CULTURAL CHARACTERISTICS OF CERATOCYSTIS FIMBRIATA ELL. AND HALST. ON DIFFERENT SOLID MEDIA CAUSING WILT IN POMEGRANATE

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Abstract
Pomegranate wilt disease caused by Ceratocystis fimbriata is one of the important diseases of pomegranate adversely affecting crop cultivation in all major growing regions of India. At present, the crop is severely affected by wilt pathogen and day by day, the wilting severity is increasing at faster rate. Growth pattern of C. fimbriata on different solid media showed that, growth type was raised and mycelial colour, white to grayish and colony margin in petriplate, regular to irregular. Oat meal agar and carrot agar supported more pathogen growth. Endoconidia and aleurioconidia abundantly produced in all media tested. Production of perithecia was observed in carrot agar, malt agar, oat meal agar, potato carrot agar, potato dextrose agar and V8 juice agar except Czapek’s agar, host leaf agar, host stem agar, Richard’s agar, V8 juice and water agar.

Key words: Pomegranate, wilt, media, Ceratocystis fimbriata and disease.

Introduction
Pomegranate wilt disease caused by Ceratocystis fimbriata is one of the important diseases of pomegranate adversely affecting crop cultivation in all major growing regions of India. At present, the crop is severely affected by wilt pathogen and day by day, the wilting severity is increasing at faster rate. It was first noticed in two areas of the Bijapur district of Karnataka, India in 1990 which rapidly spreaded in the entire Bijapur district. The cause was not identified until 1995, however, the fungus C. fimbriata was isolated from discoloured stem, root and branch tissues on wilted plants in 1996. The disease is prevalent in parts of Maharashtra, Karnataka, Andhra Pradesh, Gujarat and Tamil Nadu States in India. Pomegranate wilt results in complete wilting of plant and is characterized by the initial symptoms as yellowing and wilting of leaves on one to several branches. Initially symptoms only occurred on shoots, but later, leaves of the whole tree turned yellow and wilted, causing extensive defoliation and dieback and the xylem of the trunk turned brown to black with a star burst-like pattern. Finally, heavy infection resulting in the whole tree dying, causing severe yield losses leading to death of affected plants in a few weeks leading to loss to the farmers. The fungus derive food and energy from the substrate upon which they grow in nature, in order to culture the fungus in the laboratory, there is no universal substrate or artificial medium upon, which all the fungi can grow and reproduce. Therefore, studies were conducted in different suitable media to identify surface medium for the growth and sporulation of Ceratocystis fimbriata.

Materials and Methods
Ceratocystis fimbriata, associated with wilt was isolated from the infected stems and roots of pomegranate plant, which were collected from Ganjalli field. The sliced pieces of collected stem portions with characteristic symptoms of vascular staining were surface sterilized with 1 per cent NaHCO₃ (sodium hypochlorite) for about 2 minutes and washed in alcohol (70%) and twice with sterile water to remove traces of NaHCO₃. Pathogen isolation was made using carrot bait technique (Moller and DeVay, 1968) in which, stems were placed in between the carrot disks and kept in a humid chamber
and incubated at 25 ± 2°C under 12 hour photoperiod (Moller and DeVay, 1968). After perithecia formation, a portion of the fungi was transferred to freshly prepared PDA and oat meal agar media to allow the full development of fungi. In order to confirm the identity of the fungus, the ascospores, aeroconidia, endoconidia and perithecia were observed under the high power (40x) microscope from Raichur isolates the pure culture. The identification of studies of pathogen has done as explained by Sharma et al. (2010).

The cultural characters of C. fimbriata were studied on the following twelve different solid media viz., Carrot agar, Czapek’s agar, host leaf agar, host stem agar, malt agar, oat meal agar, potato carrot agar, potato dextrose agar, Rhichard’s agar, Sabourds agar, V8 juice agar and water agar. Twenty ml of each medium listed above was poured into the Petri dishes for solidification. Five mm discs of C. fimbriata were placed at the centre of the plate. Each set of experiment was replicated thrice and plates were incubated at 26 ± 2°C. Observations were taken on parameters such as growth type, mycelial colour, type of margin, radial growth (mm) and presence or absence of endoconidia, aeroconidia and perithecia. When the fungus covered complete petriplate in the media. The results were analyzed statistically.

Results and Discussion

The experiment on cultural characters of C. fimbriata on different solid media was taken up and characters such as colony diameter, colony color, presence or absence of endoconidia, aeroconidia and perithecia were studied in this objective and results are summarized in table 1 and plate 1. The growth type of the fungus varied from flat to raised. The carrot agar, Czapek’s agar, host leaf agar, host stem agar, malt agar, oat meal agar, potato carrot agar, potato dextrose agar, Rhichard’s agar, V8 juice agar and water agar medium recorded flat type of growth, while C. fimbriata growth was raised on Sabourauds agar medium. The mycelial colour of C. fimbriata varied differently. The media viz., carrot agar, host leaf agar, oat meal agar, potato carrot agar, potato dextrose agar and V8 juice agar recorded grayish coloured growth. White colour growth was observed in Czapek’s agar, host stem agar, Rhichard’s agar and Sabourauds agar, while only malt agar medium showed light brown colour.

The fungus produced different type of growth margins on different media tested. Type of margin varied from regular (carrot agar, Czapek’s agar, host leaf agar, host stem agar, malt agar, oat meal agar,

<table>
<thead>
<tr>
<th>S. no.</th>
<th>Media</th>
<th>Growthtype</th>
<th>Mycelial colour</th>
<th>Type of margin</th>
<th>Radial growth (mm)</th>
<th>Perithecia production</th>
<th>Aleroconidia</th>
<th>Endoconidia</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Carrot agar</td>
<td>Flat</td>
<td>Grayish</td>
<td>Smooth, circular, regular</td>
<td>84.67(67.03) *</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>Czapek’s agar</td>
<td>Flat</td>
<td>White</td>
<td>Smooth, circular, regular</td>
<td>15.00(22.79)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>Host leaf agar</td>
<td>Flat</td>
<td>Grayish</td>
<td>Smooth, circular, regular</td>
<td>17.50(24.73)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>Host stem agar</td>
<td>Flat</td>
<td>White</td>
<td>Smooth, circular, regular</td>
<td>70.00(56.79)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>Malt agar</td>
<td>Flat</td>
<td>Light brown color</td>
<td>Smooth, circular, irregular</td>
<td>81.33(53.68)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>Oat meal agar</td>
<td>Flat</td>
<td>Grayish</td>
<td>Smooth, circular, regular</td>
<td>90.00(71.57)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>Potato carrot agar</td>
<td>Flat</td>
<td>White</td>
<td>Irregular</td>
<td>22.27(28.16)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>Potato dextrose agar</td>
<td>Flat</td>
<td>Grayish</td>
<td>Irregular</td>
<td>11.00(19.37)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>9</td>
<td>Richard’s agar</td>
<td>Flat</td>
<td>Grayish</td>
<td>Smooth, circular, regular</td>
<td>30.00(33.21)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>10</td>
<td>Sabourauds agar</td>
<td>Flat</td>
<td>White</td>
<td>Regular</td>
<td>10.00(18.43)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 1 : Effect of different solid media on growth of Ceratocystis fimbriata.

C.D at 1% = 1.93

*S. Em. ± 0.49 C.D at 1%*

- Absent, + Present, * Figures in parenthesis are sine transformed value.
Cultural Characteristics of *C. fimbriata*

**Fig. 1**: Mycelial growth of *Ceratocystis fimbriata* on different solid media.

**Plate 1**: Cultural characters of *Ceratocystis fimbriata* on different solid media.
potato carrot agar, Rhichard’s agar, V8 juice agar and water agar) to irregular (Sabourd’s agar and potato dextrose agar). Among the various solid media, significantly more growth of *C. fimbriata* was observed on oat meal agar with mean colony diameter of 90 mm followed by carrot agar (84.67 mm) and least mean colony diameter of 10.00 mm was observed in water agar. The results indicated that endoconidia and aleurioconidia abundantly produced in all media (carrot agar, Czapek’s agar, host leaf agar, host stem agar, malt agar, oat meal agar, potato carrot agar, potato dextrose agar, Rhichard’s agar, V8 juice agar, water agar media and Sabourauds agar medium). Production of perithecia was observed in carrot agar, malt agar, oat meal agar, potato carrot agar, potato dextrose agar and V8 juice agar and Czapek’s agar did not produce perithecia. Similar findings were reported by several workers (Rokibah et al., 1988; Bachiller, 1998 and Chaudhari et al., 2016). Sonyal et al. (2015) evaluated the eight solid media, among them the best mycelia growth was on oat meal agar (9.0 cm) followed by Richards agar (8.4 cm) and potato dextrose agar (8.3 cm).

**References**


