive correlation ($p < 0.01$) between the total airborne fungal load and key indicators of hospital workload, including OPD attendance, admissions to the Gynaecology ICU and Neonatal ICU, and the number of surgical procedures performed (Table 7.5). These findings clearly confirm that hospital activity levels exert a substantial influence on the dynamics of airborne fungal contamination, underscoring the role of human occupancy and clinical operations in shaping the indoor aerofungal environment.

DISCUSSION

The study reveals that both seasonal climatic factors and hospital workload have a significant impact on indoor airborne fungal levels in a tertiary care hospital (Table 7.6). Fungal load followed a clear seasonal trend, peaking during the monsoon, decreasing in winter, and reaching its lowest levels in summer—patterns consistent with tropical aerobiological research, which links high humidity and moderate temperatures to increased fungal proliferation.

Common genera such as *Aspergillus, Penicillium, Cladosporium, Alternaria, Rhizopus*, and *Mucor* dominated the indoor air, matching global and national findings that identify these fungi as major indoor contaminants and clinical allergens (Tables 7.2 & 7.3). Previous studies also report similar fungal profiles in hospitals, especially in critical care areas and operating theatres.

Among hospital units, OPDs recorded the highest fungal counts due to heavy patient flow, frequent movement, and dust disturbance, whereas the lowest levels in OT-II reflected effective aseptic practices and controlled ventilation. Intermediate levels in the Gyn-ICU and NICU are clinically important because these units house vulnerable patients, and the presence of *Aspergillus* and *Penicillium* poses infection risks (Tables 7.3 & 7.5).

A major strength of this investigation is the integration of fungal data with hospital workload metrics. OPD visits, ICU admissions, and surgical procedures all increased during the monsoon, coinciding with higher concentrations of fungi. Strong positive correlations ($r = 0.73–0.85$) indicate that crowding and clinical activity substantially elevate airborne fungal loads, supporting earlier findings that human movement and inadequate ventilation amplify indoor bioaerosols.

The simultaneous rise in fungal burden and hospital workload during the monsoon underscores the need for season-specific and activity-informed infection-control strategies. Overall, the study reinforces that climate, fungal community structure, and indoor human activity collectively determine hospital airborne fungal exposure and highlights the value of linking aeromycological monitoring with hospital operational data for improved risk management.

CONCLUSION

This study demonstrates that indoor airborne fungal levels in the hospital are strongly affected by both seasonal climatic changes and hospital workload. Fungal concentrations peaked during the monsoon, decreased in winter, and were lowest in summer, confirming the influ-

ence of humidity and temperature on fungal growth. *Aspergillus* emerged as the dominant genus across all seasons, followed by *Penicillium, Cladosporium, Alternaria, Rhizopus,* and *Mucor*, many of which are clinically important allergens and opportunistic pathogens.

Among hospital sites, OPDs showed the highest fungal load due to heavy patient flow and constant air disturbance, whereas OT-II recorded the lowest levels, reflecting effective sterilization and ventilation. Moderate fungal concentrations in the Gyn-ICU and NICU are concerning because of the vulnerability of neonates and post-operative patients.

A strong positive correlation was observed between fungal load and key workload indicators—OPD attendance, ICU and NICU admissions, and surgical procedures—indicating that increased hospital activity significantly elevates airborne fungal burden. The overlap of high workload and monsoon conditions represents the period of greatest risk for hospital-acquired fungal exposure.

RECOMMENDATIONS

Seasonal fungal surveillance should be intensified during the monsoon and early winter, with strict monitoring in high-risk areas such as OPDs, Gyn-ICU, and NICU. Proper ventilation through well-maintained HEPA filters and AHUs, along with indoor humidity control below 60%, is essential. Hospital workload should guide staffing and disinfection schedules, especially during peak OPD and surgical periods. Extra protection is required for vulnerable patients, and regular staff training on fungal risks and hygiene must be ensured. Overall, seasonal climate and hospital activity together influence indoor fungal levels, making continuous aeromycological monitoring vital for effective infection control.

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Research Article

BASELINE PALYNOLOGICAL PROFILING OF COMMON SUBSTRATES AND THEIR POTENTIAL APPLICATIONS IN FORENSIC INVESTIGATIONS

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Forensic palynology—the study and application of pollen and spores in legal investigations—offers a valuable means of linking suspects, victims and crime scenes through trace evidence. The present study examines techniques for the collection and analysis of pollen grains and spores from a range of forensic samples, including air filters, fruits, clothing and hair. Samples were collected from several locations in Telangana State, India, namely Karimnagar, Wanaparthy, Hyderabad, Alampur and the Osmania University campus. The durability, abundance, dispersal mechanisms and morphological diversity of pollen grains make them particularly effective as trace evidence. Their distinctive micro-morphological characteristics allow identification of the parent plant taxa, which can be correlated with specific habitats or geographical regions relevant to an investigation. Although forensic palynology is still an emerging discipline in India, its integration with other forensic techniques demonstrates considerable potential for resolving complex criminal and civil cases, including homicide, terrorism, forgery, theft, rape, arson, drug trafficking, assault and wildlife poaching.

Key Words: Forensic palynology, pollen and spores, trace evidence, air filters, criminal investigation, Telangana, India.

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INTRODUCTION

Forensic palynology involves the identification and analysis of pollen grains, plant spores, and fungal spores for legal and investigative purposes. It provides valuable evidence by linking suspects, victims, and crime scenes through pollen traces preserved on materials such as clothing, hair, and objects. This approach has proven useful in various cases, including homicide, terrorism, forgery, theft, sexual assault, arson, drug trafficking, and wildlife poaching.

Pollen is an excellent forensic marker due to its microscopic size, abundance, and exceptional resistance to physical and chemical degradation. Its durable outer wall (exine), composed of sporopollenin, allows long-term preservation under diverse conditions[1,2]. Pollen dispersal mechanisms affect their forensic value: wind-

dispersed (anemophilous) pollen are abundant and widely distributed, whereas animal-pollinated (zoophilous) pollen are larger and sticky and tend to remain localised, making them especially useful for linking evidence to specific habitats.

The spatial distribution of airborne pollen, or "pollen rain," forms distinctive regional signatures that can help to determine the origin or movement of materials and individuals. Since its first forensic application in the 1950s, palynology has become a well-established investigative tool, especially in countries such as Australia, New Zealand, and the United Kingdom[3–6]. Landmark cases include Erdtman's[3] identification of crime scene displacement in Sweden, Klaus's use of fossil hickory pollen in Vienna[4], and Mildenhall's[5] work linking a suspect to a sexual assault in Auckland. Bryant *et al*.[6] and Frei[7] further demonstrated its use in homicide investigations and artifact provenance, respectively. In the United States, pollen evidence has even been used to trace the origin of narcotics[8]. In India, the field is still emerging, though several studies have underscored its potential. Dev Kapil[9], Kaur & Garg[10], and Garg and Sachan11 discussed its forensic applications, while later works by Mayuri Kumari *et al*.[12], Ammna Rakshanda and Jayarama Reddy[13], and Tejesh Kumar and Tina Sharma[14] expanded its scope through methodological and case-based analyses. Recently, Bhawana Saini and Surya Shekhar Daga[15] established a pollen profile for Jaipur City, reinforcing its forensic value.

Despite these efforts, the forensic utility of palynological evidence in India remains under explored. The present study aims to develop an experimental database of pollen and spores from diverse materials—air filters, fruits, clothing, and hair—collected across Telangana State. This database will support the application of forensic palynology in crime investigations and promote its integration into multidisciplinary forensic research in India

MATERIALS AND METHODS

Study Area

The study was conducted across Telangana State, India, encompassing varied vegetation and climatic zones suitable for palynological investigation. Samples were collected from Karimnagar, Wanaparthy, Hyderabad, and Alampur districts between January – December 2024 to establish a baseline database for forensic palynomorph analysis under Indian environmental conditions (Table 8.1).

Sample Collection

Samples were collected following standard forensic procedures to minimize contamination and ensure proper chain of custody[16]. Based on Locard's Exchange Principle[17], potential pollen-bearing materials such as air filters, fruits, clothing, and human hair were obtained from selected locations. All samples were sealed in sterile containers, labeled, and transported to the Palynology and Plant Systematics Laboratory, Osmania University, Hyderabad, for analysis.

Extraction of Palynomorphs

Palynomorph extraction was done following the method of Horrocks[18] with minor modifications. Dust, soot, and hair samples were preserved in

70% ethanol and refrigerated. Hair and fabric samples were cleaned with mild detergent, agitated for 60 min, and centrifuged at 3000 rpm. The residues were sequentially treated with 10% KOH (70°C), 10% HCl (24 h), and 40% HF (100°C) to remove organic, carbonate, and siliceous impurities, respectively, followed by repeated washing with distilled water.

Acetolysis and Mounting

Pollen purification was performed using the acetolysis method of Erdtman[3]. Residues were treated with glacial acetic acid and an acetolysis mixture (acetic anhydride: sulfuric acid, 9:1 v/v), heated in a boiling water bath for 5 min, centrifuged, and washed with 50% glycerin. The final residue was mounted in glycerin jelly on glass slides for microscopic analysis[18].

Microscopic Analysis and Identification

Prepared slides were examined under a binocular microscope, and images were captured using a DSLR camera. Pollen and spore identification was based on comparison with authenticated reference slides and published identification keys[19]. All specimens and reference material were catalogued and archived at the Palynology and Plant Systematics Laboratory for future forensic research and database development.

RESULTS

Palynological analyses of samples collected from different sources—air filters, fruits, cloths, and human hair—revealed diverse pollen assemblages representing both herbaceous and arboreal taxa (Table 8.2, 8.3 & Plate-I). These results demonstrate the capacity of each material type to serve as an efficient trap for airborne

Table 8.1: Summary of samples collected for palynological investigation in Telangana State (Total samples analyzed: 23)

Sample Type	Sample Code(s)	No. of Samples	Location(s)	Description / Remarks
Air Filter Dust	A1–A4	4	Karimnagar, Wanaparthy	Dust and soot from vehicle air filters collected from different vegetation zones.
Fruits	F1–F4	4	Hyderabad	Apple, pomegranate, and dragon fruit samples obtained from the Hyderabad fruit market.
Cloths	CL1–CL5	5	Osmania University, Hyderabad	Fabric samples collected during June 2024 from the Osmania University campus.
Human Hair	HHK1–HHK5, HHW1–HHW5	10	Alampur, Wanaparthy	Human hair samples collected from two distinct localities during May–June 2024.

Table 8.2: Summary of dominant pollen taxa identified from various sample types and their indicative forensic significance

Sample Type	Dominant Pollen Taxa (identified)				Un-identified	Indicative Environment/ Forensic Relevance
	Dominant pollen types	Species level	Genus level	Family level		
Air Filters	Ageratum conyzoides, Fabaceae, Leucaena leucocephala, Ledebouria revoluta	9%	42%	37%	12%	Urban/rural vegetation; vehicular movement across mixed landscapes
Fruits	Punica granatum, Ehretia aspera, Neltuma juliflora	42%	20%	25%	13%	Orchard or semi-arid environments; exposure to open-air markets
Cloths	Asteraceae, Grewia asiatica, Holoptelea integrifolia, Kalanchoe pinnata		13%	30%	39%	18% Contact with tropical/ sub-tropical vegetation; field or garden exposure
Hair	Volkameria inermis, Poaceae, Azadirachta indica, Parthenium hysterophorus	5%	28%	42%	25%	Outdoor exposure; roadside or forested regions; temporal environmental record

pollen and fungal spores, with varying pollen density and diversity reflecting their exposure and surface characteristics (Fig. 8.1).

Air Filter Dust and Soot

Air filter samples collected from Karimnagar and Wanaparthy exhibited the highest pollen

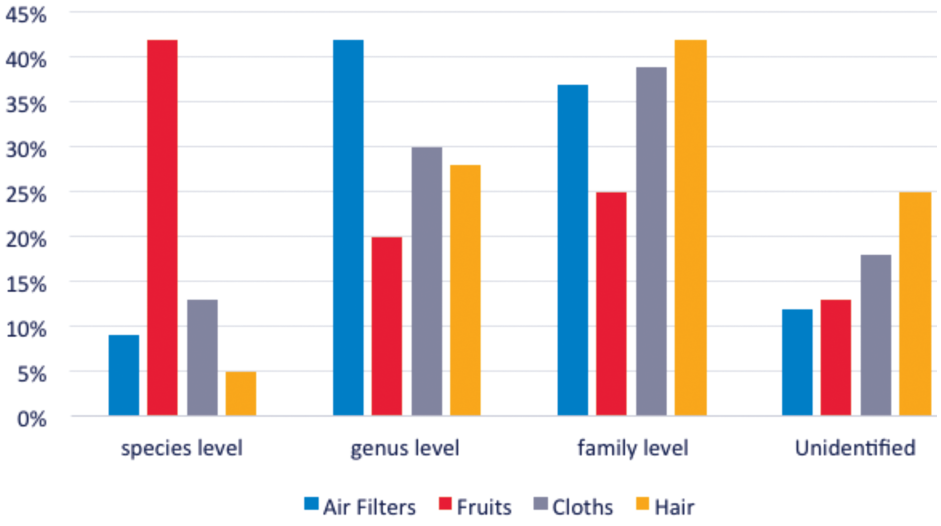


Fig. 8.1: Distribution of palynomorph categories across various forensic sample types

diversity among all substrate types examined. Of the total palynomorph assemblage, 9% of grains were identifiable to the species level, while 42% corresponded to genus-level identi-

Plate 8.1: Pollen and fungal spores recorded from various forensic samples

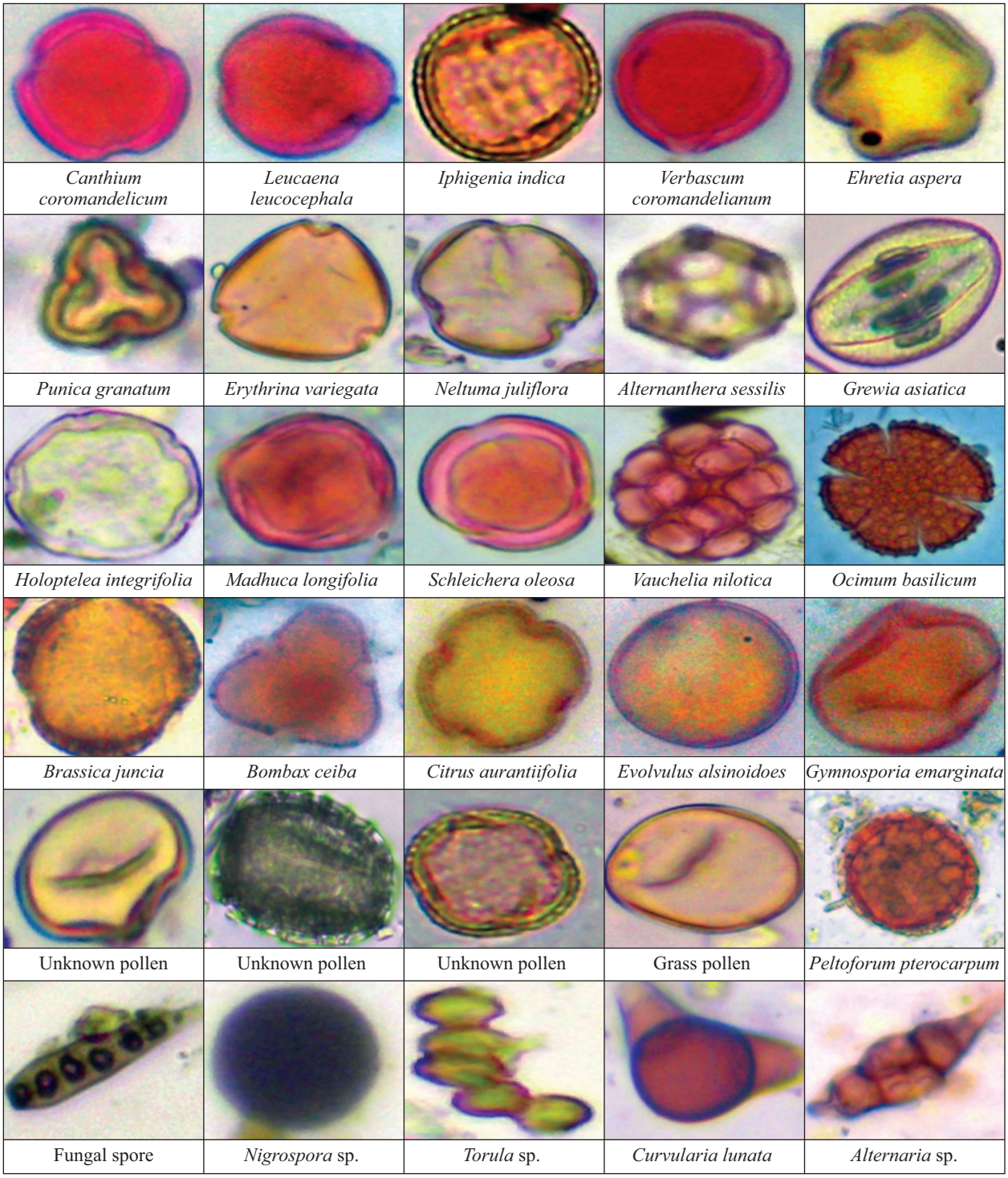


Table 8.3: List of pollen grains collected from the Plant species

Sl. No.	Botanical Name	Family	Flowering Season
1.	*Hygrophila auriculata* Schumach.	Acanthaceae	Oct-April
2.	*Rostellularia procumbens* (L.) Nees.	Acanthaceae	June-March
3.	*Alternanthera sessilis* (L.) R Br ex DC.	Amaranthaceae	Dec-March
4.	*Crinum lorifolium* Roxb.	Amaryllidaceae	Oct-Dec
5.	*Lannea coromandelica* (Houtt.) Merr.	Anacardiaceae	Jan-March
6.	*Ledebouria revoluta* (L.f.) Jessop	Asparagaceae	April-sept
7.	*Ageratum conyzoides* L.	Asteraceae	Oct-Dec
8.	*Parthenium hysterophorus* L.	Asteraceae	Aug-Sept
9.	*Dolichandrone falcata*, (Wall. ex DC.) Seem.	Bignoniaceae	April-May
10.	*Ehretia aspera* Willd.	Boraginaceae	Jan-May
11.	*Brassica juncea* (L.) Czern.	Brassicaceae	Nov-Jan
12.	*Caesalpinia pulcherrima* (L.) Sw.	Fabaceae	Oct-Dec
13.	*Leucaena leucocephala* (Lam.) de Wit.	Fabaceae	Oct-Dec
14.	*Neltuma juliflora* (Sw.) Raf.	Fabaceae	Aug-Oct
15.	*Peltophorum pterocarpum* (DC.) Baker ex Heyne	Fabaceae	May-Aug
16.	*Prosopis cineraria* (L.) Druce	Fabaceae	Nov-Jan
17.	*Vachellia nilotica* (L.) P. J. H. Hurter & Mabb.	Fabaceae	March-May
18.	*Gymnosporia emarginata* (Willd.) Thwaites	Celastraceae	Nov-Dec
18.	*Gloriosa superba* L.	Colchicaceae	Aug-Sep
20.	*Iphigenia indica* (L.) A. Gray ex Kunth.	Colchicaceae	Nov-Dec
21.	*Evolvulus alsinoides* (L.)L.	Convolvulaceae	Aug-Nov
22.	*Kalanchoe pinnata* (Lam.) Pers.	Crassulaceae	Jan-April
23.	*Coccinia grandis* (L.) Voigt	Cucurbitaceae	June-Nov
24.	*Cyperus exaltatus* Retz.	Cyperaceae	March-Nov
25.	*Erythrina variegata* L.	Fabaceae	April-May

Contd.

Contd. Table 8.3

Sl. No.	Botanical Name	Family	Flowering Season
26.	*Anisomeles indica* (L.) Kuntze	Lamiaceae	July-Aug
27.	*Ocimum basilicum* L.	Lamiaceae	June-Sep
28.	*Volkameria inermis* L.	Lamiaceae	Aug-Jan
28.	*Punica granatum* L.	Lythraceae	Round the year
30.	*Bombax ceiba* L.	Malvaceae	Feb-March
31.	*Grewia asiatica* L.	Malvaceae	April-May
32.	*Azadirachta indica* A. Juss.	Meliaceae	April-May
33.	*Canthium coromandelicum* (Burm f.) Alston	Rubiaceae	June-July
34.	*Catunaregam spinosa* (Thunb.) Tirveng.	Rubiaceae	Round the year
35.	*Citrus aurantiifolia* (Christm.) Swingle	Rutaceaee	Aug-Sep
36.	*Schleichera oleosa* (Lour.) Oken	Sapindaceae	Feb-Aug
37.	*Madhuca longifolia* (L.) J. F. Macbr.	Sapotaceae	April-May
38.	*Verbascum coromandelianum* (Vahl.) Hub.-Mor.	Scrophulariaceae	Mar-Aug
39.	*Solanum anguivi* Lam.	Solanaceae	Aug-Nov
40.	*Tamarix dioica* Roxb. ex Roth	Tamaricaceae	Aug-Dec
41.	*Holoptelea integrifolia* (Roxb.) Planch.	Ulmaceae	Feb-March
42.	*Vahlia digyna* (Retz.) Kuntze	Vahliaceae	Feb-April

fications and 37% to the family level. Only 12% of grains remained unidentified, indicating that vehicular airflow provides an efficient mechanism for trapping airborne pollen with relatively low loss to ambiguity. Dominant taxa included *Ageratum conyzoides, Leucaena leucocephala,* Fabaceae members, and *Ledebouria revoluta*, reflecting exposure to a mosaic of urban, peri-urban, and semi-natural vegetation along road networks. The taxonomic richness and broad ecological representation captured by air filters suggest that vehicular systems act as mobile pollen samplers, integrating palynological signals from a wide spatial range.

Fruit Samples

Fruit surfaces—particularly those of *Punica granatum, Ehretia aspera, and Neltuma juliflora* —retained a distinct pollen signature. Unidentified pollen accounted for 13% of the total assemblage, followed by 42% identified at the species level, 20% at the genus level, and 25% at the

family level. The predominance of *Punica granatum* pollen reflects close contact during harvesting and handling, while the presence of semi-arid indicators such as *Neltuma juliflora* suggests fruit exposure during transportation or open-market storage. Frequent fungal spores further indicate high humidity and organic interaction typical of market environments. These results demonstrate the capability of fruit surfaces to adsorb airborne pollen due to their textured epidermal layers and natural stickiness.

Cloth Samples

Textile material collected from Osmania University retained a substantial and diverse pollen load. The assemblage was dominated by species-level identifications 13%, followed by genus-level 30% and family-level 39% taxa. Unidentified grains constituted only 18%, indicating high diagnostic visibility of pollen on textile fibers. Representative taxa included Asteraceae, *Grewia asiatica*, Poaceae members, *Holoptelea integrifolia*, and *Kalanchoe pinnata*. The fibrous microstructure of the fabric facilitated selective retention of sculptured and sticky pollen types, supporting the role of cloth as a stable short-term reservoir for environmental palynomorphs. Fungal spores were also frequently observed, suggesting exposure to open or vegetated areas.

Hair Samples

Human hair samples from Alampur and Wanaparthy displayed a diverse palynomorph profile, with the highest proportion of family-level identifications 42% among all sample categories. Species-level and genus-level assignments accounted for 5% and 28%, respectively, while unidentified grains were 25%, indicating effective capture and clear visibility of pollen on hair strands. Detected taxa included *Volkameria inermis*, Poaceae, *Azadirachta indica*, *Parthenium hysterophorus*, *Caesalpinia pulcherrima*, and *Terminalia* species. The microstructural properties of hair—such as cuticular scales—promote adherence of fine particles, enabling the retention of pollen reflective of recent environmental exposure. The presence of fungal spores further supports the sensitivity of hair to ambient biological particulates.

DISCUSSION

The baseline palynological data generated from diverse material types—vehicular air filters, fruits, textiles, and human hair—demonstrate the strong capacity of these substrates to accumulate and retain airborne pollen and spores. Although not derived from forensic casework, the patterns observed offer valuable insights into how such materials may function as passive collectors of environmental particulates, with implications for future forensic applications[20,21]. The distinct pollen assemblages associated with each material type reflect differences in exposure duration, mobility, and environmental context, providing foundational evidence for constructing regional pollen reference frameworks.

Air filters exhibited the highest taxonomic diversity, underscoring the efficiency of vehicular airflow in trapping airborne palynomorphs. Dominant taxa such as *Ageratum conyzoides*, *Leucaena leucocephala*, and members of Fabaceae indicate exposure to disturbed urban margins, roadside vegetation, and mixed rural

habitats. The presence of *Ledebouria revoluta*, a taxon typical of semi-natural landscapes, further suggests that vehicular filters integrate pollen signatures accumulated across broad spatial gradients. Such baseline data reinforce existing literature demonstrating the value of mechanical air-intake systems as mobile pollen samplers capable of approximating routes traversed by vehicles[20].

Fruit surfaces also retained characteristic pollen types, with *Punica granatum*—a cultivated orchard species—appearing as a dominant taxon. Concurrent detection of *Neltuma juliflora*, common in semi-arid and degraded terrains, indicates exposure of fruits during harvesting, handling, or transit through open markets and arid landscapes. These findings highlight how pollen on perishable goods can serve as indicators of geographic origin or environmental handling conditions, a concept previously emphasized in provenance studies and agri-trade verification research.

Textile materials demonstrated strong pollen adherence, consistent with previous reports that fibrous matrices trap and preserve pollen even after washing or prolonged handling[22]. The representation of Asteraceae and Poaceae suggests general outdoor exposure, whereas taxa such as *Grewia asiatica* and *Holoptelea integrifolia* point toward contact with tropical and subtropical vegetation typical of the study region. These results support the potential of clothing as a sensitive substrate for capturing fine-scale environmental information, with implications for reconstructing human movement histories in future forensic settings.

Hair samples also retained diverse pollen assemblages, albeit with a comparatively higher proportion of unidentified grains. The detection of *Azadirachta indica, Parthenium hysterophorus,* and Poaceae signifies exposure to urban and peri-urban vegetation, while the occurrence of other taxa suggests interactions with locally abundant flora. Although the high percentage of unassigned pollen types emphasizes the need for more comprehensive regional palynological reference collections, the results align with previous studies demonstrating hair as a reliable short-term environmental sensor[23].

Taken together, these baseline findings confirm that different substrates capture complementary palynological signatures reflective of their environmental exposure histories. Air filters and textiles exhibit high taxonomic richness and can integrate exposure across broader spatio-temporal scales, whereas fruits and hair provide more immediate, context-dependent pollen assemblages. The recovery of fungal spores across all sample types further contributes to environmental interpretation by indicating humidity levels and biological activity within the sampled surroundings. Although this study does not involve forensic case analysis, the results underscore the potential of combining palynological evidence with other trace materials—such as soil, fibers, and particulate residues—to enhance environmental reconstruction in forensic applications. For India, where forensic palynology remains nascent, the development of standardized protocols, multidisciplinary collaborations, and extensive regional pollen reference libraries will be essential for translating baseline research into operational forensic tools.

CONCLUSION

This baseline investigation demonstrates that commonly encountered substrates—vehicular air filters, fruits, textiles, and human hair—serve as effective passive collectors of airborne pollen and spores, each preserving distinct environmental signatures reflective of their exposure pathways. Across all material types, the palynomorph assemblages show clear relationships with the vegetation and landscapes characteristic of the sampling regions in Telangana, offering strong potential for future forensic and environmental applications.

Air filter samples exhibited high pollen diversity with dominant representation of *Ageratum conyzoides*, Fabaceae members, *Leucaena leucocephala*, and *Ledebouria revoluta*. These taxa are abundant along urban margins, village roads, and mixed agricultural–semi-natural corridors typical of Karimnagar and Wanaparthy. Their presence confirms that vehicular movement captures a composite record of urban/rural vegetation, supporting the use of air-intake systems as reliable samplers for reconstructing route traversal or vehicle-associated environmental history. Fruit samples, primarily sourced from Hyderabad markets, showed strong representation of *Punica granatum, Ehretia aspera,* and *Neltuma juliflora*. These pollen types are characteristic of orchard landscapes, semi-arid scrub vegetation, and open trading environments, respectively. Their combined occurrence indicates exposure during harvesting, transportation, and market handling, suggesting that fruit surfaces can provide meaningful information regarding the provenance of agricultural produce or the environmental context of commodity movement. Cloth samples collected from Osmania University exhibited high proportions of Asteraceae, Poaceae, *Grewia siatica*, and *Holoptelea integrifolia*, taxa widely distributed in tropical and subtropical urban green spaces. This pattern aligns with the university's open fields, roadside vegetation, and landscaped gardens, confirming that textiles effectively retain pollen associated with field activity, campus movement, and general outdoor exposure. Hair samples from Alampur and Wanaparthy retained pollen from Poaceae, *Azadirachta indica, Parthenium hysterophorus,* and *Terminalia* species. These reflect environments ranging from roadside vegetation and open scrublands to forest–field ecotones, all of which are characteristic of these locations. The combination of these taxa reinforces the sensitivity of human hair as a short-term collector of local environmental contact, capable of capturing temporal exposure records relevant for reconstructing human movement or location history.

Collectively, the results demonstrate that each substrate carries a unique yet complementary pollen signature reflecting its interaction with local landscapes. While this study does not involve forensic casework, the patterns observed highlight how palynological evidence can support geographical attribution, environmental reconstruction, and contextual interpretation in future forensic investigations. To advance the operational use of forensic palynology in India, the development of region-specific pollen reference databases, standardized analytical protocols, and stronger interdisciplinary collaboration will be essential. As environmental "fingerprints," pollen assemblages hold exceptional promise as a supplementary tool for modern forensic science and environmental intelligence

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Research Article

ASSESSMENT OF SOME MULTI-DRUG-RESISTANT BACTERIA FROM HOSPITALS AND VETERINARY SITES OF RAJNANDGAON, CENTRAL INDIA

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The overuse of antibiotics during and after Covid-19 pandemic has dramatically increased the problem of resistant bacteria, which created a global health challenge. Apart from the pandemic, climate change, weakened immunity, and pollution are some other reasons for the use of antibiotics by people around the globe. Combating antibacterial resistance is a difficult task for the scientific community. Identifying resistant bacteria from the environment is preliminary work toward the control of this health risk. The sites where antibiotics are frequently used for therapeutic purposes are a major source of these bacteria; therefore, the present study aims to test the above hypothesis. Airborne bacteria have been sampled, and pure cultures are utilized for the assessment of antibiotic resistance in the agar plate method. The identification of resistant bacteria has been done by the sequence of their 16S ribosomal RNA. The plasmid of resistant bacteria has been extracted and analyzed with agarose gel as a confirmation of the presence of the drug resistance gene in the plasmid DNA. The study concluded that the antibiotic-resistant bacteria are present in the area where it utilizes. We recommend analyzing public areas like residential colonies, food zones, educational institutes, and public and private offices for the presence of bacteria.

Key Words: Antibiotic disc; Bacterial Plasmid; Covid-19 pandemic; Hospital born flora; *Enterococcus faecalis*.

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INTRODUCTION

In 2011, the World Health Organization warned that without urgent action to combat antimicrobial resistance, the world could enter a "POST-ANTIBIOTIC ERA" in which common infections would no longer be curable[1]. Presently, antimicrobial resistance (AMR) poses a major threat to case treatment as it leads to increased morbidity and mortality, increased hospital stay, and severe economic loss to the case and nation[2]. The clinical isolates similar as *Pseudomonas*

aeruginosa, Methicillin Resistant *Staphylococcus aureus* (MRSA), Enterococci, especially Vancomycin Resistant *Enterococci* (VRE), and members of the Family Enterobacteriaceae, for illustration, *Klebsiella pneumoniae, E. coli*, and *Proteus* sp., quickly develop antibiotic resistance and spread in the hospital environment. Multidrug resistance (MDR) was defined as acquired nonsusceptibility to at least one agent in three or more antimicrobial orders[3].

Antibiotics frequently act by inhibiting the conformation of a bacterial cell, conformation of proteins, DNA, RNA, by a membrane-disorganizing agent, or by other specific conduct[4]. Antibiotics may also enter the cell wall of the bacteria by binding to them, using the energy-dependent transport mechanisms in ribosomal spots, which latterly leads to the inhibition of the protein conflation[5]. Increased demand for antibiotics across numerous sectors has allowed for less precious and off-label uses of medicines. Again, the enormous and reckless use of antibiotics has contributed significantly to the emergence of the resistant strains[6]. In the former days, the product of new antibiotics was directly commensurable to the development of resistant strains. Bacteria come 'resistant' and continue to multiply in the presence of remedial situations of the antibiotics. Bacteria, when replicates indeed in the presence of the antibiotics, are called resistant. In its wake, the U.S. National Strategy for Combating Antibiotic-Resistant Bacteria. The 2019 description assessed that at least 2 million people were sick with antibiotic-resistant microbes each year in the United States and that at least 23,000 persons died as a result[7].

There's presently no approved pharmaceutical product for the treatment of COVID-19. Still, antibiotics are presently being used for the treatment of COVID-19 cases in numerous settings, either to treat coinfections or for the treatment of COVID-19 itself. In this commentary, we point out that the increased rates of antimicrobial prescribing for COVID-19 cases could further worsen the burden of antimicrobial resistance[8]. Admission and release of cases from tertiary hospitals have risen dramatically in the once time due to the COVID-19 epidemic. Because the operation of COVID-19 is increasingly getting dependent on pharmacological interventions, there's a lesser threat for accelerating the elaboration and spread of antimicrobial resistance. The use of anti-parasite, antiviral, antibacterial, and anti-inflammatory medicines for preventing secondary infections in cases with COVID-19 during a prolonged epidemic will inescapably invite unborn complications, including aggravation of antimicrobial resistance. Numerous fresh pieces of information have defined an increase in multidrug-resistant organisms (MDROs) through the COVID-19 prevalent[9-11]. A review study set up that the occurrence of carbapenem-resistant Enterobacterales colonisation increased from 6.7 in 2019 to 50 in March – April 2020[12]. The initial reasons included the high intensity of care needed for cases.

Antibiotic resistance in the health practice institution is a serious public issue because health workers may affect direct or indirectly. If a similar bacterial pathogen transmits from the health practice area to the domestic and marketable area, it'll be more dangerous and delicate to control. Thus, the present study aims to find

the presence of medicine-resistant bacteria in the health practice institution of Rajnandgaon city. To achieve this end following objects have been taken: sample collection from mortal and veterinary health practice spots; isolation of bacteria from collected samples; morphological and Molecular Identification of dominant Bacteria; and antibiotic resistance test.

MATERIALS AND METHODS

Study Area

The literature reviewed suggests the circumstance of multi-resistant bacteria may be observed in the medicine/drug exercising spots like hospitals, individual centres, flesh husbandry point, old age homes, veterinary and parentage spots etc.[13-14]. Thus, an airborne sample has been collected from hospitals and flesh husbandry point (Table 9.1, Figure 9.1). An indigenous or public position veterinary centre isn't available in Rajnandgaon, and no application of medicine (antibiotics and other) in individual centres is a reason for the rejection of these centres from our study point.

Sample collection and isolation of drug-resistant bacteria

The bacteria have been isolated from an air sample. The graveness plate system has been applied to isolate bacteria. Nutrient agar media (NAM) will be used to isolate and perform other trials related to the culture of bacteria. The shielded bacteria were protected in an NAM slant in a freezer (4 degree lecherous) till for the practice. The system is based on a minor revision of the original[15].

Identification of Bacterial Isolates

The isolated bacteria have been undergoing a microbial test for their shape, Gram staining, Motility, and endospore-forming property. After that, biochemical tests were performed in the laboratory such as an H_2S production test, gelatine liquefaction test, amylase test, MR/VP test, and catalase for observation of biochemical properties of bacteria[16].

Some selected isolates were identified at the sequencing facility of the NCMR, National Centre for Cell Science, Pune, (Maharashtra), India, for authentication[17], followed by PCR amplification of the 16S rRNA gene using universal primers 16F27 [5'-CCA GAG TTT GAT CMT GGC TCA G-3'] and 16R1492 [5'-TAC GGY TAC CTT GTT ACG ACT T-3']. The amplified 16S rRNA gene PCR product was cleansed by the precipitation method and sequenced on DNA sequencer. Assembly was carried out using the Lasergene package,

Table 9.1: Sample collection sites

Sl. No.	Location type	Site	Geographical Location
1.	Human	Site A - District Hospital Basantpur, Rajnandgaon	21°05'10.5"N 81°01'47.9"E
		Site B - Medical College Pendri, Rajnandgaon	21°06'07.3"N 80°59'53.7"E
2.	Poultry	Site C - IB Group Poultry Farm	21°05'44.2"N 80°57'18.6"E



A a

B b

C c

Fig. 9.1: A, B, and C Map of the sample collection site, a, b, and c real image of sites

followed by identification using the EzBio-Cloud database[18].

Finding of antimicrobial-resistant bacteria

The preserved bacteria isolated from various sources will undergo testing for their ability to resist single or multiple drugs. For this objective NAM agar plate has been used to grow the test bacteria at 37 degrees Salacious for 24 two 48 hours. After the growth of bacteria multidrug disc (DE-008, HiMedia made) was applied to the surface of the media having bacterial colony. Discs with different antibiotics are presented in the Table No. 9.2. After a further

Table 9.2: An antibiotic disc utilized for the drug resistance test			
Sl. No.	**Antibiotic**	**Code**	**Amount in the disc**
1.	**Ampicillin**	**AMP**	**10 µg**
2.	**Cefuroxime**	**CXM**	**30 µg**
3.	**Cefadroxil**	**CFR**	**30 µg**
4.	**Augmentin**	**AMC**	**30 µg**
5.	**Penicillin**	**P**	**10 units**
6.	**Cefotaxime**	**CTX**	**30 µg**
7.	**Cefaclor**	**CF**	**30 µg**
8.	**Azithromycin**	**AZM**	**15 µg**
9.	**Erythromycin**	**E**	**15 µg**
10.	**Cefoperazone**	**CPZ**	**75 µg**
11.	**Clarithromycin**	**CLR**	**15 µg**
12.	**Ciprofloxacin**	**CIP**	**5 µg**

24-48 hrs. The agar plate has been examined for clear vision ability in the zone around the antibiotic disc. The zonal scale was used to record the zone of inhibition in mm. A lower or no zone around the antibiotic disc confirms the resistance of bacteria to the particular antibiotic[19].

Isolation of plasmid DNA from bacteria

Plasmid DNA of bacteria is isolated using HiMedia HiPer® Plasmid DNA Extraction Teaching Kit (Product Code HTBM003). The DNA extraction concept involves disrupting the cell wall and releasing DNA, proteins, and other components. The bacterial solution is first subjected to a powerful anionic detergent (SDS) at a high pH, and additional content is denatured. The plasmid's highly supercoiled confirmation prevents full strand disruption throughout the procedure. The isolated DNA was separated using 0.8% agarose gel powered by an electric field of 100–120 volts and 90 mA until dye markers had migrated an appropriate distance. The DNA was further visualized by UV Trans-illuminator[20].

RESULTS

Bacterial isolates

Possible drug-resistant bacteria have been isolated from three locations as mentioned in the methodology section, viz. Basantpur district hospital, Pendri Govt. Medical college and IB group poultry farm. Hospitals and colleges are

related to the use of drugs in human health practice, while the third site is related to the use of drugs in poultry. A total of 19 bacterial colonies have been isolated from Basantpur district hospital; 25 colonies from Pendri Govt. Medical college and 18 colonies isolated from the IB group poultry farm.

General characteristics of isolated bacteria

Out of which 04-04 bacterial colonies (Figures 9.2, 9.3 and 9.4) have been selected for morphological and microscopic examination on the basis of their frequent observation in nutrient media. Bacterial shape, Gram-negative cell wall, pathogenicity, formation of endospore, and presence of flagella were investigated using laboratory experiments. Table 9.3 reports the outcome of morphological examination.

Identification of bacteria

Based on morphological characteristics, further 01-01 bacteria have been selected from each location for their molecular identification and antimicrobial resistance test. Bacteria have been selected on the basis of pathogenicity, endospore formation, and motility tests. PCR amplification of the 16S rRNA gene followed by sequencing of the amplified region gives rise to a 1521bp amplicon for BP HO3 isolates; 1466bp for P HO1 and 1145bp for P O2. Basic local alignment search of the above DNA sequences confirmed the bacterial strain, viz. *Enterococcus faecalis, Clostridioides difficile*, and *Campylobacter jejuni*.

Table 9.3: General characteristics of isolated bacteria

Sl. No.	Bacteria code	Shape	Gram	Pathogenicity (acid-fast stain)	Endospore formation	Motility
Site A						
1.	BP HO1	Cocci	Negative	Negative	Negative	Negative
2.	BP HO2	Cocci	Positive	Negative	Negative	Negative
3.	**BP HO3**	**Cocci**	**Positive**	**Negative**	**Negative**	**Negative**
4.	BP HO4	Cocci	Negative	Negative	Negative	Negative
Site B						
1.	**P HO1**	**Rod**	**Positive**	**Negative**	**Positive**	**Positive**
2.	P HO2	Rod	Negative	Negative	Positive	Positive
3.	P HO3	Cocci	Positive	Negative	Negative	Negative
4.	P HO4	Cocci	Positive	Negative	Negative	Negative
Site C						
1.	P O1	Rod	Positive	Negative	Positive	Positive
2.	**P O2**	**Spiral Rod**	**Negative**	**Negative**	**Negative**	**positive**
3.	P O3	Rod	Negative	Negative	Positive	Negative
4.	P O4	Cocci	Negative	Negative	Negative	Negative

The strain used for further study is highlighted in bold

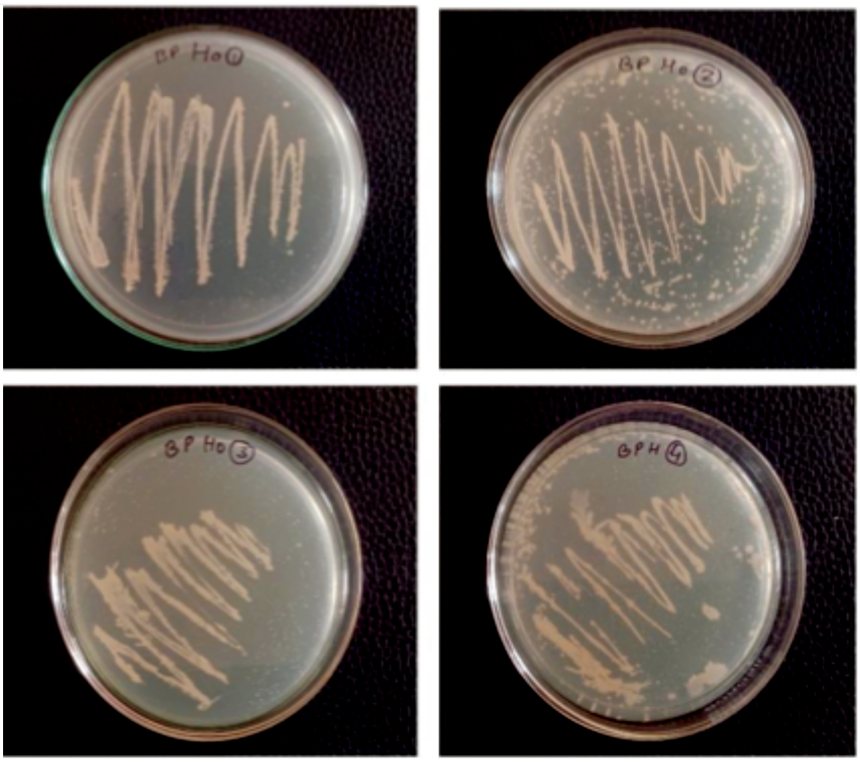


Fig. 9.2: Bacterial isolates from Basantpur district hospital (BP Ho 01 to 04)

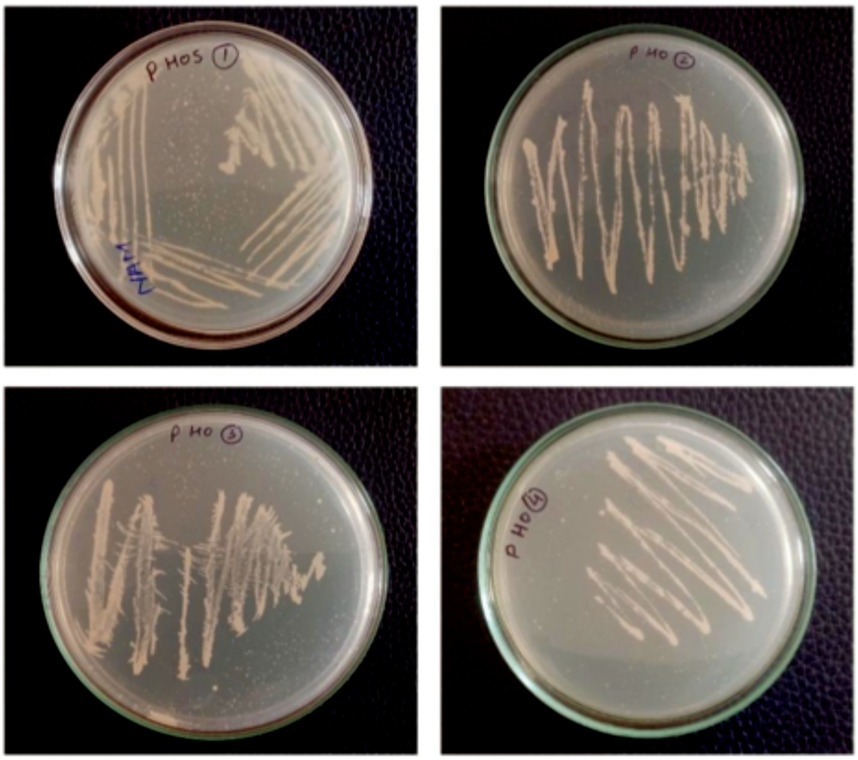


Fig. 9.3: Bacterial isolates from Medical College Pendri (P Ho 01 to 04)

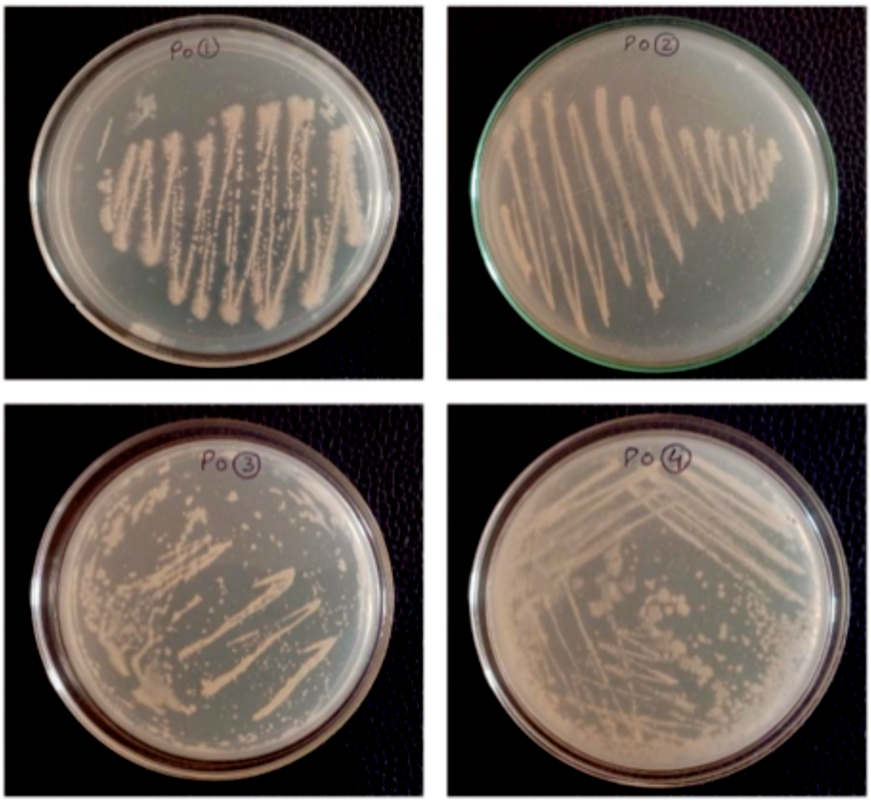


Fig. 9.4: Bacterial isolates from IB group poultry farm (Po 01-04)

Molecular Identification

Selected three bacterial have been subjected to PCR amplification of the 16S rRNA gene, give rise following amplicon:

Enterococcus faecalis – 1521bp

GAGTTTGATCCTGGCTCAGGACGAACGCTGGCGGCGTGCCTAATACATGCAAGTC-
GAACGCTTCTTTCCTCCCGAGTGCTTGCACTCAATTGGAAAGAGGAGTGGCG-
GACGGGTGAGTAACACGTGGGTAACCTACCCATCAGAGGGGGATAACACTTGGAAAC
AGGTGCTAATACCGCATAACAGTTTATGCCGCATGGCATAAGAGTGAAAG-
GCGCTTTCGGGTGTCGCTGATGGATGGACCCGCGGTGCATTAGCTAGTTGGTGAG-
GTAACGGCTCACCAAGGCCACGATGCATAGCCGACCTGAGAGGGTGATCGGCCACACT
GGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCG-
GCAATGGACGAAAGTCTGACCGAGCAACGCCGCGTGAGTGAAGAAGGTTTTCG-
GATCGTAAAACTCTGTTGTTAGAGAAGAACAAGGACGTTAGTAACTGAACGTCCCCTG
ACGGTATCTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACG-
TAGGTGGCAAGCGTTGTCCGGATTTATTGGGCGTAAAGCGAGCGCAGGCGGTTTCT-
TAAGTCTGATGTGAAAGCCCCCGGCTCAACCGGGGAGGGTCATTGGAAACTGGGAGA
CTTGAGTGCAGAAGAGGAGAGTGGAATTCCATGTGTAGCGGTGAAATGCGTA-

GATATATGGAGGAACACCAGTGGCGAAGGCGGCTCTCTGGTCTGTAACTGACGCT-
GAGGCTCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCG-
TAAACGATGAGTGCTAAGTGTTGGAGGGTTTCCGCCCTTCAGTGCTGCAGCAAACGCA
TTAAGCACTCCGCCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAG-
GAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGC-
GAAGAACCTTACCAGGTCTTGACATCCTTTGACCACTCTAGAGATAGAGCTTTCCCTTC
GGGGACAAAGTGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGAT-
GTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATTGTTAGTTGCCATCATT-
TAGTTGGGCACTCTAGCGAGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACG
TCAAATCATCATGCCCCTTATGACCTGGGCTACACACGTGCTACAATGGGAAGTA-
CAACGAGTCGCTAGACCGCGAGGTCATGCAAATCTCTTAAAGCTTCTCTCAGTTCG-
GATTGCAGGCTGCAACTCGCCTGCATGAAGCCGGAATCGCTAGTAATCGCGGATCAGC
ACGCCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCACGA-
GAGTTTGTAACACCCGAAGTCGGTGAGGTAACCTTTTTGGAGCCAGCCGCCTAAG-
GTGGGATAGATGATTGGGGTGAAGTCGTAACAAGGTAGCC

Clostridioides difficile – 1466bp

ATCATKGCTCABGATGAACGCTGGCGGCGTGCCTAACACATGCAAGTTGAGCGATT-
TACTTCGGTAAAGAGCGGCGGACGGGTGAGTAACGCGTGGGTAACCTACCCTGTACA-
CACGGATAACATACCGAAAGGTATGCTAATACGGGATAATATATTTGAGAGGCATCTCTT
GAATATCAAAGGTGAGCCAGTACAGGATGGACCCGCGTCTGATTAGCTAGTTGGTAAG-
GTAACGGCTTACCAAGGCGACGATCAGTAGCCGACCTGAGAGGGTGATCGGCCA-
CATTGGAACTGAGACACGGTCCAAACTCTACGGGAGGCAGCAGTGGGGAATATTGCA
CAATGGGCGAAAGCCTGATGCAGCAACGCCGCGTGAGTGATGAAGGCCTTCGGGTCG-
TAAAACTCTGTCCTCAAGGAAGATAATGACGGTACTTGAGGAGGAAGCCCCGGC-
TAACTACGTGCCANCAGCCGCGGTAATACGTAGGGGGCTAGCGTTATCCGGATTTACTG
GGCGTAAAGGGTGCGTAGGCGGTCTTTCAAGTCAGGAGTGAAAGGCTACGGCTCAAC-
CGTAGTAAGCTCTTGAAACTGGGAGACTTGAGTGCAGGAGAGGAGAGTGGAATTCC-
TAGTGTAGCGGTGAAATGCGTAGATATTAGGAGGAACACCAGTTGCGAAGGCGGCTCT
CTGGACTGTAACTGACGCTGAGGCACGAAAGCGTGGGGAGCAAACAGGATTAGATAC-
CCTGGTAGTCCACGCTGTAAACGATGAGTACTAGGTGTCGGGGGTTACCCCTTCGGT-
GCCGCACTAACGCATTAAGTACTCCGCCTGGGAAGTACGCTCGCAAGAGTGAAACTCA
AAGGAATTGACGGGGACCCGCACAAGTAGCGGAGCATGTGGTTTAATTCGAAG-
CAACGCGAAGAACCTTACCTAAGCTTGACATCCCAATGACATCTCCTTAATCGGA-
GAGTTCCCTTCGGGGACATTGGTGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCG
TGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGTCTTTAGTTGCCATCAT-
TAAGTTGGGCACTCTAGAGAGACTGCCAGGGATAACCTGGAGGAAGGTGGGGAT-
GACGTCAATCATCATGCCCCTTATGCTTAGGGCTACACACGTGCTACAATGGGTAGTAC

AGAGGGTTGCCAAGCCGTAAGGTGGAGCTAATCCCTTAAAGCTACTCTCAGTTCG-
GATTGTAGGCTGAAACTCGCCTACATGAAGCTGGAGTTACTANTAATCGCAAT-
CAAAATGCTGCGGTGAATGCGTTCCCGGGGTCTTNTACACACCGCCCGTCACACCACG
GGAGTTGGAAACGCCCGAAGCCGAATTATCTAACCTTTTGGAANAANTCSTC-
GAAAGTGGAATCAATAACTTGGGTNAANTCGTAACAAGGTAACCGTATCGGAAGGT

Campylobacter jejuni – 1145bp

GATGACACTTTCGGAGGCGGTAACTCCTTTTCTAGGAAGAATCGACGTACTAGGATAG-
CACGCTACTCGTGCAGCAGCCGCGGTAATACGGAGGGTGCAAGCGTACTCGGAAT-
CACTGGGCGTAAAGGGCGCGTAGGCGATTATCAAGTCTCTGTGAAATCTAATGGCTTAA
CATAAACTGCTTGGGAAACTGATAGTCTAGAGTGAGGGAGAGGCAGATGGAATTG-
GTGGTGTAGGGGTAAAATCCGTAGATATCACCAAGAATACCCATTGCGAAGGCGATCT-
GCTGGAACTCAACTGACGCTAAGGCGCGAAAGCGTGGGGAGCAAACAGGATTAGATA
CCCTGGTAGTCCACGCCCTAAACGATGTACACTAGTTGTTGGGGTGCTAGT-
CATCTCAGTAATGCAGCTAACGCATTAAGTGTACCGCCTGGGGAGTACGGTCGCAA-
GATTAAAACTCAAAGGAATAGACGGGGACCCGCACAAGCGGTGGAGCATGTGGTTTA
ATTCGAAGATACGCGAAGAACCTTACCTGGGCTTGATATCCTAAGAACCTTTTAGA-
GATAAGAGGGTGCTAGCTTGCTAGAACTTAGAGACAGGTGCTGCACGGCT-
GTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCACGT
ATTTAGTTGCTAACGGTTCGGCCGAGCACTCTAAATAGACTGCCTTCGTAAGGAGGAG-
GAAGGTGTGGACGACGTCAAGTCATCATGGCCCTTATGCCCAGGGCGACACACGTGC-
TACAATGGCATATACAATGAGACGCAATACCGCGAGGTGGAGCAAATCTATAAAATATG
TCCCAGTTCGGATTGTTCTCTGCAACTCGAGAGCATGAAGCCGGAATCGCTAG-
TAATCGTAGATCAGCCATGCTACGGTGAATACGTTCCCGGGTCTTGTACTCACCGCC-
CGTCACACCATGGGAGTTGATTTCACTCGAAGCCGGAATACTAAACTAGTTACCGTCCA
CAGTGGAATCAGCGACTGGGGTGAAGTCGTAACAAGGTAGCCGTAGAAGGGC-
GAATTCTGCAGATATCCATCACACTGGCGGCCGCTCGAGCATGCATCTAGAGGGCC-
CAATCGCCCTATAGAGTCTTACCC

Table 9.4: Antibiotic resistance of some bacteria

Sl. No.	Antibiotic	Code	EF	CD	CJ
1.	Ampicillin	AMP	Sensitive	**Resistance**	Sensitive
2.	Cefuroxime	CXM	**Resistance**	**Resistance**	**Resistance**
3.	Cefadroxil	CFR	Sensitive	Sensitive	Sensitive
4.	Augmentin	AMC	**Resistance**	Sensitive	**Resistance**
5.	Penicillin	P	**Resistance**	Sensitive	**Resistance**

Contd.

Contd. Table 9.4

Sl. No.	Antibiotic	Code	EF	CD	CJ
6.	Cefotaxime	CTX	Sensitive	Sensitive	Sensitive
7.	Cefaclor	CF	**Resistance**	Sensitive	**Resistance**
8.	Azithromycin	AZM	Sensitive	Sensitive	Sensitive
9.	Erythromycin	E	Sensitive	Sensitive	Sensitive
10.	Cefoperazone	CPZ	Sensitive	**Resistance**	**Resistance**
11.	Clarithromycin	CLR	Sensitive	Sensitive	Sensitive
12.	Ciprofloxacin	CIP	Sensitive	Sensitive	Sensitive

EF - *Enterococcus faecalis;* CD - *Clostridioides difficile;* CJ - *Campylobacter jejuni*

Antibiotic resistance

Antibiotic resistance of *Enterococcus faecalis* (EF), *Clostridioides difficile* (CD), and *Campylobacter jejuni* (CJ) has been tested against some antibiotics using the HiMedia DE008 kit. Based on the agar plate growth of bacteria, the above disc clearly provides the information on bacterial resistance over particular antibiotic. *Enterococcus faecalis* is resistant to Cefuroxime, Augmentin, Penicillin, and Cefaclor. *Clostridioides difficile* is resistant against Ampicillin, Cefuroxime, and Cefoperazone, while *Campylobacter jejuni* has been recorded as resistant against Cefuroxime, Augmentin, Penicillin, Cefaclor, and Cefoperazone. Bacterial resistance indicates growth of microbial colonies over the

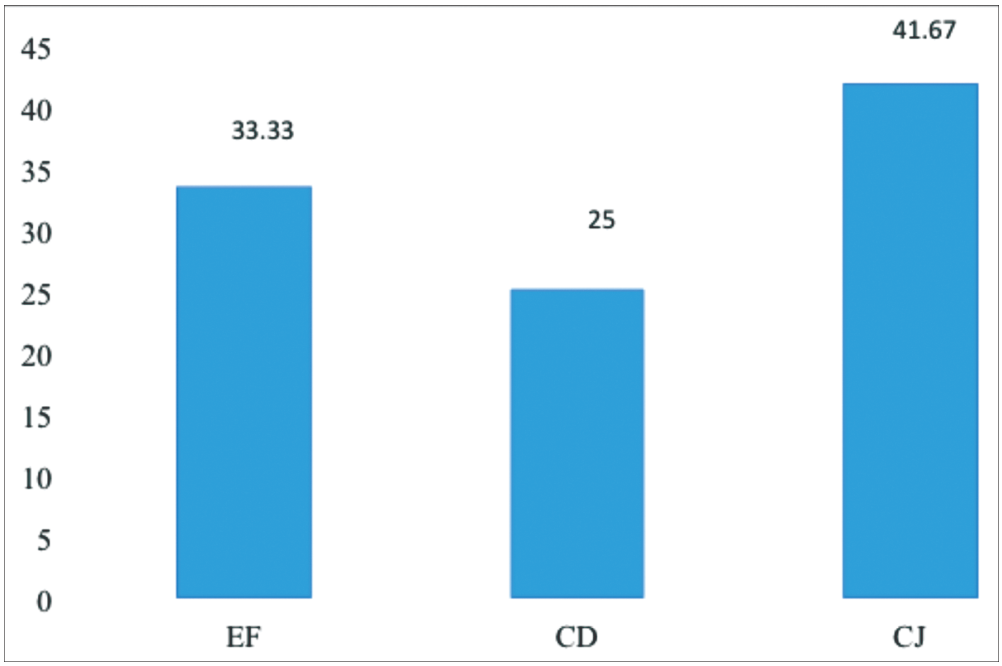
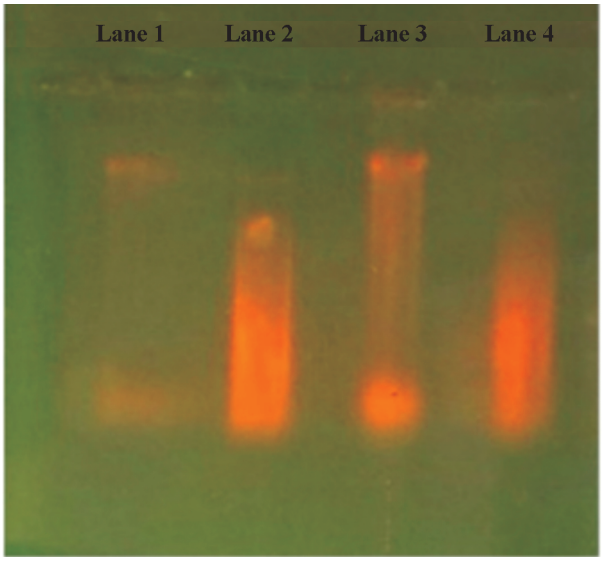


Fig. 9.5: The percentage of antibiotics for which different bacteria have shown microbial resistance

antibiotic disc, and sensitivity indicates no growth zone around the antibiotic disc. Table 9.4 represents the resistance and sensitivity of test organisms against twelve antibiotic discs. Figure 9.5 represents the percentage of bacterial resistance.

Plasmid DNA of Antibiotic resistance bacteria

Antibiotic resistance is generally a characteristic of any bacteria expressed by the gene located in plasmid DNA. Plasmid DNA of *Enterococcus faecalis, Clostridioides difficile*, and *Campylobacter jejuni* has been isolated and separated by gel electrophoresis. Figure 9.6 is showing plasmid DNA of all the above bacteria confirm the presence of plasmid and the possible presence of antibiotic resistance gene.



Plasmid DNA isolated from antibiotic resistant bacterias. Lane 1 – Ladder DNA, Lane 2 – Enterococcus faecalis, Lane 3 – Clostridioides difficile, Lane 4 – Campylobacter jejuni.

Fig. 9.6: Plasmid DNA of antibiotic-resistant bacteria, along with some impurities of RNAs and Proteins

DISCUSSION

Microbial antibiotic resistance is a serious public health issue because, after its resistance, it is not possible to control that organism until a new drug discovery. Antibiotic resistance is not new. Penicillin resistance in *Staphylococcus* became a global public pandemic in the 1940s after its invention in 1928 by Alexander Fleming. Soon, a new drug, Methicillin, was discovered to combat Penicillin-resistant *Staphylococcus* in 1959, which was again resistant to bacteria in just one year. The story continuous many antibiotics being discovered and becoming resistant to so many pathogenic microorganisms. The modern health system emerges, and traditional healing, ayurveda, siddha, Unani, homeopathy, like treatment systems, decline, leading to a large number of antibiotic resistances due to the use of antibiotics in the 1980s and 1990s. Recent Covid-19 pandemic led to the use of a huge number of antibiotics, expected again so many more antibiotic-resistant microorganisms. The present study, therefore, reports a post-pandemic investigation of antibiotic-resistant bacteria in the specific area where antibiotics are utilized for the treatment of humans and poultry. Isolated bacteria from sample sites were found to have antibiotic resistance, confirmed with the microbiological laboratory investigation, as well as molecular study of the presence of a plasmid that generally contains resistance genes. Hospitals and poultry sites have been previously reported as a major source of antibiotic-resistant bacteria. *Enterococcus faecalis, Clostridioides difficile*, and *Campylobacter jejuni* have also reported as antibiotic-resistant bacteria, according to the data of the present investigation. Similarly, plasmid DNA and antibiotic resistance genes have been investigated in isolated bacteria previously.

Several studies have been conducted to find out drug-resistant bacteria from the hospital environment, and the outcome of previous studies supports the findings of the present investigation. *Campylobacter* spp. and *Staphylococcus* spp. obtained from the airborne particulate matter of the hospital environment (China) by Zhou *et al*.[21]. Antibiotic resistance genes such as fluoroquinolone (vatC-02, mexD) and β-lactams (blaZ, mecA) have been recorded in both organisms. Zahlane *et al*.[22] have determined clinical risk factors, epidemiology, and the causative agents of multidrug-resistant bacteria in the ICU of the University Hospital in Marrakesh, Morocco. Valuation of bacterial infection and their drug resistance in the air of different areas of selected teaching clinics has been studied by Montazer *et al*.[23]. *Staphylococcus aureus* and *Escherichia coli* have been found resistant against several types of antibiotics such as Oxacillin, Ceftazidime, Imipenem, and Sulfamethoxazole. *Acinetobacter* spp, *Acinetobacter baumannii*, and *Staphylococcus* spp has been reported as the most predominant β-lactam-resistant bacteria from hospitals of Isfahan University of Medical Sciences, Isfahan, Iran, by Mirhoseini *et al*.[24]. *Stenotrophomonas maltophilia* and *Enterobacter cloacae* have been isolated from the air sample of hospital environment in Canada[25]. Erythromycin resistance genes were detected in all air samples collected, whereas tetracycline resistance genes were also reported. Fahim[26] has determined the prevalence of multidrug-resistant organisms in the environment of Ain Shams University Hospitals (ASUHs), Egypt, followed by the study of the resistance profile of the common pathogens. The most commonly isolated pathogens were *Klebsiella* spp. (22.5%), *Escherichia coli* (13.4%), and Coagulase-negative *Staphylococci* (12.5%).

Similarly, various findings also reported for the presence of drug-resistant bacteria from veterinary sites. *Aerococcus viridans, Corynebacterium xerosis*, and *Acinetobacter lwoffii* have been isolated from the dairy farms located in Shandong Province, China by Cui *et al*.[27]. These bacteria were found to be resistant against lincomycin and sulfadiazine. Multi-drug resistant *Escherichia coli* has been isolated from the animal farm of Zhanjiang, China by Peng *et al*.[28]. *Escherichia coli* was observed to be resistant against amoxicillin, colistin, doxycycline, and oxytetracycline. Multidrug-resistant *Escherichia coli* has been investigated from the Egyptian poultry, dairy, and meat farms by Samy *et al*.[29]. The bacteria were found resistant against amoxicillin and oxytetracycline. Multi-drug resistant *E. coli* was isolated from nine commercial broiler chicken farms located in the Fraser Valley of British Columbia, Canada, by Furtula *et al*.[30]. The bacteria have been found to be resistant against β-lactam antibiotics (amoxicillin, ceftiofur), tetracyclines, and sulfonamides.

The present study therefore represents a modest contribution toward understanding antibiotic resistance in society. While it provides valuable insights, it also highlights that much broader and more extensive investigations are still required to explore the issue in greater depth.

CONCLUSIONS, LIMITATIONS, AND PROSPECTS

The present study concluded that several bacteria have been isolated from hospital and poultry

sites; the isolated bacteria showed antibiotic resistance in the plate assay; ribosomal RNA sequencing reveals the presence of *Enterococcus faecalis, Clostridioides difficile*, and *Campylobacter jejuni* bacteria from the study area. The plasmid of the above bacteria confirmed the presence of gene-resistant DNA in bacteria. The study is limited to shorten area of study with a few sampling sites. Only selected bacteria have been chosen for the identification and resistance study. In the future, we are aiming to isolate bacteria from residential, commercial, educational institutions, and restaurants to get information about the penetration and spreading of antibiotic-resistant bacteria in other parts of society. The upcoming study also aims to get the antibiotic resistance gene from an isolated plasmid as a more precise confirmation of bacterial characteristics. Will also target to use some medicinal plant extracts and phytochemical compounds for breaking antibiotic resistance in bacteria.

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