MATERIALS AND METHODS
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Aerobiological sampling methods are diverse based on different scientific principles and vary according to individual's interest in component of the aeromicrobiota. The equipment used for such trapping purposes or for air monitoring is called as air sampler. The selection of sampler depends on its efficiency in collecting the components of airspora and the components that we want to investigate thoroughly.

Various methods have been proposed to trap the fungal spores, pollen grains, plant parts, protozoan cysts etc. These airborne components are trapped using the Tilak air sampler, which was devised by Tilak (1968) (Plate I). This sampler was awarded the import substitute prize by the Government of India in the year 1972. Present investigation, the trapping of mycosporophytes was done by operating continuously this air sampler over sunflower field. The Technical description and method of working of the sampler is as under:

Tilak Air Sampler (Plate I) :

The Tilak Air sampler is an electrically operated machine which runs on electric power supply (AC 230 V.) and provides a continuous air sampling data for eight days. The apparatus consists of two mechanical systems, the clock mechanism and the exhaust fan system. The electric clock fitted in the bottom of the synchronized with drum of air sampler, which revolves slowly and completes one rotation in eight days. Air is sucked through the orifice of the projecting tube at the rate of 5 liters per minute and it impinges on the transparent cellophane tape on the outer surface of the drum. The cellophane tape, which is 1.5 cm in breadth, is stuck
on the slowly rotating drum. The drum is fitted on the clock system and rotates in anticlockwise fashion and completes one rotation in eight days thus giving a trace of catches for eight days.

The cellophane tape was coated with white petroleum jelly as an adhesive. The tape thus coated with adhesive, faces the orifice of outward projecting tube 0.5 cm. away from it. When sampler is operated, the drum rotates continuously with clock mechanism giving continuous air sampling data for eight days.

Before the tape is mounted on the glass slides, at the end of eight days, it is divided into equal eight parts with graduation on the surface of the drum. Thus each part corresponds to 24 hours trace measuring 8.4 cm. in length. Such parts are again divided into two equal parts thus giving sixteen equal segments, each measuring 4.2 cm. Each piece of the tape now obtained represents the twelve hours sampling area for a day or night accordingly. Each cellophane tape segment is mounted on date labeled glass slide with glycerine jelly used as mountant.

During air sampling, the air is sucked through the orifice tube with the help of exhaust fan having three prongs and fixed in the circular cavity in the cover lid of air sampler. Exhaust fan forces air out of the collection chamber, causing the negative pressure. An exhaust hole measuring 6 x 2.7 cms. is kept in the lid of the apparatus.

The sampler has 75% collection efficiency, great retention capacity, portable, economical and provides continuous data of air sampling for eight days. Its special merit lies in providing volumetric data (numbers of spores/m³ of air) which enables to analyse microbial population both quantitatively and qualitatively. As it provides continuous sampling, the diurnal (circadian) periodicity studies can be
carried out in greater details.

**Sampling Method:**

The practical experiment of air sampling was carried out by operating continuously Tilak Air Sampler with its orifice kept at a constant height of 1.5 meter above the ground level in the sunflower field. The apparatus was covered with polythene cover to protect from rains and it did not affect the efficiency of sampler, during sampling. The air was sampled at the rate of 5 liters per minute and the transparent cellophane tape fixed on the drum coated uniformly with white petroleum jelly as adhesive. The drum was changed after every eight days at about 8 a.m. The complete tape was cut into eight equal segments, again each segment into two equal parts, each representing 12 hours trace area of day and night accordingly. The cellophane tape pieces were then mounted on glass slide by using glycerine jelly as mountant. Glycerine jelly has the best optical properties for visual examination.

Glycerine jelly can be prepared in the laboratory, its composition is as follows:

- Gelatin - 40 gm.
- Glycerine - 120 ml.
- Distilled water - 140 ml.
- Phenol - 0.5 gm.

Measured amounts of glycerine and distilled water are mixed in a beaker and heated in a water bath for 2 to 3 hours. While heating this mixtures, jelatine is added slowly by stirring with glass rod to avoid clumping. After complete dissolution of gelatin, phenol crystals are added as preservative and metabolic
inhibitor. This glycerine jelly is used for the preparation of permanent slides. While mounting, jelly is melted by keeping the flask of jelly on hot water bath as and when required.

**Scanning:**

Scanning was done regularly after slide preparation. Before scanning the mounted slide, tape segment was divided into six equal parts by marking on the glass slide. Each portion corresponds to the two hours sampling period. Area of 9600 sq. microns of the total area of the trace obtained is scanned under 10x X 45x eye piece objective combination of binocular research microscope.

**Conversion factor :-**

The conversion factor for Tilak air sampler is 14.2, to avoid confusion and for easily calculations "14" has been used as round figure conversion factor. Assuming the trapping efficiency to be 75% with the help of conversion factor the spore concentration /m\(^3\) of air can be calculated. This conversion factor is constant.

The sampler being volumetric, the number of spores/m\(^3\) of air can be calculated in the following way:

1) Sampled area
   \[= 8.4 \text{ cm.} \times 1 \text{ cm.} = 8.4 \text{ cm}^2\]
   \[= 84,000,000 \text{ mm}^2\]

2) Scanned area
   \[= 20 \times 20 \times 24\]
   \[= 9,600 \text{ mm}^2\]

3) Volume of air sampled per minute.
   \[= 5 \text{ litres.}\]

4) Volume of air sampled
in 24 hours. \[= 5 \times 24 \times 60\] 
\[= 7,200\] litres

5) To convert one litre of air into cubic meter multiply by \[= 0.001000028\]

6) Volume of air sampled in 24 hours in terms of cubic meter. \[= 7200 \times 0.001000028\] \[= 7.2\] m³

7) Volume of air sampled in the scanned area in 24 hrs. \[= \frac{9600 \times 7200}{1000000}\] \[= 69.12\] litres

8) Volume of air sampled in the scanned area during 24 hours. \[= 1000 \times 69.12\] \[= 14.4\] m³ \[= (1\ m³ = 10000\ meters)\]

Hence, the conversion factor for this sampler is 14.4, but for convenience we use “14”.

If total number of spores from catches is 17, then total number of spores per m³ of air is \[17 \times 14 = 238/m³\] of air. Assuming the trapping efficiency to be 75 % with the help of conversion factor, we can easily estimate the spore concentration /m³ of air. The conversion factor is constant irrespective of locality, season and weather. The time recorded here in the present work is Indian standard time (IST).
Composition of the Catches:

The identification of the fungal spore types was based upon

(1) Morphological character
(2) Visual identification by comparison with reference slides prepared and
(3) by exposing culture plates.

The composition of catches varies from place to place and also impacted by the surroundings. The bioparticles observed over the cellophane tape exposed in the trap varied in shape, size and composition. The common components are dust particles, fungal spores, pollen grains, hyphal fragments, insect parts, microscopic plant parts, protozoan cysts etc. Some miscellaneous particles were also caught on the cellophane tape exposed during trapping.

Sampling Site:

The aerobiological experiments were conducted by keeping Tilak Air Sampler in the Sunflower fields at Rajuri (Navgan) Tq. & Dist. Beed, Maharashtra. Rajuri (Navgan) village is located on Beed – Ahmednagar road, 14 Km. away from Beed. Nearly 4 acres of land was under Sunflower cultivation in the season mentioned earlier. In the remaining land, other traditional crops were cultivated.

Beed district, a part of the Deccan plateau, is situated in the eastern part of Maharashtra. It is bounded by Aurangabad and Jalna districts on the north, Parbhani and Latur districts on the east, Osmanabad district on the south and Ahmednagar district on the west. The total geographical area of Beed district is 10921.08 sq.kms. with 11 Tahsils and 1043 villages. It lies between 180 27’ and 190 27’ North
latitude and 740 49’ and 760 44’ East longitude. The area is at an average height of 600 meters above the sea level.

The details of the varieties grown, crop season, the dates of sowing and harvesting are presented in tabular form.

The studies were conducted over the hybrid sunflower (Mahyco) fields for two winter and summer seasons. The air sampling over sunflower fields was started one week before the sowing date and continued for one week after the harvesting of the crop. The total sampling period for two winter and summer seasons was 295 days.

<table>
<thead>
<tr>
<th>Season</th>
<th>First sampling date</th>
<th>Sowing date</th>
<th>Harvesting date</th>
<th>Last sampling date</th>
<th>Period of sampling days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Summer – I</td>
<td>05.02.03</td>
<td>12.02.03</td>
<td>04.5.03</td>
<td>11.5.03</td>
<td>96</td>
</tr>
<tr>
<td>Summer – II</td>
<td>12.02.05</td>
<td>19.02.05</td>
<td>17.5.05</td>
<td>17.5.05</td>
<td>95</td>
</tr>
<tr>
<td>Winter – I</td>
<td>20.10.03</td>
<td>27.10.03</td>
<td>13.5.04</td>
<td>20.1.04</td>
<td>93</td>
</tr>
<tr>
<td>Winter – II</td>
<td>01.10.04</td>
<td>08.01.05</td>
<td>04.1.05</td>
<td>11.1.05</td>
<td>103</td>
</tr>
</tbody>
</table>

Weather:

During the period of investigation, daily records of temperature, relative humidity, wind velocity and rainfall were obtained from Water and Land Management Institute (WALMI), Aurangabad. Month wise mean average wind velocity, mean average temperature, mean relative humidity and total monthly rainfall is shown in Fig.No.5.

Meteorology:

The climate is generally dry except in the South - West monsoon season. The climate of Beed district reveals generally four seasons in a year, March to
May is the summer season, June to September constitute the South-West monsoon season (rainy season), October to November form the post monsoon season and December to February form cold season. The environmental parameters particularly wind velocity, temperature, relative humidity and rainfall exhibited great deal of variations during different seasons of these years.

**Winds**: Winds are generally light to moderate with some increase in force in the month of May. The direction of the wind is mainly from West to North-East. During the post monsoon and the cold season, winds are very light and variable in direction. The afternoon wind direction is mostly from North-East to South-West. The winds begin to blow from the beginning of the month of May till the end of the rainy season.

**Temperature**: In Beed district, temperature begins to increase rapidly from mid of February. April and May are the hottest months with highest temperature ranging roughly between 35°C and 42°C. During the summer season and in early June, the day temperature often increases when the South-West monsoon begins. There is a slight increase in the day temperature whereas in the midnight, temperature is found to be slightly decreased. December is the coldest month with the temperature range during day time between 9°C and 30°C but during this season the night temperature sometimes decreases up to 5°C.

**Humidity**: During the rainy season (June to mid of September), the relative humidity
was found to be maximum ranging between 85 % and 95 %. On an average, the
percentage of relative humidity decreases from the post monsoon onwards. The
most dry and hot part of the year is the summer season (between 20 % and
40 %).

Rainfall:

During the period of present investigation in the year 2003, it was 645.9
mm. June and July months in the year 2003 and June and August months in
the year 2004 were the months which received heavy rainfall. The total rainfall
recorded was 633.7 mm in the year 2004.