CHAPTER 3

PHYSIOLOGICAL STUDIES

\[ \text{In vitro} \]
3. PHYSIOLOGICAL STUDIES

Physiological studies form the basis for mushroom crop management. They are prerequisite for effective planning and preparation for a successful crop production. Physiological studies include the study of responses of organism to their environment, the mechanism involved in their growth, development, reproduction and nutrient uptake. The complete physiological analysis is beyond the scope of present investigation. Therefore, only those physiological aspects which have a major and direct impact on vegetative cultivation of Pleurotus species on culture media were studied. This study includes:

- Evaluation of culture media.
- Study of growth pattern.
- Study of incubation period.
- Influence of temperature, and
- Influence of pH.

MATERIALS AND METHODS

Cultures:

The following seven species of Pleurotus were obtained from National Centre for Mushroom Research and Training (NCMRT), Chambaghat, Solan 173213, Himachal Pradesh:
1. *Pleurotus cornucopiae* P-120
3. *Pleurotus sajor-caju* P-10
4. *Pleurotus flabellatus* P-50
5. *Pleurotus membranaceus* P-90
6. *Pleurotus citrinopileatus* P-100
7. *Pleurotus fossulatus* P-80

All these *Pleurotus* species were evaluated for their suitability for cultivation in Gujarat along with *Pleurotus florida*, an existing cultivated species of Gujarat.

8. *Pleurotus florida* was obtained from the sporophore of the mushroom from a local grower. The culture was originally obtained from NCMRT.

**Media:**

Mushrooms grow on a wide variety of culture media and different agar formulations, both natural and synthetic. In the present investigation seven different solid media were utilized to evaluate their ability to support mycelial growth of *Pleurotus* species. The media employed were:

* Potato Dextrose Agar
* Malt Extract Agar
* Carrot Agar
* Cellulose Yeast extract Agar
* Sabouraud's Glucose Agar
* Czapex Dox Agar
* Martin Rose Bengal Agar
Formulation and preparation of media is placed at Appendix-I.

Sterilization of Glassware:

Corning glassware were used, after cleaning by chromic acid (mixture of 100 g potassium dichromate, 400 ml water and 600 ml concentrated sulphuric acid as suggested by Tuite, 1969), to remove alkali and spore present in new glass material. After rinsing with tap water the glassware were sterilized in an oven at 180 ± 5°C for 90 minutes before use.

Inoculation of media:

Using a sterile cupborer, 2 mm disc of actively growing mycelium was placed in the centre of the petridish (90 mm dia).

Measurement of growth:

The mycelial growth on different solid media was measured as an average diameter of growth after 10 days of incubation. The growth/day was also calculated.

Study of growth pattern:

The growth pattern of Pleurotus species was studied by observation and comparison of completely colonized Potato dextrose agar plates.

Incubation period:

The incubation period was recorded as the time required by Pleurotus species to colonize the entire surface of Potato dextrose agar plate.
Influence of temperature:
The optimum temperature for growth of *Pleurotus* species was studied by incubating the inoculated Potato dextrose agar plates at 5, 10, 15, 20, 25, 30, 35 & 40 ± 2°C temperature, and measuring average diameter of growth after 10 days of incubation. Each treatment was repeated three times.

Influence of H^+ ion concentration:
Potato dextrose agar plates having initial pH 4, 4.5, 5.0, 5.5, 6.0, 6.5 and 7.0 were inoculated and incubated at 25 ± 2°C for 10 days. The average diameter of growth after 10 days was measured.

Statistical analysis:
The data recorded were subjected to statistical analysis wherever required. The differences exhibited by the treatments in various experiments were tested for their significance (CD 0.05) by employing Randomized block design as described by Panse and Sukhatma (1967).

RESULTS AND DISCUSSION

3.1 EVALUATION OF CULTURE MEDIA:
Results presented in table 3.1 clearly indicate that rapid growth of all *Pleurotus* species was observed on Potato dextrose agar. The most rapid growth obtained was of *P. cornucopiae* and *P. florida*, both the species produced growth covering the entire surface of plate (90 mm) in 10 days of incubation. The other *Pleurotus* species namely *P. pulmonarius*,
Table 3.1: Mycelial growth of *Pleurotus* species on solid media

<table>
<thead>
<tr>
<th>Media</th>
<th><em>P. cornucopiae</em></th>
<th><em>P. pulmonarius</em></th>
<th><em>P. sajor-caju</em></th>
<th><em>P. flabellatus</em></th>
<th><em>P. membranaceus</em></th>
<th><em>P. citrinopileatus</em></th>
<th><em>P. fossulatus</em></th>
<th><em>P. florida</em></th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Potato dextrose agar</em></td>
<td>90 9.0</td>
<td>82 8.2</td>
<td>81 8.1</td>
<td>83 8.3</td>
<td>74 7.4</td>
<td>70 7.0</td>
<td>61 6.1</td>
<td>90 9.0</td>
</tr>
<tr>
<td><em>Malt extract agar</em></td>
<td>86 8.6</td>
<td>75 7.5</td>
<td>81 8.1</td>
<td>83 8.3</td>
<td>51 5.1</td>
<td>49 4.8</td>
<td>51 5.1</td>
<td>84 8.4</td>
</tr>
<tr>
<td><em>Carrot agar</em></td>
<td>72 7.2</td>
<td>62 6.2</td>
<td>82 8.2</td>
<td>81 8.1</td>
<td>46 4.6</td>
<td>42 4.2</td>
<td>46 4.6</td>
<td>71 7.1</td>
</tr>
<tr>
<td><em>Cellulose Yeast Extract agar</em></td>
<td>70 7.0</td>
<td>56 5.6</td>
<td>80 8.0</td>
<td>80 8.0</td>
<td>63 6.3</td>
<td>43 4.3</td>
<td>35 3.5</td>
<td>70 7.0</td>
</tr>
<tr>
<td><em>Sabouraud's Glucose Agar</em></td>
<td>60 6.0</td>
<td>43 4.3</td>
<td>51 5.1</td>
<td>70 7.0</td>
<td>34 3.4</td>
<td>20 2.0</td>
<td>22 2.2</td>
<td>53 5.3</td>
</tr>
<tr>
<td><em>Czapek Dox agar</em></td>
<td>43 4.3</td>
<td>46 4.6</td>
<td>36 3.8</td>
<td>31 3.1</td>
<td>23 2.3</td>
<td>46 4.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Martin Rosebengal agar</em></td>
<td>36 3.8</td>
<td>22 2.2</td>
<td>28 2.8</td>
<td>20 2.0</td>
<td>14 1.4</td>
<td>29 2.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>C.D. (0.05)</em></td>
<td>1.2</td>
<td>1.2</td>
<td>0.8</td>
<td>0.7</td>
<td>0.9</td>
<td>0.5</td>
<td>0.7</td>
<td>0.9</td>
</tr>
</tbody>
</table>

(a): average diameter (mm) of radial growth after 10 days (average of 3 replications); b: growth/day; Incubation temperature: 25±2°C
P. sajor-caju and P. flabellatus also produced relatively faster growth admeasuring 81 to 83 mm. The growth of P. membranaceus and P. citrinopileatus was found to be moderate measuring 74 and 70 mm respectively while P. fossulatus produced very slow growth admeasuring only 61 mm after 10 days of incubation.

The growth on malt extract agar was statistically at par with the growth on Potato dextrose agar measuring 86, 84, 83, 81 and 75 mm in respect of P. cornucopiae, P. florida, P. flabellatus, P. sajor-caju and P. pulmonarius. The other species also produced moderate growth on malt extract agar measuring 48-51 mm.

On carrot agar and cellulose yeast extract agar, P. sajor-caju, and P. flabellatus produced rapid growth measuring between 80-82 mm, it was statistically at par with their growth on Potato dextrose agar and malt extract agar. P. cornucopiae, P. florida, P. pulmonarius, P. membranaceus and P. citrinopileatus produced moderate growth ranging from 42-72 mm. However cellulose yeast extract agar was found to be the second best medium for P. membranaceus.

On Sabouraud's glucose agar P. flabellatus produced relatively significant growth measuring 70 mm, whereas P. citrinopileatus and P. fossulatus produced very poor growth measuring 20 and 22 mm respectively, the other Pleurotus species produced moderate growth ranging from 34 to 60 mm.

The growth on Czapex Dox agar was found to be between
23-48 mm whereas the growth of *Pleurotus* species on Martin rose bengal agar was between 14-38 mm.

Further analysis of data on individual basis in respect of *P. cornucopiae* has revealed that the growth rates were statistically at par on Potato dextrose agar and Malt extract agar. Slightly slow and statistically similar growth was observed on carrot agar and cellulose yeast extract agar whereas growth on semisynthetic media was slow. The growth rate pattern on various media with respect to *P. florida*, *P. pulmonarius*, *P. membranaceus*, *P. citrinopileatus* and *P. fossulatus* was almost same as that of *P. cornucopiae* except *P. membranaceus* which produced a significant growth on cellulose yeast extract agar. However the growth pattern of *P. sajor-caju* and *P. flabellatus* was slightly different, showing statistically similar growth on all four natural media, moderate growth on Sabouraud's glucose agar and poor growth on Czapex dox agar and Martin rose bengal agar.

Looking to the above mentioned data the natural media tested were found to be better than the semisynthetic media in supporting the growth of *Pleurotus* species. Potato dextrose agar was found to be superior than all other media producing rapid and better mycelial growth of all *Pleurotus* species. These findings are in accordance with the findings of Mehta (1985) for *P. sapidus*. Sharma (1984) has also reported both Potato dextrose agar and yeastal Potato dextrose agar as better media for different isolates of *P. eryngii*. Suharban
and Nair (1991) reported potatodextrose broth as the best medium for the cultivation of *Pleurotus* species. Quimio *et al.* (1990) described Potato dextrose agar as the simplest and most popular medium for growing the mycelium of most cultivated mushrooms.

In the present investigation malt extract agar was found to be the second best medium for all *Pleurotus* species except *P. membranaceus*. Similar results have been reported by Mehta (1985) for *P. sapidus* and Suherban and Nair (1991) for *Pleurotus* species. However Zadrazil (1978) has reported superiority of Malt extract medium for the cultivation of *Pleurotus* species. Dudka *et al.* (1978) and Jong and Peng (1975) reported 3% malt extract agar as the best medium for the growth of *P. cystidiosus*. Semisynthetic media were found to be moderate to poor for the growth of *Pleurotus* species. These results almost agree with the findings of Bukhalo (1973) and Jandaik and Kapoor (1975a).

Thus, the present findings clearly indicate the superiority of Potato dextrose agar for producing rapid and readily available growth of *Pleurotus* species and therefore, Potato dextrose agar was used for further studies, as well as for cultivating *Pleurotus* species as inoculum for spawn.

### 3.2 STUDY OF GROWTH PATTERN

As is evident from results presented in table-3.2 and photographs 3.1-3.4, three distinct patterns of growth were observed. *P. cornucopiae*, *P. florida*, *P. sajor-caju* and *P.
Table 3.2: Growth pattern and incubation period of *Pleurotus* species on Potato Dextrose Agar

<table>
<thead>
<tr>
<th>Name</th>
<th>Pattern of growth</th>
<th>Incubation period (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. cornucopiae</em></td>
<td>Appressed and white</td>
<td>10</td>
</tr>
<tr>
<td><em>P. pulmonarius</em></td>
<td>Less dense &amp; transparent</td>
<td>11</td>
</tr>
<tr>
<td><em>P. sajor-caju</em></td>
<td>Appressed and white</td>
<td>11</td>
</tr>
<tr>
<td><em>P. flabellatus</em></td>
<td>Appressed and white</td>
<td>11</td>
</tr>
<tr>
<td><em>P. membranaceus</em></td>
<td>Less dense and transparent</td>
<td>12</td>
</tr>
<tr>
<td><em>P. citrinopileatus</em></td>
<td>Less dense and transparent</td>
<td>13</td>
</tr>
<tr>
<td><em>P. fossulatus</em></td>
<td>Dense, white and fluffy</td>
<td>15</td>
</tr>
<tr>
<td><em>P. florida</em></td>
<td>Appressed and white</td>
<td>10</td>
</tr>
</tbody>
</table>
Photograph 3.1: Growth of *P. Sajor-caju* (P-10) and *P. Cornucopiae* (P-20) on potato dextrose agar.

Photograph 3.2: Growth of *P. flabelatus* (P-50) and *P. citrinopileatus* (P-100) on potato dextrose agar.
Photograph 3.3: Growth of *P. pulmonarius* (PP) and *P. membranaceus* (P-90) on potato dextrose agar

Photograph 3.4: Growth of *P. fossulatus* (P-80) and *P. florida* on potato dextrose agar
flabellatus produced white growth with a little aerial hyphae, which is described as appressed growth. The growth of P. pulmonarius, P. membranaceus and P. citrinopileatus was less dense and transparent, whereas growth of P. fossulatus was white, dense and with abundance of extensive aerial mycelium, described as fluffy growth. The growth pattern is the characteristic of an individual species and it may vary according to environmental conditions and are species dependent.

3.3 Study of Incubation Period:

It is obvious from data presented in table 3.2 that shortest incubation period of 10 days was recorded for P. cornucopiae and P. florida and 11 days of incubation period was recorded for P. pulmonarius, P. sajiro-caju and P. flabellatus. However, P. membranaceus took 12 days and P. citrinopileatus required 13 days whereas P. fossulatus showed highest incubation period of 15 days. Thus, P. cornucopiae, P. florida, P. pulmonarius, P. sajiro-caju and P. flabellatus are characterized by rapidity of growth and high saprophytic colonizing ability. This attribute facilitates a speedy penetration of the substrate which simplifies cultivation as a whole. Similar findings of rapidity of growth are reported for P. ostreatus, P. florida and P. salmoneostramineus by Zadrazil (1978).

3.4 Influence of Temperature:

The results presented in figure 3.1 clearly indicate that the temperature requirements of P. fossulatus was quite
Fig. 3.1: Effect of Temperature on mycelial growth

P. cornucopiae

\[ \text{Average dia mm} \]

\[ \begin{array}{c}
\text{Temperature } ^\circ \text{C} \\
5 & 10 & 15 & 20 & 25 & 30 & 35 & 40 \\
\end{array} \]

\[ \begin{array}{c}
0 & 20 & 40 & 60 & 80 & 100 \\
\end{array} \]

\[ \text{CD (0.05) : 8} \]

P. pulmonarius

\[ \text{Average dia mm} \]

\[ \begin{array}{c}
\text{Temperature } ^\circ \text{C} \\
5 & 10 & 15 & 20 & 25 & 30 & 35 & 40 \\
\end{array} \]

\[ \begin{array}{c}
0 & 20 & 40 & 60 & 80 & 100 \\
\end{array} \]

\[ \text{CD (0.05) : 9} \]

P. sajor-caju

\[ \text{Average dia mm} \]

\[ \begin{array}{c}
\text{Temperature } ^\circ \text{C} \\
5 & 10 & 15 & 20 & 25 & 30 & 35 & 40 \\
\end{array} \]

\[ \begin{array}{c}
0 & 20 & 40 & 60 & 80 & 100 \\
\end{array} \]

\[ \text{CD (0.05) : 8} \]

P. flabellatus

\[ \text{Average dia mm} \]

\[ \begin{array}{c}
\text{Temperature } ^\circ \text{C} \\
5 & 10 & 15 & 20 & 25 & 30 & 35 & 40 \\
\end{array} \]

\[ \begin{array}{c}
0 & 20 & 40 & 60 & 80 & 100 \\
\end{array} \]

\[ \text{CD (0.05) : 9} \]

Contd...
P. membranaceus

P. citrinopileatus

CD (0.05) : 8

P. fossulatus

P. florida

CD (0.05) : 6

CD (0.05) : 9
different from other *Pleurotus* species. No growth was recorded at 5, 30, 35 and 40°C temperatures. The growth was scanty at 10°C, which accelerated at 15°C, reached its peak at 20°C and decelerated subsequently. It is therefore, clearly established that the optimum temperature for the growth of *P. fossulatus* was 20 ± 2°C. This result is in keeping with the optimum temperature range of 18-22°C for the growth of *P. fossulatus* as reported by Upadhyay (1990b).

The other *Pleurotus* species exhibited no growth at 5°C and 40°C and scanty growth at 10°C. At 15°C the growth recorded was between 12-36 mm. At 20°C growth accelerated and at 25°C maximum growth of all the species was recorded. Most of the species showed relatively better growth at 30°C and poor growth at 35°C.

Further analysis of data has revealed that *P. cornucopiae* produced substantial growth at 20 and 25°C admeasuring 82 and 90 mm respectively. The growth at 30°C was observed to be relatively less measuring, 34 mm. *P. florida* and *P. pulmonarius* also produced similar results. Thus, the optimum temperature for the growth of these species was 25 ± 2°C and the temperature range for the significant growth was between 20-25°C. These findings are supported by Bahukhandi (1989), Delmass and Mamoun (1983) and Zadrazil and Schneidereit (1972).

*P. sajor-caju, P. flabellatus, P. membranaceus* and *P. citrinopileatus* exhibited moderate growth at 20°C, significant
growth at 30°C and maximum growth at 25°C temperature. Thus, the optimum temperature for the growth of these species was 25 ± 2°C and temperature range for significant growth was between 25-30°C. These findings are in agreement with the findings reported by Upadhyay (1990b).

Thus, all Pleurotus species except P. fossulatus produced maximum growth at 25 ± 2°C. Although the optimum temperature for the growth of P. fossulatus was found to be 20 ± 2°C, significant growth at 25 ± 2°C was also observed. Therefore, 25 ± 2°C was considered as ideal incubation temperature for all the Pleurotus species under study.

3.5 INFLUENCE OF pH:

Figure 3.2 represents the effect of pH on the growth of Pleurotus species. The perusal of data suggests that the growth at pH 4.0 and pH 7.0 was scanty for all Pleurotus species. The growth at pH 4.5 was relatively better. At pH 5.0 significant growth of all Pleurotus species was observed. P. citrinopileatus, however, exhibited maximum growth at this pH. The rest of the Pleurotus species exhibited maximum growth at pH 5.5. Good growth was also recorded at pH 6.0 for all Pleurotus species. P. cornucopiae and P. fossulatus showed identical growth at pH 5.5 and pH 6.0. Moderate growth was observed for all Pleurotus species at pH 6.5. These findings are similar to the findings of Hashimoto and Takahashi (1976), Zadrazil (1976, 1978) and Rangad and Jandaik (1977).
Fig. 3.2: Effect of pH on mycelial growth

P. cornucopiae

P. pulmonarius

P. sajor-caju

P. flabellatus

CD (0.05): 10

CD (0.05): 8

CD (0.05): 9

CD (0.05): 7

Contd...
P. membranaceus

Average dia mm

P. citrinopileatus

Average dia mm

P. fossulatus

Average dia mm

P. florida

Average dia mm

CD (0.05) : 9

CD (0.05) : 10

CD (0.05) : 10

CD (0.05) : 11

66
Thus, the optimum pH for all *Pleurotus* species except *P. citrinopileatus* was found to be pH 5.5. Although the optimum pH for *P. citrinopileatus* was 5.0, it however produced good growth at pH 5.5. Therefore, pH 5.5 was considered ideal pH for the cultivation of all *Pleurotus* species under study.