CHAPTER TWO

VARIATION OF PHYLLOPLANE MICROFLORA
IN RELATION TO SEASON.
VARIATION IN PHYLLOPLANE MICROFLORA IN RELATION TO SEASON.

The weather, Rainfall, percentage relative humidity and temperature (Minimum and Maximum) were recorded in the year 1983. Rainfall in mm., was recorded from the rain gauge installed in the campus of 'Aurangabad Airport'. Data regarding percentage relative humidity and temperature were obtained from meteorological centre of Aurangabad Airport. Data is presented in fig.1. The maximum temperature in 1983 ranged between 26°C to 41°C and minimum from 10°C to 28°C. Percentage relative humidity ranged between 13 to 48 for the first 5 months i.e. Jan. to May. It increased to 79 in June and July onwards. Humidity remained high by mid October. Percentage relative humidity in this period ranged between 56 to 95. This period coincides with the rainy season. Percentage humidity dropped below 50 during November/December. Rains were received in the month of June, July, August, September and October. The maximum rainfall recorded in the year was 126.5 mm. on 1st of September and minimum was 0.2 mm. in the month of August. Three distinct seasons thus can be made out from the data given in Fig.1.
Fig. 1

SEASONAL VARIATIONS IN RELATION TO TEMPERATURE, HUMIDITY AND RAINFALL YEAR 1983.
Croton Aucubaefolium, Croton Katonii, Croton Daisy O, Croton Appendiculatum and Croton Capt Kidd were the varieties selected for the study of the effect of seasonal variation on phylloplane microflora. The criteria behind the selection of these varieties were based on the normal distribution of blotches on leaf surface. Croton Aucubaefolium had dense distribution of blotches, Croton Katonii and Croton Capt Kidd had moderate distribution. Croton Appendiculatum had sparse with mixed pigmentation (Red and Yellow blotches) and Croton Daisy O had a rare distribution. A look at fig.2, which represents total bacterial count, clearly indicated that in all the five varieties there was increase in number of bacteria from Jan. to May. In the month of May maximum colony forming units were recorded. The bacterial flora then declined in all the varieties. Croton Aucubaefolium supported maximum number of bacteria throughout the year. Croton Capt Kidd came next with 2150 to 2950 colony forming units throughout the year. Excepting April, May & June, colony forming units were between 2550 to 3200. Katonii gave 1810 to 2400 colonies per sq. inch of leaf blade for the remaining 9 months of the year. Croton Appendiculatum and Croton Daisy O were poor substrates for phylloplane bacteria. In that colony
Fig. 2

SEASONAL VARIATION IN TOTAL BACTERIAL PHYLLOPLANE

POPULATION OF FIVE CROTON VARIETIES.

1. ●—● Croton *Aucubaefolium*
2. ○—○ Croton *Katonii*
3. △—△ Croton *Daisy O*
4. △—△ Croton *Appendiculatum*
5. ○—○ Croton *Capt Kidd.*
forming units in the former ranged between 880 to 1390 and in the latter from 310 to 810.

A study of Gram positive bacilli on five croton varieties throughout the year was made. When screened through appropriate tests, it was observed that 15 species belonging to genus *Bacillus* were the dominant components.

1. *Bacillus subtilis*
2. *Bacillus pumilus*
3. *Bacillus licheniformis*
4. *Bacillus cereus*
5. *Bacillus megaterium*
6. *Bacillus polymyxa*
7. *Bacillus circulans*
8. *Bacillus firmus*
9. *Bacillus stearothermophilus*
10. *Bacillus brevis*
11. *Bacillus sphaericus*
12. *Bacillus badius*
13. *Bacillus freudenreichii*
14. *Bacillus insolitus*
15. *Bacillus aneurinolyticus*.

Data presented in fig. 3 indicate that *Croton Aucubaefolium* harboured maximum gram positive bacilli on its leaves. This variety was followed by *Croton Capt Kidd*, *Croton Katonii*, *Croton Appendiculatum* and *Croton Daisy Q*. Maximum counts were obtained in the month of April, May
FIG. 3

SEASONAL VARIATION IN GRAM POSITIVE BACTERIAL (BACILLI) PHYLLOPLANE POPULATION OF FIVE CROTON VARIETIES.
and June. This period is characterized by high temperature, prolonged days and low humidity.

Variation in gram negative bacilli on the phylloplane of crotons is presented in relation to season fig. 4. Peak counts were evident in the month of January in all the varieties. Second peak was associated with high rains in August and September. The months March and May were characterized by lowest counts of gram negative bacilli, in all the test varieties. The erratic fluctuations in populations were evident throughout the year. In general the counts for gram negative bacilli were too low in comparison with gram positive.

The gram positive cocci gave a pattern distinct from the earlier two groups. Croton Capt Kidd gave maximum colony forming units (400 colonies per sq.inch of leaf blade) and was followed by Katonij, Aucubaefolium, Appendiculatum and Daisy O. Wide fluctuations in populations were evident; however uniformity in behavior was expressed through high counts in the month of May (Fig. 5).

Seasonal variation in fungal population:

The fungi associated with five croton varieties were isolated through leaf washings throughout the year. In all twelve fungi were found to be associated with the phylloplane of crotons.
FIG. 4

SEASONAL VARIATION IN GRAM NEGATIVE BACTERIAL (BACILLI) PHYLLOPLANE POPULATION OF FIVE CROTON VARIETIES.
SEASONAL VARIATION IN GRAM POSITIVE BACTERIAL (COCCI) PHYLLOPLANE POPULATION OF FIVE CROTON VARIETIES.
1. \textit{Trichoderma lignorum}
2. \textit{Cladosporium sp.}
3. \textit{Aspergillus niger}
4. \textit{Aspergillus versicolor}
5. \textit{Aspergillus fumigatus}
6. \textit{Paecilomyces varioti}
7. \textit{Fusarium oxysporum}
8. \textit{Curvularia lunata}
9. \textit{Penicillium frequentan}
10. \textit{Rhizopus species}
11. \textit{Mucor species}
12. \textit{Alternaria tenuis}.

The total counts obtained were however very low. The maximum being 150 colony forming units per sq. inch of the leaf blade. With \textit{Croton Capt Kidd} the count increased February onwards, reached a peak in June and then there was a decline. Another peak was obtained in October. The counts of \textit{Croton Katonii} were fluctuating maximum in May. \textit{Croton Daisy O} gave a continuous increase in fungal epiphytes from January to May, which suddenly dropped in June. Another peak was evident in October. Phylloplane fungi of \textit{Croton Appendiculatum} exhibited no definite pattern. With \textit{Croton Aucubaefolium} three peaks in fungal counts were observed, one in February, the other in May-June and third in Sept/Oct. (Fig.6).
Fig. 6

SEASONAL VARIATION IN FUNGAL PHYLOPLANE
POPULATION OF FIVE CROTON VARIETIES.
Thus maximum counts in fungal flora were associated with summer and October, both the periods characterized by high temperature. Of 12 fungi isolated, \textit{Alternaria} is a wellknown foliar pathogen. \textit{Cladosporium} has been reported as dominant resident of many plant species. The analysis of individual fungi in relation to season reveals that \textit{Aspergilli} were the most dominant fungal epiphytes on all the crotons tested. \textit{Penicillium sp.} followed \textit{Aspergilli} in dominance and were followed by \textit{Curvularia}, \textit{Fusarium} and \textit{Cladosporium} in turn.

Using Glycerol Aspergine agar medium, epiphytic actinomycetes were isolated by plating and washings of selected croton varieties for the entire year (Table 25). In general actinomycetes gave low counts upto 40 colony forming units/inch sq. of leaf. No definite pattern of variation was evident.

\textbf{Effect of change in season on the fluctuations in the Bacterial phylloplane population of selected croton varieties:}

From the observations recorded in Table 26 it is very clear that the fluctuation in the bacterial phylloplane population, from January to December was minimum in \textit{Croton Daisy 0} and \textit{Croton Appendiculatum} i.e. 50 and 51 colony
**TABLE NO.25**

Seasonal variation in population of Actinomycetes on Croton leaves.

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Croton</th>
<th>JAN</th>
<th>FEB</th>
<th>MAR</th>
<th>APR</th>
<th>MAY</th>
<th>JUN</th>
<th>JUL</th>
<th>AUG</th>
<th>SEPT</th>
<th>OCT</th>
<th>NOV</th>
<th>DECEMBER</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Croton <em>Aucubaefolium</em></td>
<td>30</td>
<td>20</td>
<td>10</td>
<td>30</td>
<td>30</td>
<td>40</td>
<td>10</td>
<td>20</td>
<td>10</td>
<td>10</td>
<td>20</td>
<td>10</td>
</tr>
<tr>
<td>2.</td>
<td>Croton Katonii</td>
<td>20</td>
<td>10</td>
<td>30</td>
<td>10</td>
<td>-</td>
<td>10</td>
<td>30</td>
<td>10</td>
<td>20</td>
<td>30</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>3.</td>
<td>Croton capt kidd</td>
<td>-</td>
<td>10</td>
<td>10</td>
<td>20</td>
<td>-</td>
<td>-</td>
<td>20</td>
<td>-</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>20</td>
</tr>
<tr>
<td>4.</td>
<td>Croton Appendiculatum</td>
<td>30</td>
<td>20</td>
<td>20</td>
<td>10</td>
<td>10</td>
<td>-</td>
<td>-</td>
<td>10</td>
<td>20</td>
<td>20</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>5.</td>
<td>Croton Daisy O.</td>
<td>20</td>
<td>10</td>
<td>20</td>
<td>20</td>
<td>-</td>
<td>10</td>
<td>-</td>
<td>20</td>
<td>20</td>
<td>30</td>
<td>-</td>
<td>10</td>
</tr>
</tbody>
</table>
forming units per petriplate respectively. However it was 170 in Croton Aucubaefolium and 166 in Croton Katonii. Fluctuation was found to be significant in the croton varieties with dense blotches whereas insignificant in the varieties with rare blotches.

**Effect of change in season on the fluctuations in the Fungal phylloplane population of selected croton varieties.**

From the observations recorded in Table 27 it is very clear that the maximum fluctuation in the fungal population was observed in Croton Capt Kidd i.e. by 11 colony forming units per petriplate. Other croton varieties did not show any significant fluctuation in their fungal flora. It indicated that the seasonal variation did not have any profound influence on the phylloplane fungal population.

**Effect of change in season on the fluctuations in the Actinomycetee phylloplane polulation of selected croton varieties.**

Fluctuations in the population of Actinomycetes were almost constant in all varieties (Table 28).
TABLE NO. 26

FLUCTUATION IN THE BACTERIAL PHYLLOPLANE
POPULATION OF THE CROTON VARIETIES IN THE
YEAR 1983.
<table>
<thead>
<tr>
<th>S. No.</th>
<th>Croton Aucubaefolium</th>
<th>Croton Kadonii</th>
<th>Croton Capt. Kidd</th>
<th>Croton Appendiculatum</th>
<th>Croton Daisy Q</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Dense</td>
<td>Moderate</td>
<td>Moderate</td>
<td>Sparse</td>
<td>Rare</td>
</tr>
<tr>
<td>2</td>
<td>419</td>
<td>347</td>
<td>295</td>
<td>139</td>
<td>081</td>
</tr>
<tr>
<td>3</td>
<td>249</td>
<td>181</td>
<td>215</td>
<td>088</td>
<td>050</td>
</tr>
<tr>
<td>4</td>
<td>170</td>
<td>166</td>
<td>080</td>
<td>051</td>
<td>050</td>
</tr>
<tr>
<td>5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

TABLE NO. 26

Concentration of bacterial colonies per sq. inch area of the leaf surface petri-plate. Minimum and maximum bacterial count per population, fluctuation in bacterial population.
**TABLE NO. 27**

FLUCTUATION IN THE FUNGAL PHYLLOPLANE

POPULATION OF THE CROTON VARIETIES IN

THE YEAR 1983.
<table>
<thead>
<tr>
<th>S. No.</th>
<th>C R O T O N</th>
<th>Concentration of blotches per sq. inch area of the leaf surface</th>
<th>Maximum fungal count per petriplate</th>
<th>Minimum fungal count per petriplate</th>
<th>Fluctuation in fungal population</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Croton Aucuboefolium</td>
<td>Dense</td>
<td>9</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>2.</td>
<td>Croton Katonii</td>
<td>Moderate</td>
<td>11</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>3.</td>
<td>Croton Capt kidd</td>
<td>Moderate</td>
<td>15</td>
<td>4</td>
<td>11</td>
</tr>
<tr>
<td>4.</td>
<td>Croton Appendiculatum</td>
<td>Sparse</td>
<td>8</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>5.</td>
<td>Croton Daisy Q</td>
<td>Rare</td>
<td>9</td>
<td>2</td>
<td>7</td>
</tr>
<tr>
<td>S. No.</td>
<td>Croton</td>
<td>Concentration of blotches per sq. inch area of the leaf surface</td>
<td>Maximum Actinomycetes count per petriplate</td>
<td>Minimum Actinomycetes count per petriplate</td>
<td>Fluctuation in Actinomycetes population</td>
</tr>
<tr>
<td>-------</td>
<td>----------------</td>
<td>---------------------------------------------------------------</td>
<td>------------------------------------------</td>
<td>------------------------------------------</td>
<td>----------------------------------------</td>
</tr>
<tr>
<td>1.</td>
<td>Croton Aucubaefolium</td>
<td>Dense</td>
<td>4</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>2.</td>
<td>Croton Katonii</td>
<td>Moderate</td>
<td>3</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>3.</td>
<td>Croton Capt. Kidd</td>
<td>Moderate</td>
<td>3</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>4.</td>
<td>Croton Appendiculatum</td>
<td>Sparse</td>
<td>3</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>5.</td>
<td>Croton Daisy O</td>
<td>Rare</td>
<td>2</td>
<td>0</td>
<td>2</td>
</tr>
</tbody>
</table>
TABLE NO. 28

FLUCTUATION IN THE ACTINOMYCETES
POPULATION OF THE CROTON VARIETIES
IN THE YEAR 1983.