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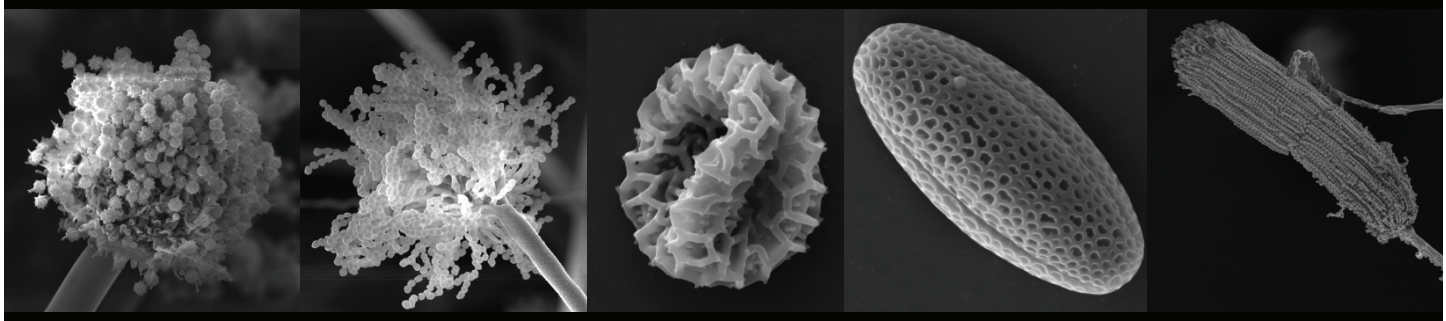

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Contents

POLLEN ANALYSIS OF UNIFLORAL HONEYS FROM BHUPALPALLY DISTRICT, TELANGANA STATE, INDIA WITH REFERENCE TO PHYSICAL PROPERTIES AND HEAVY METALS

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Ten honey samples produced by the *Apis florea* and *Apis dorsata* collected from different mandals of Bhupalpally district, Telangana state, India were examined for their botanical sources, physical properties, and heavy metal contents. The honey samples were acetolyzed and identified microscopically as unifloral. Predominant pollen types found in the samples were *Gardenia lucida* (72%), *Eucalyptus globulus* (84%), *Sphaeranthus indicus* (73%), *Leucaena leucocephala* (78.8%), and *Prosopis juliflora* (74%) from *Apis florea* honey samples, whereas *Aspidopterys indica* (62%), *Capsicum frutescens* (80%), *Legerstroemia parviflora* (70%), *Ocimum tenuiflorum* (45%) and *Zea mays* (48%) from *Apis dorsata* honey samples. Altogether 28 pollen types referable to 25 families were recorded. The physical parameters i.e, the pH value, moisture content and electrical conductivity of the honey samples were also measured to evaluate the quality of honey and the heavy metal contents (Zn, Pb, Cd and Cu) identified by using Atomic Absorption Spectrometry after wet digestion. The present result showed the relationship between bee hive and atmospheric quality of the surroundings. .

Key Words: Heavy metals, pollen analysis, unifloral honey, Bhupalpally district, Telengana.

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INTRODUCTION

Melissopalynology is one of the important branches of Palynology used as a very significant tool in the field of apiculture and production of quality honey. Qualitative and quantitative pollen analysis of honey provides the only means of codifying honey and recognizing the bee pasturage plants in any locality. Honey is the most popular and most recognized natural food produced by the various honey bee species from nectar and honey dew[1]. Normally, honey consists mainly of glucose and fructose sugars and also includes about 200 bioactive substances, viz., minerals, vitamins enzymes, organic acids, proteins, and phytochemicals[2].

The composition and quality of honey is affected by contributions of the nectar sources, vegetation patterns, location, climate and environmental conditions[3,4]. Pollen quantitative analysis provides some significant information regarding honey extraction, filtration and fermentation[5], some kinds of adulteration[6] and hygienic aspects such as contamination with mineral dust, soot, or starch grains[7]. In order to have a beneficial effect, honey must be free of every contaminating agent in general and any contaminants such as heavy metals in particular present in honey above the permissible limits by normal pollution standards, are threats to human health[8]. Honey bees are continuously exposed to potential pollutants present in widespread foraging areas and the more usage of pesticides and highway roads on bee health has been widely documented[9,10]. The forage range of bees is very large and during the nectar gathering process bees come into contact not only with air but also

with soil, water and various heavy metals within the forage radius, which could be transported to the colony. Moreover, air and water from pesticides and vehicle smoke contain heavy metals, which can also contaminate the bee colony and its products. Some studies confirm that honey produced from nectar collected in areas exhibiting high environmental contamination with heavy metals meets residue limits for contamination with elements, minerals[11]. Some studies showed that metal contamination levels are lower in honey compared to those in the bodies of bees, indicating that bees can filter and purify nectar by removal of honey heavy metals during honey production[12].

MATERIAL AND METHODS

The ten samples were collected from *Apis florea* and *Apis dorsata* from various mandals (local government area) of Bhupalapally district, Telangana State (Fig. 1.1) in India. Honey samples were collected between September and January 2020-21.

The mandals from which the *Apis florea* honey samples were collected include two samples from Gaddiganipalli village (Bhupalapally mandal), whereas one from Mahadevpur and another from Mogullapally mandal.

Five *Apis dorsata* honeys were also collected (two samples from Chelpur, two samples from Azamnagar and one from Kataram mandal) from Bhupalpally district. The ten honey samples were acetolyzed to study the pollen grains within them, by following standard method[13].

The Honey samples were further analyzed to measure the moisture content, sugar levels, electrical conductivity, and pH value. Electric conductivity measured by Digital auto ranging conductivity meter (EQ-664 A) with magnetic stirrer according to standard method[14,15].

Fig. 1.1: Map showing Honey extracting locations of Bhupalapally District, Telengana, India.

The measurement of pH was carried out at 27°C in aqueous solution and the results were articulated in micro-siemens/cm using controller pH meter (EQ-621). The honey samples were dissolved in 10% distilled water and moisture content was measured by calculating the refractive index using refractometer. The refractive index proportionally decreases with increase of water content. The sugar content present in honey was also measured by refractometer.

Then the concentration of heavy metals was determined by Atomic Absorption Spectrometer (AAS-model No. AA-6300). The samples were subjected to acid digestion using HNO_3 and HCl and 50 ml of de-ionized water was added to dissolve the content and then it was filtered. The honey was examined for the concentration of heavy metals[12,16]. The results indicated different levels of heavy metals depending on the honey variety, plant species and region of collection shown in Table 1.1 and

Table 1.1: Frequency of pollen grains in squeezed honey samples of Bhupalapally District of Telengana state

Sl. No.	Honey sample name and date of collection	Name of the Predominant Pollen Type (>45%)	Secondary Pollen type (16-44%)	Name of the Important minor pollen (3-15)	Name of the minor pollen (below 3%)
1.	*Apis florea* Gaddiganipally November 2019	*Gardenia lucida* (Rubiaceae) 72%	Grass (Poaceae) 17%	*Schleichera oleosa* (Sapindaceae) 10.5%	Nil
2.	*Apis florea* Venkatapur December 2019	*Sphaeranthus indicus* (Asteraceae) 73%	*Alangium salvifolium* (Cornaceae) 24%	*Sesamum indicum* (Pedaliaceae) 6%	*Coccinia grandis* (cucurbitaceae) 2% *Acacia chundra* (Fabaceae) 0.16%
3.	*Apis florea* Mahadevpur forest December 2019	*Eucalyptus globulus* (Myrtaceae) 84%	Nil -	*Hygrophila auriculata* (Acanthaceae) 6%	*Grass* (Poaceae) 2% *Sida acuta* (Malvaceae) 0.6%
4.	*Apis florea* Gaddiganipalli January 2020	*Leucaena leucocephala* (Fabaceae) 78.8%	Nil	*Brassica nigra* (Brassicaceae) 13% *Melilotus alba* (Fabaceae) 8.28%	Nil
5.	*Apis florea* Mogullapally January 2020	*Prosopis juliflora* (Fabaceae) 74%	*Carum capticum* (Apiaceae) 17.34%	*Sesamum indicum* (Pedaliaceae) 8% *Bombax ceiba* (Bombaceae) 5%	Nil
6.	*Apis dorsata* Azamnagar February 2020	Capsicum frutescens (Solanaceae) 80%	Nil	*Zea mays* (Poaceae) 16%	*Sesamum indicum* (Pedaliaceae) 2% *Hibiscus rosasinensis* (Malvacea) 0.8%
7.	*Apis dorsata* Azamnagar October 2019	*Aspidopterys indica* (Malphigiaceae) 62%	*Allium sepa* (Liliaceae) 24%	Grass (Poaceae) 12%	*Hibiscus rosasinensis* (Malvaceae) 0.3%
8.	*Apis dorsata* Chelpur January 2020	*Lagerstroemia parviflora* (Lythraceae) 73%	Nil	*Terminalia arjuna* (Combretaceae) 12% *Cyperus rotundus* (Cyperaceae) 14%	Nil
9.	*Apis dorsata* Chelpur March 2020	*Ocimum tenuiflorum* (Lamiaceae) 45%	*Hygrophila auriculata* (Acanthaceae) 32% *Cucumis sativus* (Cucurbitaceae) 22%	Nil	Nil
10.	*Apis dorsata* Katarum March 2020	*Zea mays* (Poaceae) 48%	*Hygrophila auriculata* (Acanthaceae) 24%	*Celosia argentea* (Amaranthaceae) 17% *Allium cepa* (Liliaceae) 9%	*Sida acuta* (Malvaceae) 0.73%

Table 1.3. Moreover, significant differences in the content of particular heavy elements, which were measured in the same honey sample variety having different origins were observed. A similar tendency was reported during the testing of heavy metals in different types of honey samples[17].

RESULTS

Ten honey samples collected from the hives of *Apis florea* and *Apis dorsata* were used in the present study for palynological analysis. The study confirmed a wide variability of pollen content in honey samples, which represent their plant sources as herbs, shrubs, trees, and some cultivated plants[18].

Table 1.1 shows the total 28 pollen types from 25 plant families (Fig. 1.2), which are identified in the honey samples with various percentages. The pollen types *Gardenia lucida* (Rubiaceae, 72%) in AF-01 honey, *Sphaeranthus indicus* (Asteraceae, 73%) in AF-02 honey, *Eucalyptus globulus* (Myrtaceae, 84%) in AF-03 honey, *Leucaena leucocephala* (Fabaceae, 79%) in AF-04 honey, *Prosopis juliflora* (Fabaceae, 74%) in AF-05 honey, *Capsicum frutescens* (Solanacae, 80%) in AD-06 honey, *Aspidopterys indica* (Malpighiaceae, 62%) in AD-07 honey, *Legerstoemia parviflora* (Lythraceae, 73%) in AD-08 honey, *Ocimum tenuiflorum* (Lamiaceae, 45%) in AD-09 honey and *Zea mays* (Poaceae, 48%) in AD-10 honey, were identified as predominant pollen, which are considered as unifloral honeys. The pollen types *Alangium salvifolium* (Cornaceae, 24%) in AF-01 honey, *Carum copticum* (Apiaceae, 17.3%) in AF-05 honey, *Allium cepa* (Liliaceae 24%) in AD-07 honey, *Hygrophila auriculata* (Acanthaceae, 32%) and *Cucumis sativus* (Cucurbitaceae, 22%) in AD-09 honey, *Hygrophila auriculata* (Acanthaceae, 24%) and *Celosia argentea* (Amaranthaceae 17%) in AD-10 honey were recorded as secondary pollen types. Remaining pollen types were recorded (Table 1.1) as important minor and minor pollen types[19].

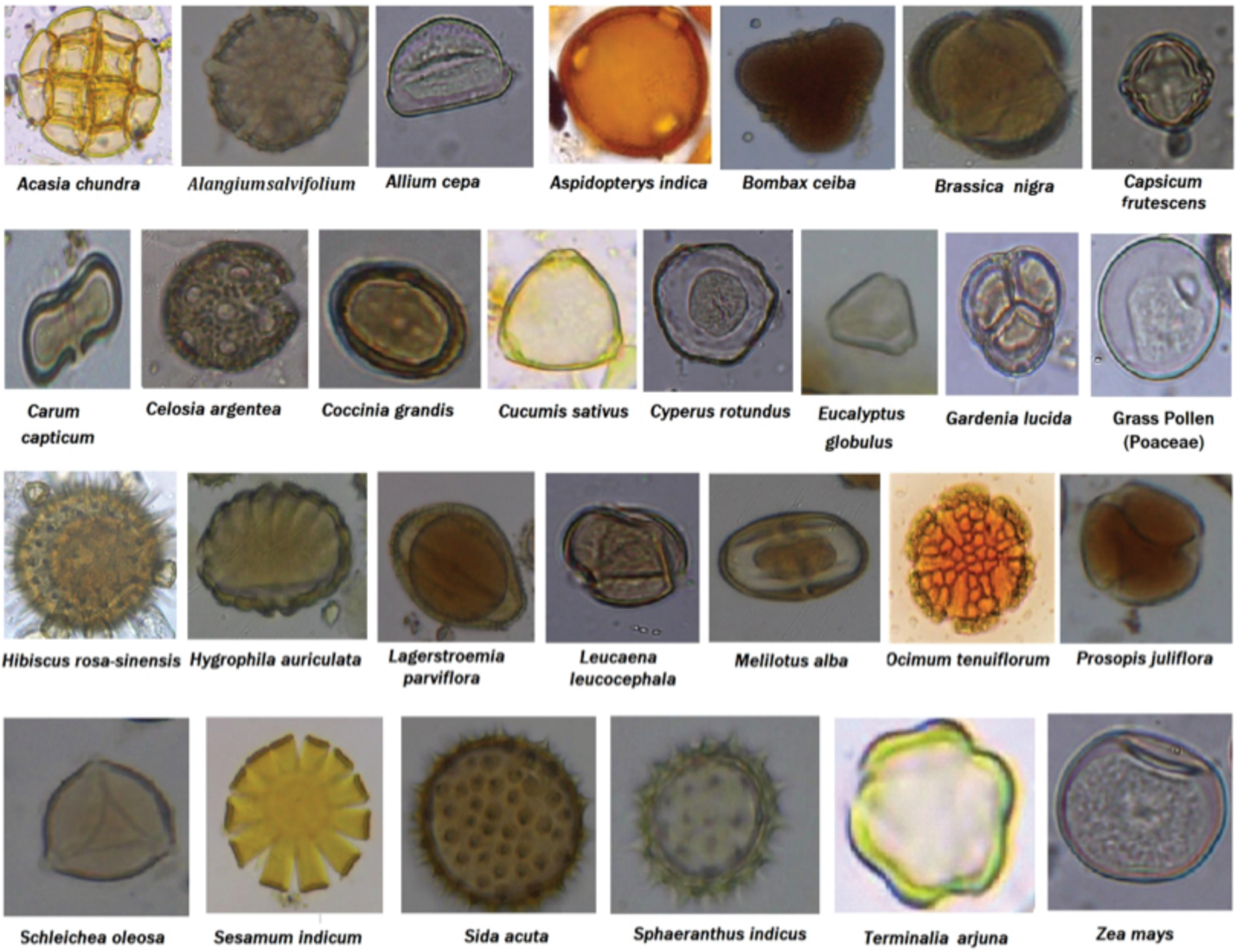

Fig. 1.2: Photo micrographs of important pollen grains retrieved from the Honey samples of Bhupalpally district of Telengana state

Table 1.2. Physical parameters of unifloral honey samples

Sl. No.	Name of the honey	Nature	pH index	Refractive	Sugar 27°C	Electrical conductivity mS/cm
1.	AF-01	UF	4.56	1.493	81.45	0.507 mS
2.	AF-02	UF	3.99	1.492	80.63	0.69 mS
3.	AF-03	UF	3.95	1.491	80.21	0.99 mS
4.	AF-04	UF	4.60	1.487	78.7	0.57 mS
5.	AF-05	UF	4.30	1.484	79.39	0.59 mS
6.	AF-06	UF	4.61	1.490	78.8	0.60 mS
7.	AF-07	UF	4.59	1.483	79.33	0.51 mS
8.	AF-08	UF	3.69	1.480	79.33	0.71 mS
9.	AF-09	UF	4.57	1.479	78.9	0.57 mS
10.	AF-10	UF	4.42	1.482	7.93	0.48 mS

AF-01 – *Apis florae* (Gaddiganipally)
AF-02 – *Apis florae* (Venkatapur)
AF-03 – *Apis florae* (Mahadevpur)
AF-04 – *Apis folrea* (Gaddiganipally)
AF-05 – *Apis florae* (Mogullapally)
AF-06 – *Apis dorsata* (Azamnagar)
AF-07 – *Apis dorsata* (Azamnagar)
AF-08 – *Apis dorsata* (Chelpur)
AF-09 – *Apis dorsata* (Chelpur)
AF-10 – *Apis dorsata* (Kataram
UF – Unifloral.

Table 1.3: Heavy metal (*µ*g/g) analytical data of ten honey samples

Sl. No.	Name of the honey	Zn	Pb	Cd	Cu
1.	AF-01	1.32 ±0.12	0.165 ±0.01	0.17 ±0.01	2.45 ±0.15
2.	AF-02	8.02 ±0.91	1.36 ±0.12	0.94 ±0.04	0.15 ±0.01
3.	AF-03	3.45 ±0.84	1.85 ±0.24	1.03 ±0.15	2.12 ±0.08
4.	AF-04	2.13 ±0.95	2.01 ±0.14	0.78 ±0.03	1.25 ±0.12
5.	AF-05	4.50 ±0.72	1.49 ±0.01	1.19 ±0.14	1.78 ±0.15
6.	AF-06	3.14 ±0.57	5.46 ±0.43	1.45 ±0.18	0.18 ±0.01
7.	AF-07	3.78 ±0.42	20.82 ±0.72	3.32 ±0.23	1.99 ±0.29
8.	AF-08	1.13 ±0.55	2.32 ±0.13	0.79 ±0.01	0.12 ±0.01
9.	AF-09	0.97 ±0.24	2.9 ±0.75	1.14 ±0.09	2.19 ±0.31
10.	AF-10	4.36 ±0.34	27.13 ±0.49	4.45 ±0.17	0.84 ±0.01

Mean and Sd values are significant at $p<0.005$ (n = 5)

Table 1.2 shows the pollen frequencies in all ten honey samples based on their plant families. The present Melissopalynological data is important as it revealed the variability in pollen content and availability of preferred bee pasturage plant sources for *Apis florea* and *Apis dorsata* within the foraging range of the hives.

The pH study of ten honey samples confirmed the acidic nature of honey. It ranges between 3.69-4.61 as shown in Table 1.2. The EC (Electrical conductivity)[15,20] value was ranged between 0.48-0.99 mS/cm. Whereas the refractive index was ranged between 1.472-1.493 and the sugar content of honeys was ranged between 79.3-81.45, indicating the purity of the honey.

The level of zinc (Zn) was ranged from 0.97-8.02 μg/g in both *Apis florea* and *Apis dorsata* honey samples. The higher levels (8.02) were found to be in AF-02 honey sample collected from Gaddiganipally village, which is higher than the permissible limits compare to the limits of Codex Alimentarius Commission (5 μg/g). The lead (Pb) concentration was ranged from 0.16-27.13 μg/g in all honey samples analysed and represented higher than permissible limits in AD-06, AD-07 and AD-10 honey samples, which were extracted from the hives of *Apis dorsata* (Codex Alimentarius 0.3, and Prevention of Food Adulteration Act – 2.50 μg/g). The cadmium (Cd) levels were ranged from 0.17-4.45 μg/g and found to be more than permissible limits[16] (1.5 ug/gm) in samples AD-07 and AD-10 collected from the *Apis dorsata* honey combs (Codex Alimentarius 0.05, and Prevention of Food Adulteration Act). The levels of copper (Cu) were ranged from 0.12-2.19 μg/g, which are below the permissible limits in all the ten honey samples studied indicating no Cu contamination of honey samples collected from the various localities of Bhupalapally District.

DISCUSSION

Pollen analytical studies have revealed that all the honey samples were composed of predominant pollen types, which classified them as unifloral honeys. However, presence of secondary pollen types in AF-01, AF-02, AF-05, AD-06, AD-07, AD-09 and AD-10 honey samples shows the copious nectar availability in the studied areas. The physical parameters showed that all the honey samples are acidic in nature. While analysing the heavy metal content, the higher levels of zinc, lead and cadmium were found only in few *Apis dorsata* honey samples. The values could be due to the use of pesticides, fertilizers in agriculture fields, industries and vehicle smoke nearer to the honey combs. These findings show that the plant sources can alter the pollen composition of the honeys. The presence of heavy metals in the environment could lead to the bio-magnifications, i.e., uptake of the metal by plants from contaminated water, air and soil resulted in the different concentrations of heavy metals in honeys which may have lethal effect on consumption.

CONCLUSION

The results showed that the heavy metals in honey samples collected from some areas of Bhupalpally district of Telengana state may lead to the adverse health effects in consumers. The pH of ten honey samples confirmed acidic in nature and the ten tested honey samples were found to be unifloral in nature.

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ISOLATION AND IDENTIFICATION OF PSYCHROPHILIC FUNGI FROM REGIONS OF JAMMU AND KASHMIR, INDIA

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Studies on psychrophilic (cold loving) microorganisms that inhabit cold environment were carried out for the economic value of their cold-active properties for novel industrial applications. Extreme environments include those with either high (55 to 121°C) or low (–2 to 20°C) temperature, high salinity (2-5 M NaCl), high alkalinity (pH>8) or high acidity (pH<4). In the current study, two fungi were isolated and identified. The psychrotolerant fungus BPF4 isolated from soil of Baramulla, Jammu and Kashmir was characterized at morpho-molecular levels and identified as *Panicillium canescens*. The psychrophilic fungal strain BPF6 also isolated from Baramulla soil was characterized at morphological level and identified as *Pseudogymnoascus roseus* var. *roseus*

Key Words: Pychrophilic fungi, Morpho-molecular identification, Jammu and Kashmir

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INTRODUCTION

Psychrophilic (cold-loving) or psychrotolerant (cold-adapted) microorganisms are found in the low temperature environments of the Earth, including polar regions, high mountains, glaciers, ocean deeps, shallow sub-terranean systems (i.e. caves), the upper atmosphere, refrigerated appliances and the surfaces of plants and animals living in cold environments, where temperatures never exceed 5°C. These psychrophiles are able to degrade a wide range of polymeric substances such as starch, cellulose, xylan, pectin, chitin, protein and lipid and produce enzymes like amylase, cellulase, xylanase, pectinases, chitinase, protease and lipase. Compared to proteins from mesophiles, psychrophilic proteins show decreased ionic interactions and hydrogen bonds, possess less hydrophobic groups and more charged groups on their surface and longer surface loops. The present work was undertaken due to the ability of psychrophilic enzymes to catalyse reactions at low or moderate temperatures that offers great industrial and biotechnological potential.

MATERIALS AND METHODS

Media

(a) *Potato Dextrose Agar (PDA)*: 200 g potatoes were peeled, grated and soaked in distilled water (DW). It was kept in refrigerator for overnight. The supernatant was filtered and used as potato extract. 200 ml of this potato extract was supplemented with 10 g of dextrose and 20 g of agar powder and the volume was made to 1000 ml with DW and pH was adjusted to 6. The

medium was autoclaved for 20 min at 15 lbs. Chloramphenicol powder was added to the above medium in molten state to the final concentration of 2 mg ml^{-1} for inhibition of bacterial growth.

(b) *Minimal Media*: This was prepared by adding $(NH_4)_2SO_4$ – 5g, KH_2PO_4 – 1 g, $MgSO_4$ – 500 mg, $CaCl_2$ – 100 mg, $NaCl_2$ – 100 mg, Glucose (carbon source) – 10 g and Agar – 20 g in 1000 ml of distilled water, then autoclaved.

(c) *Glycerol Stock Solution*: This solution was prepared for the preservation and maintenance of isolates or cultures. Each 100 ml of solution contains 1.5 g of yeast extract, 2.0 g of peptone and 1.0 g of glucose with glycerol – 10 ml.

Buffers

(a) Citrate Buffer of pH3 was prepared by addition of 7 ml 0.05 M sodium citrate or tri-sodium citrate in 94 ml of 0.05 M citric acid.

(b) Citrate Buffer of pH4 was prepared by addition of 33 ml 0.05 M sodium citrate or tri sodium citrate in 65 ml of 0.05 M citric acid.

(c) Citrate Buffer of pH5 was prepared by addition of 39 ml 0.05 M tri-sodium citrate in 61 ml of 0.05 M citric acid.

(d) Phosphate Buffer of pH7 was prepared by addition of 0.2 M sodium hydroxide to 50 ml of 0.2 M sodium dihydrogen phosphate and diluted it to 100 ml.

(e) Tris-HCl Buffer of pH8 was prepared by addition of 29 ml of 0.2 M HCl in 71 ml of 0.2 M Tris.

(f) Tris-HCl Buffer of pH9 was prepared by addition 6 ml of 0.2 M HCl in 94 ml of 0.2 M Tris.

(g) Glycine-NaOH Buffer of pH11 was prepared by adding 0.2 M of glycine to 0.2 M of NaOH.

Microbes

The psychrotolerant fungus BPF4 and the psychrophilic fungus BPF6 were obtained from laboratory stocks. The psychrophilic and psychrotolerant fungi were studied by their growth rate which was calculated in terms of mm/day after centrally point inoculating each of the cultures on PDA plate and incubating at 4°C, 25°C and 37°C. The diameter of the growing colony was measured routinely up to 5 days. The average of these readings was taken as diameter of the colony. Psychrophilic organisms showed fast growth at temperature of 10°C or lower, but cannot survive at temperature above 20°C, whereas psychrotolerant strains showed fast growth rate above 20°C up to 40°C[3].

Identification of Fungi

The fungi were identified to the generic or specific level on the basis of macromorphological and micromorphological characteristics using suitable media, slide cultures and the most updated keys for identification.

(a) *Macroscopic study*

Colonies of Himalayan fungi were cultivated on potato dextrose agar at corresponding isolated temperature for 7 days. The following morphological characteristics were evaluated: colony growth (length and width), presence or absence of aerial mycelium, colony color, presence of wrinkles and furrows, pigment production etc.

(b) *Microscopic study*

Microscopic observation of spore and their arrangement is very important in the classification of fungi. Fungal spores were cultivated on potato dextrose medium. The germination and growth of mycelium was observed daily under a light microscope. The microscopic examination was made by observing needle mount preparations and slide cultures after staining with lactophenol cotton blue. All microscopic identification was carried out by using Olympus microscope and photographs of each fungal strain were taken by means of MIGS (Magnous Image Projection System).

(c) *Molecular study*

BPF4 was identified on the basis of ITS sequence data and its comparison with ITS data available in the Genbank (NCBI) as well as at Bhat Biotech Pvt. Ltd. (Bangalore). Fungal DNA extraction was done from fungus using fungal genomic DNA extraction kit (Bhat Biotech Pvt. Ltd.).

PCR Amplification: Amplification of the ITS region (ITS1, ITS2, and 5.8 S rRNA Gene) was performed using the primers ITS1 and ITS4 and the conditions described in literatures[3,10].

Primer ITS: 5′ – TCCGTAGGTGAACCTGCGG-3′

Primer ITS4: 5′ – TCCTCCGCTTATTGATATGC-3′

The ~600 bp PCR product was purified by gel elution and used for sequencing.

Sequencing: Both strands of the rDNA region amplified by PCR were sequenced by automated DNA sequencer -3037 × l DNA analyzer from Applied Biosystems using BigDye® Terminator v3.1 cycle sequencing Kit (Applied Biosystems). For sequencing, same primers NL-1 and NL-4 were used. Sequence data were aligned and dendrograms were generated using Sequence Analysis software version 5.2 from Applied Biosystems. The sequences obtained for upper and lower strands were manually aligned before performing the analysis.

Bioinformatics analysis: Sequences were compared to the non-redundant NCBI database by using BLASTN, with the default settings used to find the most similar sequence and were sorted by the E score. A representative sequence of 10 most similar neighbours was aligned using CLUSTAL W2 for multiple alignments with the default settings. The multiple-alignment file was then used to create a neighbour-joining phylogram with CLUSTAL W2.

RESULTS

Effect of temperature on growth of fungal strains

The fungal strains were cultivated at temperatures between 4°C and 35°C on agar medium and under submerged conditions. According to the temperature range for growth, the fungal isolates can be classified as psychrophilic or psychrotrophic. Both the fungal strains (BPF4 and BPF6) grew at temperatures between 4°C to 25°C, with an optimum growth temperature of 15°C. Neither of them showed any trace of growth above 25°C.

Identification of BPF4

Macromorphic characters: The colony was compact or dense, center somewhat raised, sulcate, velutinous, margin entire and white in colour. The old colony showed greenish sectors. The growth rate of colony was moderate (Fig. 2.1).

Micromorphological characters: The mycelium was thin-walled; penicilli mostly biverticillate, often monoverticillate; metulae in verticils of 2-3, roughened, phialides ampulliform, in verticils of 5-10, smooth to finely roughened, mostly short; conidia subspheroidal, less often ellipsoidal, finely roughened, thin walled, borne in loose, disordered, entangled chains (Fig. 2.2). Hence the fungal strain BPF4 has been identified as *Penicillium canescens*.

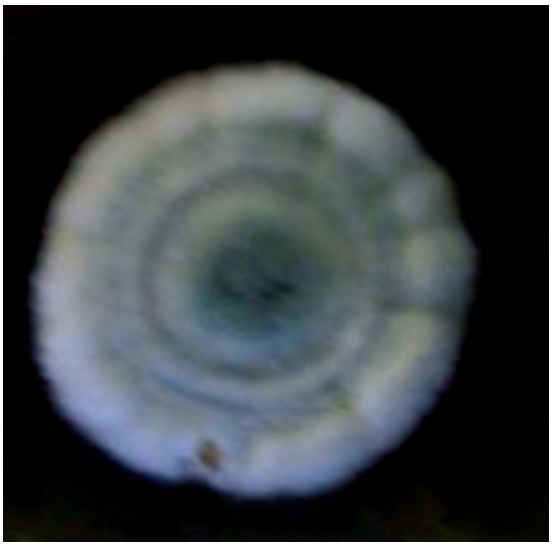

Fig. 2.1: Colony morphology of BPF4 (*P. canescens*)

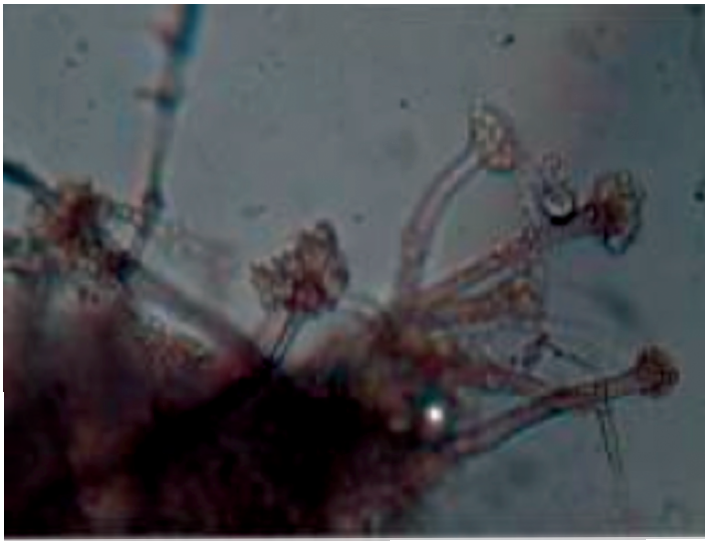

Fig. 2.2: Conidiophores of *P. canescens*

Phylogenetic tree of BPF4

The ITS sequences of ten closely related taxa (Table 2.1) of *Penicillium canescens* were retrieved from GenBank and evolutionary history was inferred using the Neighbor-Joining method. The optimal tree with the sum of branch length = 0.02129630 is shown (next to the branches). The evolutionary distances were computed using the p-distance method and are in the units of the number of base differences per site. The analysis involved 11 nucleotide sequences. All positions containing gaps and missing data were eliminated. There were

Table 2.1: Sequence analysis of the ITS region of 18S rRNA gene showed that the Sample BPF4 is *Penicillium canescens*

ID	Description	% similarity
BPF4	Analysed Sample	
FJ025212	*Penicillium canescens* strain QLF83	100%
JN585940	*Penicillium canescens* strain Cs/6/3	99%
JQ356543	*Penicillium canescens* isolate E17	98%
HQ607858	*Penicillium canescens* isolate ATT146	99%
AY373901	*Penicillium canescens* strain FRR 910	99%
AJ608965	*Penicillium canescens* isolate GFI 48 %	99%
AJ608947	*Penicillium canescens* isolate FFI 42 %	99%
FJ025214	*Penicillium canescens* strain QLF80	99%
JN246033	*Penicillium canescens* isolate P39	99%
GU565104	*Penicillium canescens* strain GZUBCECD24	99%

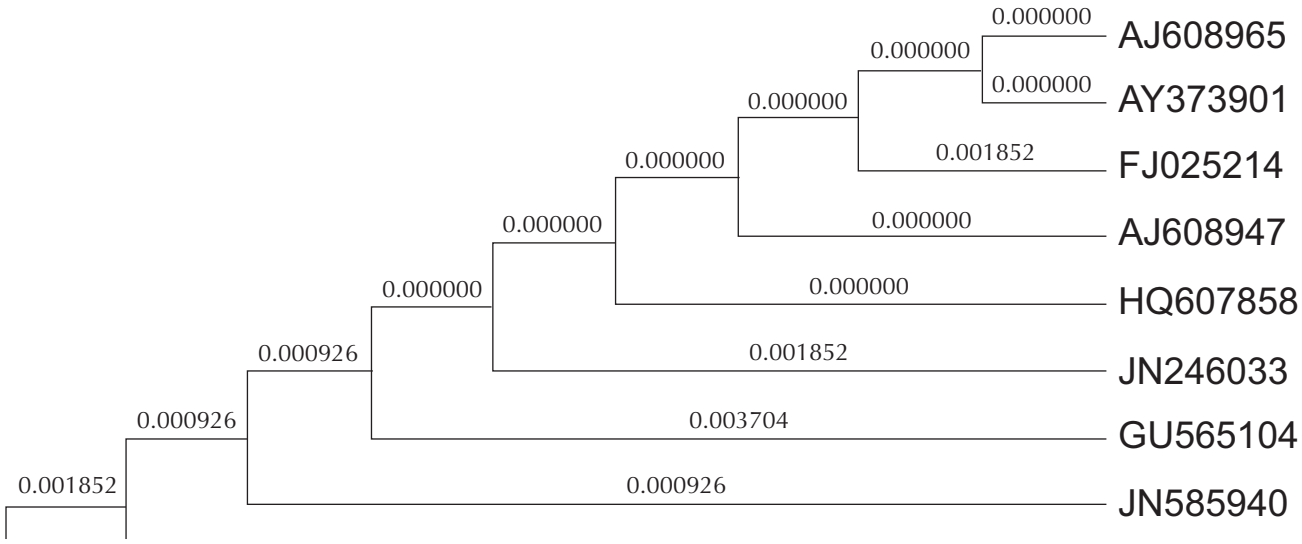

Fig. 2.3: Dendrogram showing phylogenetic relationship with most closely related strains of the isolate BPF4.

a total of 540 positions in the final dataset. Evolutionary analyses were conducted in MEGA5. The dendrogram is shown in Figure 2.3.

Identification of BPF6

The colonies were pinkish white; the reverse of the colony was not yellow. The conidia were smooth and spherical. The fungus produced ascomata which were covered with net like wall so that asci could be seen from outside. The peridial hyphae were rough-walled, red to red-brown, bearing short, sub-hyaline, thin- and rough-walled, unbranched appendages. Ascospores are smooth and lacking a longitudinal rim. Therefore, the fungal strain BPF6 has been identified as *Pseudogymnoascus roseus* var. *roseus* (Fig. 2.4 and 2.5).

Fig. 2.4: Colony morphology of BPF6 (*Pseudogymnoascus roseus* var. *roseus*)

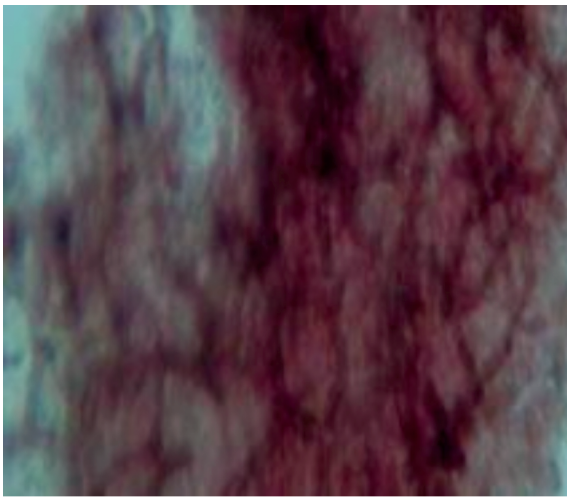

Fig. 2.5: Ascus structure of *Pseudogymnoascus roseus* var. *roseus*

DISCUSSION

Microorganisms requiring extreme environments for growth are called extremophiles and the enzymes they produce are called extremozymes. Extreme environments include those with either high (55°C to 121°C) or low (–2°C to 20°C) temperatures, high salinity (2 to 5 M NaCl), high alkalinity (pH>8) or high acidity (pH<4)[1,2]. Psychrophilic (cold-loving) or psychrotolerant (cold-adapted) microorganisms are found inhabiting the low temperature environments of the Earth, including polar regions, high mountains, glaciers, ocean deeps, shallow subterranean systems (i.e. caves), the upper atmosphere, refrigerated appliances and the surfaces of plants and animals living in cold environments, where temperatures never exceed 5°C. The potentials of psychrophiles and psychrophilic enzymes have been reviewed[4,5]. Many psychrophiles live in biotopes having more than one stress factors, such as low temperature and high pressure in deep seas (piezo-psychrophiles), or high salt concentration and low temperature in sea ice[5]. Hardly, there is any report of the microbiology of temperate regions encountering zero to subzero climate during some months with mesophilic climate in the rest of the months. In India, the Himalayan state of Jammu and Kashmir for example, has many regions that remain covered with snow during three to four winter months. The microbiological studies have shown that this area may also be important habitat for psychrophiles[6]. *Penicillium* species are moulds found commonly associated with soil, decaying organic matter, and as storage rots or pathogens of fruits and vegetables. They are usually asexual members of the Eurotiales in the Ascomycota, although some species also produce a sexual state. Morphologically, the species are distinguished by their brush-like sporulating structures, which produce long green chains of dusty, single celled spores. They are equally well known for biodeterioration of organic matter, for their production of antibiotics such as penicillin and griseofulvin, toxic metabolites (mycotoxins) such as ochratoxin A, patulin, and penitrem A in food and grain, and their starring roles in camembert and blue cheeses. *Penicillium canescens* grows in soil and can degrade plant matter through abundant xylanase and glucosidase production. *P. canescens* is a moderate calcium phosphate solubilizer, but has potential as an organic phosphate releaser through its plant degrading activities. *P. canescens* has been reported from Meghalaya earlier[7]. Nuclear ribosomal 5.8S DNA and the internal transcribed spacers (ITS) sequences have been used to identify fungi[8] and *P. canescens* has also been identified on the basis of the characteristics of ITS sequences earlier. The same fungus has earlier been used to isolate various enzymes, such as alpha-glucanase[9], endo-(1-4)-beta-glucanase[10] and xylanases[11] earlier. The species, however, has not been assessed as source of cold-active enzyme. The taxon *Pseudogymnoascus* was created to accommodate the teleomorphic fungi with transparent cleistothecia. It was Raillo[12] who first designated the taxon *Pseudogymnoascus* (Gymnoascaceae) for two species (*P. roseus* and *P. vinaceus* Raillo) isolated from soil in the Soviet Union. Later, two additional species were described from soil in the Soviet Union and Canada before 1980 namely, *P. caucasicus* Cejp & Milko and *P. bhatti* Samson[13]. All four have smooth ascospores and Geomyces anamorphs which are considered as diagnostic characteristics for the genus[13,14]. Currah[14] considered the four names synonymous and gave priority to *P. roseus*. In 1982, *P. dendroideus* Locquin- Linard from cow dung in Algeria and *P. alpines* Muller & von Arx from the rhizosphere of *Erica carnea* were described[15]. Both had ridged ascospores and poorly developed anamorphs. Udagawa *et al*.[16] created *Gymnostellatospora* to accommodate species with ornamented ascospores and absent or poorly developed anamorphs. Between 1993 and 2000 five species were described from Japanese and Russian soils and from rotting wood in Canada namely, *G. japonica* Udagawa, Uchiyama & Kamiya, *G. frigida* Uchiyama, Kamiya & Udagawa, *G. canadensis* Lumley, Sigler & Currah, *G. subnuda* Sigler, Lumley & Currah and *G. parvula*

Udagawa & Uchiyama[16,17,18]. It was later on found that the formerly known taxon *Geomyces* is anamorph of *Pseudogymnoascus* which was known as a psychrophilic fungus. In the taxon *Pseudogymnoascus, Pseudogymnoascus destructans* (formerly known as *Geomyces destructans*) is also a psychrophilic (cold-loving) fungus that causes white nose syndrome (WNS), a fatal disease that has decimated bat populations in parts of the United States and Canada. *Pseudogymnoascus destructans* grows very slowly in artificial media and cannot grow at temperatures above 24°C. *Pseudogymnoascus roseus* var. *roseus* has been reported from India (see Biodiversity portal), but there is hardly any report of its assessment as source of enzymes.

Conflict of interest

The authors have no conflicts of interest to declare that are relevant to the content of this article.

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DOCUMENTATION OF FLORA REPORTED WITH TOXIC EFFECTS IN THE NAVI MUMBAI REGION OF MAHARASHTRA

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An extensive botanical field survey was conducted in the Navi Mumbai region of Maharashtra during the years 2020-2021. A list of the plants reported to have poisonous effect, growing in Navi Mumbai has been prepared for the first time. The present investigation will contribute to the identification of toxic plants in the study area. This sort of study will be useful to generate awareness, especially for protection of the kids and the cattle from these harmful and toxic plants. A total of 16 toxic or poisonous plant species belonging to 12 families were recorded. Several plants of poisonous nature were identified that cause a variety of diseases in humans and animals. Poisonous substances can be found in a variety of plant parts including the bark, stem, leaves, fruits, latex, and tubers. Pollen grains of many such plants are reported to be airborne as well as allergenic.

Key Words: Toxic plants, poisonous effect, Navi Mumbai, Maharashtra

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INTRODUCTION

Plants have been used by the indigenous people for food, clothing, and shelter from the prehistoric period of ancient civilization. Plant kingdom is also one of the principal sources of various types of traditional medicine. Ancient people extracted numerous chemical compounds in primitive ways and used them to treat a variety of ailments. Herbal medications derived from various sections of plants are frequently used in modern medicine to treat a variety of diseases. The pharmaceutical industry is dependent on various chemical ingredients derived from plants. Some of these plants have also been observed to be harmful to man and his domestic animals in specific circumstances. Poisonous plants are defined as plants that, when consumed or brought into contact with a living thing in any way, produce harmful effects or even death, either immediately or through the cumulative action of the toxic property due to the presence of known or unknown chemical substances in them and not through mechanical action[1].

A poison is any substance that, when ingested, causes harm in a non-thermal or mechanical manner, causing death or serious health consequences. While it is common to think of toxic chemicals as being eaten whole, this is not always the case. Some compounds are not poisons at all, but break down into distinct substances during digestion, one or more of which could be poisonous[2].

Plants with glucosides, acids, or alkaloids are utilised to make treatments. When consumed in excess, they might have negative consequences. If used in excess, the latex, white or coloured sap found in the Apocynaceae, Asclepiadaceae, Sapotaceae, Euphorbiaceae, and Papaveraceae families is poisonous. Plants of the Araceae family contain calcium carbonate or oxalate crystals, which induce significant mouth and throat irritation, as well as enlargement of the throat and intestinal walls. This might result in suffocation or death. When certain plants containing orthophosphoric acids come into touch with the skin or mucous membrane, they induce severe irritation and eruption.

Several toxic plants, such as *Nerium* (oleander), *Digitalis*

purpurea (Foxglove), *Cestrum diurnum* (Jasmine berries), common decorative plants, *Calatropis* and *Datura*, commonly recognized weed species, can be found in our surroundings. These plants have the potential to be harmful to cattle and people[3].

These potentially poisonous plants include physiologically active chemicals that cause systemic and local toxicity in cardiac, nervous, muscular, and vascular tissues. These poisonous compounds have been found in intact plant parts such as seeds (*Abrus precatorius* and *Datura stramonium*), roots (*Aconitum napellus*), and leaves (*Calotropis gigantea*), among others. The degree of plant toxicity is affected by plant shapes and their growing conditions[4].

Young kids tend to be particularly sensitive to deadly plants ingested by accident. Deaths from poisonous plant intake are documented in pieces of information, but a consistent pattern of such occurrences, particularly in India, is hard to come by. Poisonous plants are the third most common sources of toxin in the world. Plant toxicoses exist everywhere, but the rich flora of India places the location high on the list of possible exposure to deadly plants. Accidental plant ingestion resulting in acute poisoning is more common in pre-school children and is believed to be more prevalent in countries where plant-based traditional remedies are widely utilised.

The main concern in phytomedicine and other situations where potentially toxic plants are consumed is phytotoxicity. Plants, in addition to their medicinal properties, produce harmful by-products such as tannins, glycosides, toxa-albumin, and alkaloids, among others, which can have antagonistic toxic effects in humans[5].

Toxic plants are the third most common type of toxin in the world[6]. A study revealed the medicinal use of toxic plants such as *Abrus precatorius* seed paste (for joint pain relief); *Argemone mexicana* seed oil mixed with *Ricinus communis* oil (for treating skin irritation and wounds)[6-8]. Poisonous plants are those that cause a harsh problem or even death when a small number of their leaves, seeds, stem, fruits, and roots are injected, ingested, inhaled, and direct contact[9].

According to the literature, there are approximately 700 plant species with poisonous effect[10] in India. Plant poisoning in children is different from plant poisoning in adults, as children are naturally curious and chew on anything that is easily accessible, including attractive berries or fruit. Plants can differ in terms of toxicity, and many sources categorize plants as extremely, moderately, or minimally toxic. As a result, some cases are not generally fatal due to the low toxicity of plants, or else only vomiting occurs, whereas other cases become serious when not managed by owners and veterinary specialists[11].

Angiosperm plants can be toxic to humans, livestock, insects, and fish under certain conditions[12]. Plant poisoning is not common in India, but it is reported regularly. Toxic plant species such as *Datura, Calotropis, Croton, Thevetia, Abrus,* and *Ricinus* are common in India. According to previous research, plant poisoning is often lethal to humans, but death can occur if the LD50 is too low. It typically occurs when there is insufficient knowledge of potentially toxic plants[13].

This observation will contribute to the identification of toxic plants in the area. Several authors[14-17] reported on various Indian plants and their toxicological consequences as deadly doses. Some hazardous Indian plants are described by Viswanathan *et al*.[18]. In Haryana, Siwach and Gupta[19] worked on poisonous plants and Singh *et al*.[20] recorded some poisonous plants from the Chandigarh zone from the state of Punjab. Treatments for different poisonous plants are also reported in different literature sources[17, 21].

In the present study, it was predicted that, several hazardous plants are grown in the vegetation in the study area of Navi Mumbai. The residents of this area do not have the expertise to determine which plants were harmful. Hence, the goal of this article is to show people how to recognize these plants, so that they can prevent the unavoidable hazards of simply touching or eating them. For this, a scientific list of the plants reported to have poisonous effect (whole plant or any specific part), growing in Navi Mumbai has been prepared for the first time on the basis of vegetation survey.

MATERIALS AND METHODS

Location of the study

Navi Mumbai is a planned city in Mumbai, Maharashtra, India, on the west coast of the state. It began as a new urban township for Mumbai in 1972 and has since grown to become the world's largest planned metropolis. The elevation of Navi Mumbai is 29 meters above sea

level with tropical climate. The average annual temperature in Navi Mumbai is 26.6°C and average annual rainfall is 1,920 mm.

Identification of plants

Specimens were identified using local floras and supporting material for ethnomedical purposes as published in various literature[22,23]. Specimens were dried and prepared as herbarium specimens as supporting documentation and stored permanently at the Herbarium of the Department of Botany, V. P. M.'S, B. N. Bandodkar College of Science, Thane Maharashtra.

Vegetation Survey

During 2020-2021, a total of ten survey visits to seven nodes in the Navi Mumbai area were conducted. The data was crosschecked with suitable and specialized applications of plants and plant components.

RESULTS AND DISCUSSION

Several poisonous plant species were found growing along roadsides and near homes. Exposure-related effects varied from skin rashes to death. Common suggestions about reducing toxic effects after intake included consuming milk or warm water. Some claim that boiling plant components reduces the toxicity. Many of the deadly plants were also employed in traditional medicine.

Toxic plants are the plants when touched or ingested in a sufficient quantity, can be harmful or fatal to human beings and other animals. When used in small amounts and in correct proportions, products from these plants can be utilized as drugs and toxins. The beauty of these plants hides the toxicity within them. The toxicity may differ from plant to plant and it depends on several factors, especially on the different chemicals that characterize it. Moreover, it depends on the part of the plant ingested with respect to its concentration and stage of growth.

The principal purpose of the present study is to identify the plants with toxic effects in the Navi Mumbai region, and to generate awareness, especially for protection of the kids and the cattle from these harmful and toxic plants. The protection against the poisonous plants includes avoiding the touching, smelling, or ingesting the harmful parts of these toxic plants.

Some of the studied plants, such as Oleander, *Calatropis, Gloriosa, Ricinus*, etc., were shown to be extremely harmful to human.

The easiest way to reduce unintentional toxicity from hazardous plants is to raise knowledge about them. In the event of an unintentional intake, the leftover plant should be removed from the mouth and washed with water. The plant must be preserved for identification so that the best treatment may be given. It is critical to avoid producing vomiting since it might cause glottic obstruction and suffocation.

In the present study, the Navi Mumbai region was found to be rich in toxic flora with a significant effect on human and animal health. It has16 toxic (poisonous) plant species belonging to 12 families. Botanical names and local names for plant species as well as toxic parts were recorded, identified, and discussed in this study.

1. *Abrus precatorius* L., Common Name: Bead Vine; Family: Leguminosae, (Fabaceae).

   Harmful effects: Toxin Abrin is found in the seeds' hard, water-resistant outer covering. Unless the seed is chewed and digested, or the seed coat is otherwise damaged, the poison is not released (for example, when the seeds are pierced and threaded on a string as in a necklace). Toxalbumin, a plant lectin related to ricin, inhibits cellular protein synthesis and is potentially hazardous. Seeds that have been ingested usually pass through the gastrointestinal tract unharmed, releasing no poison and producing no toxicity. The toxin is absorbed by intestinal cells if the seeds are chewed, crushed, or digested (i.e., if passage through the gastrointestinal tract is delayed), producing mild to severe gastrointestinal toxicity. Symptoms include nausea, vomiting, stomach cramping, diarrhea, and dehydration, depending on the quantity of toxin exposure. The degree to which the seeds are ground or chewed before intake may influence the level of poisoning. Even with tiny exposures, parenteral delivery (such as by injection or inhalation) or even significant ingestion might result in life-threatening systemic results, including multisystem organ failure[24-26].

2. *Caladium bicolor* (Aiton)Vent, common name: Heart of Jesus; Family: Araceae.

   Harmful effects: It produces acute mouth burning, vomiting, and other gastrointestinal irritations when consumed. Its sap causes temporary blindness when

it comes into touch with the eyes. Calcium oxalate crystals are the substance that causes inflammation[27].

3. *Calotropis gigantea* L., common name: Giant Milkweed, Family: Asclepiadaceae.

   Harmful effects: Chemical constituents found in the leaves and stalk include voruscharin, calotoxin, calotropin, uscharidin, trypsin, calactin, uzarigenin, syriogenin, and proper side isolated from latex, benzoyllineolone and benzoylisolineolone isolated from root bark, and cyanidin-3-rhamnoglucoside isolated from flowers. Many toxic components are created by latex, trypsin, calotoxin, and calotropin, which appear to be collectively responsible for plant toxicity. The latex of the plant causes blindness and skin and mucous membrane irritation. Latex can be fatal at doses of 4-5 ml. The milky liquid is a poisonous material[28].

4. *Caryota urens* L. Common Name: Fishtail Palm; Family: Palmae (Arecaceae)

   Harmful effects: The pulp of the fruit is toxic unverified proteinaceous toxin and raphides of water-insoluble calcium oxalate. Ingestion causes a painful burning sensation in the lips and oral cavity. There is an inflammatory response, which is commonly accompanied by oedema and blistering. Hoarseness, dysphonia, and dysphagia are possible side effects[29].

5. *Cassia fistula* L. Common name: Golden shower; Family: Leguminosae (Fabaceae)

   Harmful effects: The sticky fruit pulp is hazardous. The leaves and bark are not as poisonous. Anthraquinone cathartic toxin Emodin glycoside (senna) causes nausea, vomiting, abdominal discomfort, diarrhoea, and dehydration can all result after ingestion. Emodin can also induce mild urine discoloration (yellowish-brown urine in acid urine, red or violet urine in basic urine)[30].

6. *Catharanthus roseus* (L.) G. Don. Common name: Periwinkle; Family: Apocynaceae.

   Harmful effects: The most serious adverse effect is diarrhea, which is caused by an imbalance in the secretory function of the gastrointestinal tract. The principal alkaloids detected in the leaves are theirocritine (vincristine) and vincaleucoblastine[31].

7. *Dieffenbachia seguine* (Jacq.) Schott, Common Name: Dumbcane; Family: Araceae

   Harmful effects: The entire plant is toxic containing unverified proteinaceous toxins and raphides of water-insoluble calcium oxalate. Chewing on the leaf creates severe discomfort right away[32].

8. *Ficus elastica* Roxb. ex Hornem. Common name: Rubber tree; Family: Moraceae.

   Harmful effects: Ingesting latex can cause a range of stomach problems, and large dosages can be fatal[33].

9. *Gloriosa superba* L. Common name: Climbing Lily; Family: Liliaceae

   Harmful effects: The entire plant, especially the tubers, is poisonous. Colchicine is a medication that is used to treat (*Colchicum autumnale* is a commercial source plant of this drug.) It may induce oropharyngeal pain at first, followed by severe gastrointestinal symptoms after a few hours. Abdominal pain and severe, frequent, and chronic diarrhoea may occur, resulting in severe fluid depletion and accompanying complications. Colchicine may cause peripheral neuropathy, bone marrow suppression, and cardiovascular collapse because of its use[34,35].

10. *Heliotropium indicum* L. Common name: Scorpion's Tail; Family: Boraginaceae

    Harmful effects: The plant as a whole is poisonous. Alkaloids of pyrrolizidine Acute hepatitis can result from significant short-term exposure, whereas chronic exposure to lesser doses can result in hepatic venous-occlusive disease (Budd–Chiari syndrome) and, in some cases, pulmonary hypertension[36].

11. *Jatropha curcas* L. Common name: Physic Nut; Family: Euphorbiaceae

    Harmful effects: Poisonous seeds are present. Jatrophin (curcin) is a toxalbumin (lectin) found in plants that are linked to ricin. Unlike poisoning from other plants containing poisonous lectins, symptoms (nausea, vomiting, and diarrhoea) frequently appear quickly. The loss of fluid and electrolytes, as well as the reduction of intestinal function, are likely to cause other symptoms. Ingestion of a single seed might result in severe poisoning[37].

12. *Lantana camara* L. Common name: Lantana; Family: Verbenaceae

    Harmful effects: Poisonous pentacyclic triterpenes Lantadene A, B, and C cause prolonged cholestasis in grazing animals. Immature berries are poisonous. The consumption of mature fruit has been linked to intoxication. Wolfson and Solomons[38] claimed that the leaves are hazardous to cattle.

13. *Melia azedarach* L. Common name: Persian Lilac; Family: Meliaceae

    Harmful effects: Toxin-melatonin, *Melia azedarach's* blooms, and berries are poisonous to humans. It produces severe colic, nausea, and vomiting. It produces nervousness and limb trembling in animals. By both oral and parenteral methods, the increased concentration of the extracts depresses the respiratory center significantly. This may be due to the direct effect on the respiratory centers. It was noted that death occurs owing to the stoppage of respiration in doses where mortality was seen[39].

14. *Parthenium hysterophorus* L. Common name: Carrot/Congress grass, Family: Asteraceae

    Harmful effects: Contact with the plant causes dermatitis and respiratory difficulties in people, as well as dermatitis in cattle and domesticated animals. The main cause is parthenin, a highly dangerous toxin. Bitter milk disease is caused when cattle feed becomes polluted with Parthenium leaves[40].

15. *Ricinus communis* L. Common name: Castor-Oil Plant; Family: Euphorbiaceae

    Harmful effects: Ricine and ricinin (more poisonous) are water-soluble glycoproteins present in the seed. The seed coat, leaves, and stem all contain ricin. The seed's oil is not nearly as deadly as the seed itself. The seed contains glycerides and ricinolic acid. When you ingest the seed, you will get a burning feeling in your throat and tongue, followed by exhaustion, thirst, dizziness, and a faster heartbeat. In the end, it leads to unconsciousness. The oil cake cannot be used as animal feed because it contains more ricin than usual. Death occurs swiftly if the oil is administered directly into the bloodstream. Allergies may be triggered by the seed extract[41].

16. *Thevetia nerifolia* L. Common name: Yellow Oleander, Family: Apocynaceae.

    Harmful effects: The fruit is the most poisonous part. Its chewing dries up the tongue and throat, causing muscle strain and dilatation of the eyes. The heartbeat rises and falls, and the blood supply is cut off, ending in death. Poisonous glucosides thevetin and theveresin, as well as cardiotoxic crystal particles phytosterolin, ahoein, and cocilphin, are present in the seeds[16]. Among the poisonous plant parts seed is the most poisonous part followed by fruits and root.

The findings of the recent study corroborates with the reports of the previous researchers like Prashant and Shiddamallayya[42] from Hassan district, Karnataka, Rajbhoj and Kagne from Poladpur, Maharashtra[40], Banerjee and Sinhababu[43] from West Bengal and Vishwanathan and Joshi[18] from Mumbai, Maharashtra.

Pollen grains of many of the studied plants such as *Cassia fistula, Catharanthus roseus, Lantana camara, Melia azedarach, Parthenium hysterophorus, Ricinus communis* are reported to be airborne and causing respiratory allergy and asthma (Adak *et al.*)[44].

CONCLUSION

In the present study, it was observed that, several plants are growing in the study area are reportedly poisonous to cause a variety of problems in man and animals. These plants are generally ignored by the public. Poisonous substances can be found in a variety of plant parts, including the bark, stem, leaves, fruits, latex, and tubers. The degree of the disease caused by these plants is mostly determined by the dose consumed by the affected organisms or the level of skin contact. Poisoning by plants is considered a public health issue. Educating and raising awareness among the public about these deadly plants and their parts will be a significant long-term issue. The local database of hazardous plants undoubtedly aids in public awareness and serves as a doorway for study in the fields of botany, pharmaceuticals, and other related fields.

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SENSITIVITY TO ENVIRONMENTAL ALLERGENS IN RESPIRATORY ALLERGIC PATIENTS FROM KUPWARA DISTRICT OF JAMMU AND KASHMIR, INDIA

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Allergic rhinitis and asthma are atopic diseases caused in humans due to environmental allergens, though genetic factors may also contribute towards the manifestation of allergies. The present study was aimed at finding the total and specific IgE responses in serum sample of the patients, reporting to the health centers across the Kupwara district of Jammu and Kashmir, with symptoms of respiratory allergy. Among the 257 respiratory allergic patients (tested for pollen allergens), it was found that, 47.5% of them had bronchial asthma, 12.5% had rhinitis and 40% had combination of asthma and rhinitis. As per case history, 32% of these patients had family history of allergy. Among pollen types, *Cynodon dactylon* of grass family was found as potent allergen with 30.35% positive reaction in intradermal test. The next predominant pollen allergen was *Poa pratensis* (25.29%), followed by *Rumex acetosa* (19.06%), *Morus alba* (16.34%), etc. Among fungal spores (n = 234), *Aspergillus flavus* had the highest positivity in skin reaction (66.23%), followed by *Curvularia* sp. (60.25%), *Trichoderma* sp. (57.26%), *Nigrospora* sp. (38.46%), *Rhizopus* sp. (36.75%) and *Alternaria* sp. (28.20%). Among 234 patients, 28.20% was reactive to rice grain dust, followed by wheat grain dust (27.35%), house dust (25.20%) etc. Among the patients' population, 73% suffered with respiratory symptoms round the year. The cut-off value for immunoglobulin E (IgE) level was 325 IU/ml. As far as total serum IgE is concerned, 55% of the patients suffering with symptoms had ≥325 IU/ml and 45% of patients had ≤ 325 IU/ml of serum IgE values. It was observed that patients allergic to more than 5 allergens had elevated levels of total serum IgE values. Awareness on airborne pollen/spores allergen exposure is very important for the early prediction and treatment of allergic diseases in Jammu and Kashmir, India.

Key Words: Respiratory allergy, pollen/fungal spores, IgE, Kupwara district, Jammu and Kashmir.

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INTRODUCTION

Kashmir valley, India has been witnessing an increase in allergy related disorders usually due to aeroallergens present in the environment mostly during spring and autumn seasons[1]. Allergic rhinitis and asthma are atopic diseases caused in human, often due to exposure of environmental allergens like pollen, spores, mites, foods, etc., though genetic factors may also contribute towards the manifestation of allergies[1,2]. Atopic allergy is caused due to high levels of IgE and has a multifactorial inheritance pattern[3]. Among the environmental factors, aeroallergens play a major role[4]. It is well-known that, among the sources of airborne allergen, pollen grains and spores are very important[5].

Keeping this in the viewpoint, a survey was conducted in the district of Kupwara, situated in the north of Jammu and Kashmir, India. The objective of the present study was to determine the profile of sensitivity to pollen allergens in the local population in Kupwara district of Jammu and Kashmir.

MATERIALS AND METHODS

Clinical and immunological surveys with pollen, fungal spore and dust extracts

A retrospective analysis was carried out (n = 257, at starting point) with the patients attending the Allergy Clinic at Department of Immunology and Molecular Medicine, Sheri-Kashmir Institute of Medical Sciences, Srinagar, Kashmir. Skin test sensitivity for 12 pollen types, 13 fungal spores and seven types of dust samples were recorded from the result of skin prick tests, using buffer saline as negative control and histamine phos-

phatase as positive control[6]. Immediate and late phase cutaneous responses were recorded at 20 minute and 6-8 hours after allergen challenge, respectively. Pollen antigens were selected based on the local pollen/spore calendar[7, 8].

The total IgE levels were estimated by using commercially available ELISA Kit (General Biologicals Corp. Taiwan).

Subjects

The study was carried out among 257 patients attending the Allergy clinic, Department of Immunology and Molecular Medicine, S. K. Institute of Medical Sciences, Srinagar, with the reports of asthma and allergic rhinitis. Children, pregnant ladies and women with lactation phase were excluded from the study. Case histories were collected from all the patients in order to obtain data on family history and clinical symptoms. All the patients were subjected to physical and clinical examination, assessment of total serum IgE and chest X-ray.

Intradermal skin test

Intradermal skin tests were conducted using 13 fungal spores, 12 pollen grains and seven types of dust extracts. Allergen extracts were acquired from Curewel India Limited (New Delhi, India). The dilution of the supplied extracts was at 1 : 500 ratio. With the help of tuberculin syringe, 0.01-0.02 ml of the extract was intradermally injected to the patients with relevant case history and clinical complaints. Histamine phosphate and buffer saline were used as positive and negative controls respectively. Skin test response was noted after 20 minutes.

Blood Samples

Blood samples were collected from the patients visited Department of Pulmonary Medicine and Department of Tuberculosis, District Hospital Handwara and Sub-District Hospital Kupwara, with various seasonal respiratory disorders (Fig. 4.1).

Enzyme linked immunosorbent assay (ELISA) for total serum IgE determination

Total IgE levels were measured by ELISA test using kit of General Biologicals Corp., Taiwan. The cut off value for IgE levels was 325 IU/ml[9].

Enzyme linked immunosorbent assay (ELISA) for specific serum IgE determination

After total serum IgE measurement, same patient's sera were measured by ELISA, to determine specific IgE level, using the kit provided by General Biologicals Corp., Taiwan.

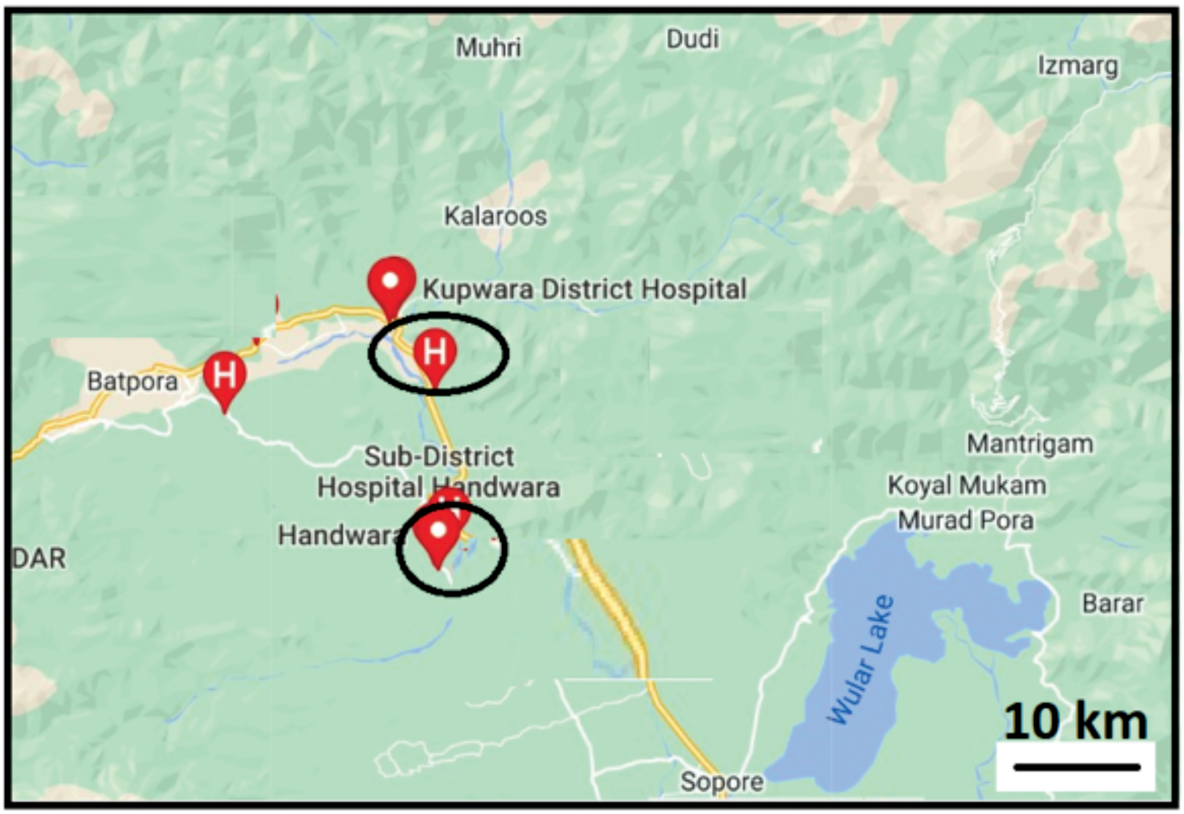

Fig. 4.1: Site map for blood sample collection at Department of Pulmonary Medicine and Department of Tuberculosis, District Hospital Handwara and Sub District Hospital Kupwara (Source: Google map).

Pollen calendar

Pollen calendar was prepared for different study sites by using Vertical Cylinder Rod sampler[10,11].

RESULTS AND DISCUSSION

Among the 257 patients (tested for pollen allergen), 124 were females and 133 were male patients with a mean age group of 34.27 ± 0.24 years. It was recorded that, 47.5% patients was asthmatics, 12.5% had rhinitis and 40% had asthma and rhinitis. According to case history, 32% patients had family history of allergy. Among pollen allergens studied, *Cynodon dactylon* (grass) found as potent allergen causing asthma and rhinitis in Kupwara region with 30.35% positivity. The next predominant pollen allergen was *Poa pratensis* (25.29%) of grass family followed by *Rumex acetosa* (19.06%), *Morus alba* (16.34%), *Plantago lanceolata* (14.78%) and others (Table 4.1). Corroborative to the present finding, the most common pollen grains reported to cause allergy in India are from grass family[12].

Table 4.1: Intradermal skin test response to pollen grain extracts (n = 257)

Sl. No.	Pollen grain types	No. of patients with positive skin reaction	Percentage
1.	*Cynodon dactylon*	78	30.35
2.	*Poa pratensis*	65	25.29
3.	*Rumex acetosa*	49	19.06
4.	*Morus alba*	42	16.34
5.	*Plantago lanceolata*	38	14.78
6.	*Robinia acetosa*	35	13.61
7.	*Abies pindrow*	28	10.89
8.	*Platanus orientalis*	22	8.56
9.	*Pinus halepensis*	18	7.0
10.	*Pinus roxburghii*	15	5.83
11.	*Chenopodium album*	12	4.66
12.	*Cedrus deodara*	8	3.11

The number of pollen grains to which patients were sensitive was found to be inversely proportional to the duration of symptoms, as observed by Raju *et al.*[13]. Additionally, these experimental results are expected to be important for understanding the relationship between the distance from the pollen source and pollen deposition[14]. Since the pollen distribution in the air and the pollen distribution bias on the sampling plate may also cause inaccurate pollen concentration evaluation[15], a new standard method for airborne pollen concentration evaluation is recommended for a more precise understanding of the airborne pollen distribution.

Among 234 patients tested with various extracts, 26.9% had breathlessness, 46.5% had sneezing, 44.87% had nose block and 42.7% had rhinitis. Some of the patients had more than one symptoms.

In the present study, intradermal skin test showed *Aspergillus flavus* to be the most predominant fungal allergen in asthma and rhinitis showing 66.23% positivity among patients (Table 4.2). The second predominant fungal allergen in asthma and rhinitis was *Curvaria* sp. (60.25%) followed by *Trichoderma* sp. (57.26%), *Nigrospora* sp. (38.46%), *Rhizopus* sp. (36.75%) and *Alternaria* sp. (28.20%). Comparison with others works with respect to fungal allergens in nasobronchial allergy, allergens such as *A. flavus, Curvularia* sp. and *Alternaria* sp. were found to be common allergens[16, 17].

Table 4.2: Intradermal skin test response to fungal spore extracts (n = 234)

Sl. No.	Spore types	No. of patients with positive skin reaction	Percentage
1.	*Aspergillus flavus*	155	66.23
2.	*Curvularia* sp.	141	60.25
3.	*Trichoderma* sp.	134	57.26
4.	*Nigrospora* sp.	90	38.46
5.	*Rhizopus* sp.	86	36.75
6.	*Alternaria* sp.	66	28.20
7.	*Helminthosporium* sp.	63	26.92
8.	*Aspergillus fumigatus*	55	23.50
9.	*Mucor mucedo*	52	22.22
10.	*Fusarium solanii*	48	20.51
11.	*Cladosporium* sp.	45	19.2
12.	*Candida albicans*	29	12.39
13.	*Aspergillus niger*	08	3.42

In this study, *Trichoderma* sp. has gained momentum as allergen in asthma and rhinitis which was hardly observed in other studies.

Among different dust allergens (n = 234) studied in nasobronchial allergy patients, rice grain dust had more prevalence (Table 4.3). The common dust extracts with notable level of IgE reactivity was observed in rice grain dust (28.20%), followed by wheat grain dust (27.35%), house dust (25.20%), paper dust (18.80%) and cotton dust (13.67%). In the earlier observation, house dust, wheat dust, paper dust, cotton dust were also reported as predominant allergens in respiratory disorders[18]. Thus in the present study a variation in dust allergens causing nasobronchial allergies was observed (Table 4.3).

Table 4.3: Intradermal skin test response using dust allergen extracts (n = 234)

Sl. No.	Dust types	No. of patients with positive skin reaction	Percentage
1.	Rice grain dust	66	28.20
2.	Wheat grain dust	64	27.35
3.	House dust	59	25.20
4.	Paper dust	44	18.80
5.	Cotton dust	32	13.67
6.	Wheat thrashing dust	25	10.68
7.	Straw dust	22	9.40

According to the information collected from the case histories, 3% of the patients suffer in summer, 5% suffer only in rainy season, 7% only in winter, 12% in rainy season as well as winter and 73% suffer with symptoms round the year. It was recorded that, 28% of the patients were positive to more than five pollen allergens, 37% were positive to more than five fungal allergens and 9% were positive to more than 5 dust allergens. The number of patients that suffered in winter and rains was on an average value. An explanation offering this may be due to fungal species as omnipresent in nature. However, the concentration of fungal spores in air changes with temperature, humidity, rainfall, wind-velocity and the vegetation of the province.

The cut-off value for immunoglobulin E (IgE) level was 325 IU/ml (Table 4.4). As far as total serum IgE is concerned, 55% of the patients suffering with symptoms had ≥325 IU/ml and 45% of patients had ≤ 325 IU/ml of serum IgE values. It has been observed that patients allergic to more than 5 allergens had elevated levels of total serum IgE values.

The IgE levels were high in 53.5% of asthma patients compared to 36.1% in urticaria patients.

Large differences in the serum IgE levels, standard deviation (SD) values, mean and median were observed in patients with different types of allergies. Chi-square test of association was not significant when the levels of IgE were tested against the number of positive pollen grains. Chi-square value with 3 degrees of freedom is 6.324 (P<0.10) (Table 4.4).

It was found that among 121 patients suffering for less than one year, 25% were positive to more than 10 pollen grains. The patients who suffered since more than one year but less than four years, 40% are positive to more than 10 pollen grains. However, no patient was tested positive to more than 10 pollen grains among the 12 patients, who suffered since childhood. The majority of the patients allergic to pollen grains suffer round the year.

Allergens other than pollen, such as house-dust mites, animal dander and molds may cause allergy to the

Table 4.4: Patients with sensitivity against the number of pollen grains positive, compared with their IgE levels

Sl. No.	Sensitivity against the no. of pollen	No. of patients	IgE level >325 IU/ml	IgE level <325 IU/ml
1.	0	31	16 (51.6%)	15 (48.3%)
2.	1	52	36 (69.2%)	16 (30.7%)
3.	<5	154	76 (49.3%)	78 (50.6%)
4.	>5	263	139 (52.8%)	124 (47.1%)

Note: Chi-square value with 3 degree of freedom in 6.324 at P<0.10.

patients. Aerobiological study revealed three predominant pollen grains like as *Poa pratensis, Cynodon dactylon and Cedrus deodara* in the air of present study area. A large number of patients have hypersensitivity to grass pollen grains, though it is assumed to be their too large size to gain access into the lower airways to trigger an asthmatic response.

However, patients who had positive reaction to a higher number (1-24) of allergens, found to have normal levels of IgE. The present study suggests that low levels of circulating IgE may not necessarily indicate the absence of allergic disease. However, specific allergen exposure may be associated with levels of sensitization to specific allergy.

In order to overcome the difficulty of wide variations in the IgE values in the evaluation of inter-group differences, the data were cast into two groups namely, those having an IgE value of less than 325 IU/ml and those having more than 325 IU/ml (Table 4.4). The mean value in rhinitis and urticaria patients was relatively low compared to those with asthma and rhinitis. Majority of the asthma patients were positive to higher number of pollen grains when compared to patients with urticaria and rhinitis. However, within the asthma group, a larger number of patients are positive to single pollen. This may act as an advantage in the selection of patients for immunotherapy since the role of allergen immunotherapy in the treatment of most patients with asthma is likely to be influenced by the selection of the patient.

In the present study, high number of patients showed total serum IgE level ≥ 325 IU/ml suggests atopic nature of the disease (Table 4.4). However, for better diagnosis and immunotherapy treatment, intradermal skin test with specific IgE levels may be fruitful. More and more studies from different places are to be conducted to specify the prevalent allergens in allergic diseases as there is a variation in allergens from region to region.

CONCLUSION

The findings of this study indicate an atopic nature in majority of the patients with asthma and rhinitis, unlike in large number of patients with urticaria. The influence of seasonal changes on allergic symptoms is marginal. As the association between skin test with pollen and serum IgE levels is inconsistent, it is crucial to search for better diagnostic parameters, or use parameters such as specific IgE. Grass pollen grains seem to be the major pollen allergens, the predominant pollen being *Poa pratensis*.

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INCIDENCE OF ALLERGIC SYMPTOMS AMONG POPULATIONS OF DIFFERENT SOCIOECONOMIC STATUS IN WEST BENGAL, INDIA

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The association of socioeconomic status in the incident of allergic diseases is uncertain. In the developing countries, the dearth of space sometimes forces to cook inside living room. Thus such household air pollution from burning biomass or fossil fuels is a major root of morbidity and/or mortality especially in low-income backgrounds worldwide. The present paper aimed to find out the prevalence of various allergic symptoms among local inhabitants of different socioeconomic status. Out of 5491 subjects, 3673 allergic subjects were studied through a questionnaire both at Bolpur sub-divisional hospital and Durgapur sub-divisional hospital in presence of the clinicians. Out of 3673 allergic subjects, about 71.4% patients were recorded from poor socioeconomic class people, while 28.6% patients were encountered in middle/higher socioeconomic class. Significant Association in Odd ratio analysis with a confidence interval of 95% was found between the economic status and the prevalence of cough 2.54 (1.987-3.24), skin diseases 2.743 (2.19-3.42), rhinitis 0.158 (0.125-0.201), conjunctivitis 10.11 (5.82-17.53), etc. Evidences acquired from the present systematic study suggest that the prevalence of allergic diseases is a bit higher in lower socioeconomic populations, thus the prevalence of allergic symptoms is significantly associated with socioeconomic status of populations.

Key Words: Allergic symptoms, Questionnaire, Hospitalization data, Odd ratio, Socioeconomic status, West Bengal, India

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INTRODUCTION

The prevalence of asthma and allergic diseases varies widely among countries/geographical regions and also within countries with different socioeconomic status[1,2]. The INSEARCH (Indian Study on Epidemiology of Asthma, Respiratory Symptoms and Chronic Bronchitis) study[3] in adults estimated that the national burden of asthma among 17.23 million people showed an overall prevalence of 2.05%. The recent Global Burden of Disease (GBD, 1990-2019) estimated that the total burden of asthma in India among 34.3 million population is accounting for 13.09% of the global burden[4].

In relation to socioeconomic status, the "hygiene hypothesis" is the most acceptable theory that features most prominently in the explanation of the association between disease prevalence and socioeconomic status[5]. In addition, the evidence on the relationship between socioeconomic status and asthma as well as allergy prevalence has been conflicting where both low and high socioeconomic status also being reported as a risk factor[6].

In some studies, the socioeconomic status was reported to have an association with asthmatic symptoms. This includes parental education and income. Evidences indicated that children from lower-income group were more prone to asthma culminating to hospitalization compared to children with asthma from higher-income group[7]. Crowded house is associated with several infectious diseases including lung and skin infections, meningococcal disease, and rheumatic fever[8].

In a Brazilian study[9] it was reported that monthly family income was a key factor for prevalence of asthma. At the beginning of the study, the patients with uncontrolled asthma had a median monthly family income of US$ 372.09, whereas those with controlled asthma had higher incomes (median = US$ 558.13). At the end of the study, this difference persisted (uncontrolled asthma: median = US$ 372.09 versus controlled asthma: median = US$ 604.65).

A health survey was conducted[10] at a rural tribal low-income community in sub-Saharan Africa. Each tribal group had distinctive practices when it came to staple

food, cooking tradition, sleeping areas, and smoking habits, which largely affected levels of exposure to biomass fuel smoke and tobacco smoke. Generally speaking, wood is the major source of biomass fuel for cooking and heating. The poorest people in the rural areas are mostly exposed to biomass fuel smoke as they cannot afford a separate cooking place and used to cook in a leaving room along with other family members. Societal roles are largely determined by gender, with the result that women are much more exposed to biomass smoke than men, starting at a young age.

Keeping the above view in mind, in the present study an attempt has been made to record the prevalence of allergic symptoms among local inhabitants in two unexplored sites of West Bengal with regard to their socioeconomic status

METERIALS AND METHODS

Hospitalization data

The health survey was conducted in two sub-divisional hospitals namely, Bolpur sub-divisional hospital and Durgapur sub-divisional hospital. A total of 5491 subjects (2826 from Durgapur and 2665 from Santiniketan) were studied covering an age limit from 5 years to 78 years at both the Durgapur and Bolpur sub-divisional hospitals from 2013 to 2016. In the present investigation, among the overall 5491 subjects a total of 3673 allergic subjects were studied after excluding those who were active smokers (n = 127), had congenital diseases (n = 38), not interested to take part to answer the questions (n = 89) and non-allergic subjects (n = 1668). Out of 3673 allergic subjects, a sum of 2623(71.4%) patients belonging to 1397 male and 1226 female were recorded among poor socioeconomic class people (Table 5.1). Among middle/higher economic class people 1050 patients (28.6%) were encountered belonging to 582 male and 478 female (Table 5.1).

The survey of 3673 allergic subjects in relation to their health hazards in the seasonal changes, together with their lifestyle, smoking habit, family history, occupation, etc., were taken into account, because such factors may enhance the chance of susceptibility to allergy-related symptoms[11-13].

Questionnaire study and Health survey

A health survey of local patients was carried out by visiting the outpatients' department of the sub-divisional hospitals in each study area. The data on the demographic and medical history of the studied allergic patients were collected in presence of the physicians using a standard questionnaire which was prepared according to WHO (2010) with some modifications based on local socio-economic conditions. The patients were clinically examined by the physician before collecting information from them. The questions on concerning allergic symptom include cough, breathlessness, allergic rhinitis, allergic conjunctivitis, allergy-related skin disease and food allergy. The pattern of symptoms, whether seasonal, perennial or irregular, worst month and time of onset of symptoms were also recorded.

RESULTS

In the present study, a total of 3673 allergic subjects were divided into two categories according to their socio-economic status. Those who have a monthly family income maximum of INR 10,000 per month have been considered as poor economic class people (2623 subjects) and those who have a family income of more than INR 10,000 per month are treated as the middle (M) or higher (H) economic class people (1050 subjects). The number of studied poor economic class subjects was higher because the study was conducted at Govt. sub-divisional hospitals where mainly economically poor class population visit for medical treatments according to present Indian socio-economic context.

A total of 25 different types of allergic diseases were recorded from both the study sites. Eleven (11) types of allergic diseases were reported among the economically middle/higher (M/H) class population, while 24 types were recorded among the poor (P) economically class population (Table 5.1). Certain diseases were highly prevalent in M/H class than that of P class people such as allergic urticaria (M/H-15.2%, P-7.9%), allergic rhinitis (H/M-27.05%, P-7.1%), undetermined insect bite allergy (M/H-11.5%, P-2.6%). Except for such types, all other diseases were highly prevalent in the poor (P) economic class population (Table 5.1). A total of eleven urticarial skin diseases were reported in poor (P) class population, while only five such diseases (urticaria, dermatographism, angioedema, skin rash, acne) were registered in the M/H class population. Acne vulgaris urticaria was only noted among M/H class

Table 5.1: Prevalence of allergic diseases among the population of study areas (Durgapur and Santiniketan) in relation to socioeconomic status.

Allergic symptoms Study subjects (N) = 3673	**Poor economic class** Study subjects (N) = 2623 [M = 543, F = 497] (Percentage)	**Middle/Higher economic class** Study subjects (N) = 1050 [M = 280, F = 211] (Percentage)
Allergic Skin Diseases	625 (23.8)	174 (16.6)
(i) Atopic Dermatitis (691.8 ICD9-CM)	52 (1.9)	
(ii) Eczema(acute/chronic)(Infantile) L20.83 ICD-10	23 (0.9)	
Dermatitis due to other radiation		
(i) Solar allergy/Polymorphous light eruption(L56.4 ICD 10)	17 (0.6)	
(ii) Dermatitis chronic	6 (0.2)	
Urticaria		
(i) Allergic urticaria (L50.0 ICD 10) (acute, atopic)	207 (7.9)	160 (15.2)
(ii) Idiopathic urticaria (L50.1 ICD 10)	14 (0.5)	
(iii) Cold and heat urticaria (L50.2 ICD 10)	12 (0.45)	
(iv) Dermatographism (L50.3 ICD 10)	18 (0.7)	4 (0.38)
(v) Chlinergic urticaria (L50.5 ICD 10)	19 (0.72)	
(vi) Chronic urticaria (L50.8 ICD 10)	85 (3.24)	
(vii) Allergic contact dermatitis (L23 ICD10)	12 (0.45)	
(viii) Angioedema	6 (0.2)	1 (0.09)
(ix) Skin rash (R21)	146 (5.6)	7 (0.7)
(x) Papular urticarial	4 (0.15)	
(xi) Pitrasis rosea	2 (0.076)	
(xii) Acne vulgaris		2 (0.18)
Cough R05	440 (16.8)	
Allergic Asthma J45	399 (15.2)	
Allergic Rhinitis J30.9	186 (7.1)	
Chronic allergic conjunctivitis H10.45 (ICD-10-CM) (perennial, seasonal)	238 (9.07)	
Undetermined insects bite allergy	68 (2.6)	
Rhinosinusitis	20 (0.76)	
Genetic allergy	6 (0.22)	
Food allergy	18 (0.7)	

N = No. of the population; **M** = Male; **F** = Female

population. Prevalence of allergic asthma was more or less similar in both the community (H/M-16.47%, P-15.2%), but the prevalence of allergic cough was higher in the poor economic class population (16.8%) than M/H class population (10.48%). Genetic allergy, rhino-sinusitis, and food allergy were not revealed in M/H class population may be due to the small sample size.

DISCUSSION

An Odds ratio (OR) is a measure of association between exposure and its outcome. The OR represents the odds that an outcome will occur given a particular exposure of a setup, compared to the odds of the outcome occurring in the absence of that exposure of the setup[14]. OR of 1 would suggest that there is no difference between the groups, OR of >1 suggests that the odds of exposure are positively associated with the adverse outcome compared to the odds of not being exposed, and OR of <1 suggests that the odds of exposure are negatively associated with the adverse outcomes compared to the odds of not being exposed. Odds ratios (OR) and 95% confidence intervals (CI) were adjusted for important potential confounders (Table 5.2). To assess the joint effect and interactions between socioeconomic status and allergic disease occurrence the subjects were stratified into four groups such as

(i) high-income status and prevalence of a particular disease (viz. relation between asthma prevalence in high-income status individual),

(ii) high-income status and absence of a particular disease,

(iii) low-income status and prevalence of a particular disease,

(iv) low-income status and absence of a particular disease.

Association between the economic status of the local inhabitants played a significant role behind some allergic diseases like cough 2.54 (1.987-3.24), skin diseases 2.743 (2.19-3.42), rhinitis 0.158 (0.125-0.201), conjunctivitis 10.11 (5.82-17.53), other diseases 0.213 (0.115-0.294), sneezing 0.744 (0.584-0.949) and food allergy 8.64 (1.151-64.94) (Table 5.2). There is a strong association between asthma prevalence with socioeconomic status 1.144 (0.915-1.43), but the relationship is not significant (p-0.236).

CONCLUSION

The lower economy class population showed the highest allergic symptoms (eg. Skin diseases, cough, asthma and eye diseases) than middle or higher economic class population. Proper maintenance of health and hygiene, cleanliness of the surrounding environment, lack of proper drainage system, cooking inside living room, use of polluted pond water for sanitation, lack of sufficient food and nutrition, lack of space, sometimes occupational hazard also responsible behind allergic prevalence which is partly associated with socio-economic status.

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Table 5.2: Association between the prevalence of allergic diseases with socio-economic status of local people

Symptoms	Chi-square	P-value	Odds ratio	95% CI	Remark
Asthma	1.40	0.236	1.144	0.915-1.43	
Cough	2.54	<0.0001	2.54	1.987-3.24	AF
Skin diseases	81.28	<0.0001	2.743	2.19-3.42	AF
Rhinitis	250.30	<0.0001	0.158	0.125-0.201	AF
Conjunctivitis	97.35	<0.0001	10.11	5.82-17.53	AF
Other diseases	11.04	<0.0001	0.213	0.155-0.294	AF
Sneezing	5.68	0.017	0.744	0.584-0.949	AF
Cold	119.30	<0.0001	0.271	0.213-0.34	AF
Food allergy	6.36	0.01	8.64	1.151-64.96	AF

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