Efficient method of sporulation of *Cercospora beticola* causing leaf spot of palak

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Sugar beet leaf extract medium

For the preparation of sugar beet leaf extract medium, 200g of sugar beet leaves were finely ground and added to 1000 ml of distilled water and boiled for 5 minutes then strained through muslin cloth. 20 g of agar was added and volume was made to 1000ml. Medium was sterilized at 1.1 kg/cm² pressure for 15 min. Twenty ml of the medium was poured in 90 mm Petriplates. Such Petriplates were inoculated with 5 mm mycelial disc of *C. beticola*, cut from periphery of actively growing culture and incubated at 25°C. Observations were recorded on colony colour, margin and sporulation.

Placing the mycelial bits on palak leaf surface

This experiment was carried out in glass house. The mycelial bits were taken from sixteen days old non-sporulating culture of *C. beticola* and placed on the leaves of palak under congenial conditions like 25°C temp. and 95% RH. Observations were taken at an interval of 12 hr for sporulation and symptom development.

Morphological studies of *Cercospora beticola*

Spores of *Cercospora beticola* were taken from 3 methods mentioned above and mounted on a clean glass slide in lactophenol thoroughly in order to obtain a uniform spread, on which cover slip was placed. The spores were measured under high power objective using Motic images 2.0 software. The average size of the spore was calculated. For comparison, spores collected from naturally infected leaves used.

Induction of sporulation

The results presented in Table 2 and Fig.1 revealed that the radial growth of *C. beticola* was maximum on modified PDA medium with 12% of dextrose (89 mm) which was significantly superior over all other treatments. Absence of dextrose in the medium resulted in minimum mycelial growth (44.3 mm) and it increased with increase in dextrose concentration. The sporulation was found in modified PDA with 4%, 6% and 8% dextrose. In 6% dextrose, sporulation was more compared to 4% and 8%, whereas, sporulation was absent in other treatments. The spores were hyaline, many celled, long, needle shaped slightly broader at one end and pointed at the other end (Fig. 1).
Spore induction and morphological characters of *C. beticola*: a = on PDA with different concentration of dextrose; b & c = conidia in PDA with 4% and 6% dextrose (40x); d = mycelial bits on leaf surface; e = symptom development; f & g = conidiophores and conidia from mycelial bits on leaf surface (40x); h & i = growth and conidia (40x) on sugar beet leaf extract medium; j = variable size of conidia from infected host (40x); k = uniform size conidia (40x); l = long size conidia (40x).

**Table 2. Effect of dextrose concentration on growth and sporulation of *Cercospora beticola***

<table>
<thead>
<tr>
<th>Dextrose concentration (%)</th>
<th>Mean colony diameter (mm)</th>
<th>Sporulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>44.3</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>53.7</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>59.7</td>
<td>++</td>
</tr>
<tr>
<td>6</td>
<td>70.0</td>
<td>+++</td>
</tr>
<tr>
<td>8</td>
<td>75.0</td>
<td>+</td>
</tr>
<tr>
<td>10</td>
<td>83.7</td>
<td>-</td>
</tr>
<tr>
<td>12</td>
<td>89.0</td>
<td>-</td>
</tr>
</tbody>
</table>

S.Em = 1.18
CD at 1% = 3.58
CV = 3.01

+++ : > 40 spores / microscopic field
++ : 10-40 spores / microscopic field
+ : < 10 spores / microscopic field
- : No sporulation

**Sugar beet leaf extract medium**

After 16 days of incubation sporulation was observed in sugar beet leaf extract medium. There were less spores per microscopic field. The conidia were hyaline, septate, needle shaped and shorter than on modified PDA with 6% dextrose (Fig. 1).

**Placing the mycelial bits on palak leaf surface**

Conidiophores of *C. beticola* started developing 24 hrs after placing the mycelial bits on palak leaf surface and sporulation was observed 36 hours after incubation. Large numbers of conidia were observed in each microscopic field. The spores were hyaline, many celled, long and needle shaped (Fig. 1).

**Morphological studies of *Cercospora beticola***

The spores of the pathogen obtained by different methods, viz., modified PDA with 6% dextrose, Sugar beet leaf extract...
medium. The mycelial bits placed on palak leaf surface and naturally infected host were observed under high power (40x). Fifty spores of pathogen were observed under microscope and measured using Motic images 2.0 software.

The results presented in Table 3 revealed that there was great variation in the size of conidia of C. beticola obtained from different methods. Average length was maximum in spores obtained from mycelial bit placed on palak leaf surface (277.96 µm x 6.6 µm) and was followed by spores from PDA with 6% dextrose. This is the first report of inducing sporulation of C. beticola on modified PDA with 6% dextrose. In the present study, less sporulation was observed in sugar beet leaf extract medium and Minimum spore length was observed in Sugar beet leaf extract medium (121.9 µm x 4.2µm). These results are in accordance with the work of Nagel (7) and Calpouzous and Stalknecht (2).

Spores obtained from mycelial bit placed on host leaf surface were of more uniform length, (297.3 µm x 8.1 µm to 143.6 x 5 µm), whereas, spores obtained from PDA with 6% dextrose showed great variation with a range of 487.6 µm – 7.0 µm to 156.1 µm x 4.0 µm.

In the present investigation, C. beticola sporulated in all the three methods tested. The maximum sporulation was observed when the mycelial bit was placed on palak leaf surface by providing favourable conditions. It may be due to presence of optimum temperature of 25°C with relative humidity of more than 95% and the susceptible host which favoured sporulation of C. beticola. This method of spore induction is the new report of sporulation of C. beticola and results in heavy sporulation. In case of modified PDA with different concentration of dextrose, the mycelial growth increased as the concentration of dextrose increased but sporulation was observed only at 4%, 6% and 8%. Heavy sporulation was observed in 6% dextrose compared to 4% and 8% dextrose. Sporulation of C. beticola is favoured by moderate level of dextrose and higher as well as lower concentration prevented sporulation of C. beticola. Higher concentration of dextrose favoured only mycelial growth. Similarly Rangaswamy and Chandrasekharan (8) observed that sugars favour good growth of Cercospora sp.

In the present investigation the maximum average size of the spores were observed when mycelial bit was placed on host leaf surface (277.96µm x 6.6 µm) followed by PDA with 6% dextrose (270.5 µm x 6.3 µm) and small sized spores were observed in sugar beet leaf extract medium (121.9 µm x 4. 68 µm) followed by infected host (137.58µm x 7.49 µm) However, the maximum length of individual spore was more in modified PDA with 6% dextrose (487.6 µm x 7.0 µm).

Present study clearly indicated that maximum sporulation and long sized conidia of C. beticola were observed when mycelial bits were kept on palak leaf surface. Minimum sporulation and short sized conidia were observed in sugarbeet leaf extract medium.

REFERENCES


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<table>
<thead>
<tr>
<th>Measurement</th>
<th>Range (µm)</th>
<th>Average (µm)</th>
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</thead>
<tbody>
<tr>
<td>Spores from Potato dextrose agar medium with 6% dextrose.</td>
<td>487.6 µm x 7.00 µm – 156.1 µm x 4.00 µm</td>
<td>270.51 µm x 6.3 µm</td>
</tr>
<tr>
<td>Spores from Sugar beet leaf extract medium</td>
<td>210.5 µm x 4.5 – 79.1 µm x 3.00 µm</td>
<td>121.9 µm x 4.2 µm</td>
</tr>
<tr>
<td>Spores from mycelial bit placed on host leaf surface</td>
<td>277.3 µm x 8.1 µm - 143.6 µm x 5 µm</td>
<td>277.96 µm x 6.6 µm</td>
</tr>
<tr>
<td>Spores from infected host</td>
<td>277.3 µm x 7.0 µm – 61.0 µm x 4.2 µm</td>
<td>137.58 µm x 6.49 µm</td>
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