Influence of cultural media on the growth of *Fusarium moniliforme* causing foot rot disease

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**Abstract:** Foot rot of rice caused by *Fusarium moniliforme* (*Fusarium verticilloides*) has become a major problem on certain cultivars of rice under Punjab conditions. The pathogen produces different types of symptoms (elongation, stunting or both and death of plants) in nursery as well as in field. The influence of the cultural media on morphological, cultural characteristics and growth rate of *F. moniliforme* on four different media: Potato dextrose agar (PDA), Soil Extract Agar (SEA), Spezieller Nahrstoffarmer (SNA) and Water Agar (WA) were studied and macro-conidial features were observed on carnation leaf Agar (CLA) medium. The pathogen was isolated from the foot rot infected Basmati plants, collected from different agro-ecological zones of Punjab. The colony color in PDA and SEA was varied between whitish to pinkish whereas in SNA and WA media it was observed to be white. The maximum colony diameter of *Fusarium moniliforme* was observed on PDA with 82.33 mm diameter and growth rate of 25.72 mm/ 48hrs, followed by SEA with colony diameter of 79.67 mm and growth rate of 19.65 mm/48hrs. Poor growth rate was observed on SNA with 16.55mm/ 48hrs growth rate and 47.98mm colony diameter. Lowest was observed on Water Agar (WA) with 35 mm colony diameter and 6.5mm/ 48hrs growth rate. Mycelial growth was dense and fluffy on both PDA and SEA. Moderate and sparse to thin growth on SNA medium was observed. No chlamydospores were observed on all the media under study. Sporulation was highest in SNA (6 x10^5 spores/ ml), followed by SEA (5.7 x10^5 spores/ ml) and PDA (4 x10^5 spores/ ml). But the culture show poor sporulation in WA.

**Key words:** Foot rot, Media, Growth rate, sporulation

**Introduction**
Fungi have been considered as an ecologically interesting source of pigments, since some fungal species are rich in stable colorants. A number of media are used for isolation and identification of different groups of fungi. These culturing media influences the colony morphology and the vegetative growth, pigmentation and sporulation depending upon the composition of specific culture medium, pH, temperature, light, water availability and surrounding atmospheric gas mixture (Kumara and Rawal, 2008). Environmental factors such as temperature, pH and water activity have a great influence on fungal growth and development. Variation in the type of carbon and nitrogen sources besides changes in pH, temperature, incubation period, shaking and inoculum size have great influenced on the growth of pathogen (Bhattacharyya and Jha, 2011). Effects of temperature, pH and carbon source on radial growth rate have been well assessed by Yadav et al. (2014). Among the fungi producing stable colorants, *Fusarium moniliforme* has been known for producing variant pigments. *Fusarium moniliforme* is a ubiquitous fungus distributed all over the world. The nature of the surface growth pattern either flat or cottony type were also influenced by the availability of nutrition, although it differs from species to species and strain to strain (Carlile, 1995). Considering the importance of the culturing media, the present study was planned to study its effects on the culture of *F. moniliforme* to ascertain the distinguishable differences appeared in the culture.

**Materials and Methods**
*Fusarium moniliforme* were isolated from the foot rot infected basmati plants collected from different agro-ecological zones of Punjab and maintained on Potato Dextrose Agar (PDA). They were subjected to pathogenicity test on susceptible cultivar Pusa 1121 and all the isolates proved positive for Koch’s postulates. The isolates were then, identified morphologically as *Fusarium moniliforme* using “The Fusarium Laboratory Manual” (Leslie and Summerell, 2006) and molecular confirmation was done using VERT1 and VERT2. The growth characters of the confirmed *F. moniliforme* isolate was studied on four different solid media viz., Potato Dextrose Agar (PDA), Soil Extract Agar (SEA), Water Agar (WA) and Spezieller Nahrstoffarmer Agar.
The study revealed the colony color to be varied between white to pink. The findings corroborate to the findings of Pannu et al. (2013) who observed white color colony on Potato Dextrose Agar and Nutrient Glucose Agar, white-light red color on Richard’s Agar medium and light pinkish white color on Czapek’s Agar medium, white color on Water Agar and Spezieller Nahrstoffarmer Agar (SNA) medium. Leslie and Summerell (2006) also reported that pigmentation in the agar varies and ranges from no pigmentation or grayish orange in some isolates to violet grey, dark violet or dark magenta (almost black) in others. Atukwase et al. (2012) also reported that colonies on PDA generally produced white/purple color interspersed with rings of different shades of purple/white colors. Kaur et al. (2014) also observed the colony color of Fusarium spp causing foot rot to be white to purplish. From the cultural characteristics observed on different media, it can be concluded that different culture media influenced the growth and pigmentation of the culture. The sporulation was highest on SNA medium, followed by SEA medium as compared to PDA. However, there was least sporulation in WA medium. Yadav et al. (2014) also observed the maximum growth in medium containing rice husk and maximum sporulations in medium containing sugarcane bagasse. The finding was supported by the fact that most fungi grow best on PDA, being rich in nutrients, supported the flush growth of mycelia leading to ultimate loss of sporulation Pradeep et al. (2012), Pannu et al. (2013), Kaur et al. (2014). However, the concept apparently failed in case of WA medium where poor sporulation was observed despite being less nutrient content. But, this can be explained by the fact that WA medium is very poor in nutrient content that it fails to support the normal growth of the fungus.

Among all the media, macro-conidia were formed only on CLA medium. Same observation has also been suggested by Leslie and Summerell (2006). This reveals that the components of the media influenced the production of macro-conidia. No literature has been found on what particular component of CLA medium triggered the production of macro-conidia but the importance and significance of the medium has been described in “The Fusarium Laboratory Manual” by Leslie and Summerell (2006). Atukwase et al. (2012) and Kaur et al. (2014) reported falcate to cylindrical macro-conidia with 3-5 septa. Yadav et al. (2014) reported micro-conidial size of 5-12X 1.5-2.5µm with 0-1 septum and hyaline, long fusoid, tapered to the ends, straight or curved macroconidia of 25-60 X 2.5-4µm with 3-7 septa. The findings also corroborate to the reports of Zainudin et al. (2008) and Bashyal et al. (2015). Ahangar et al. (2014) also reported micro-conidial size of 4-10 x 1-2 im and macro-conidial size of (20-50 x 2.0-3.5) im. F. moniliforme produced pale orange color sporodochia but normally they are absent (Pradeep et al. 2014). Hwang et al. (2013) studied the colony morphology, colony diameter on PDA and its color (whitish pink with active sporulation). They reported the production of micro and macro-conidia and in particular, micro-conidia in chains on conidiophores, which is one of the important mycological characteristics of F. fujikuroi.
Plate-1: Cultural characteristics observed on four different cultural media

1.1 Cultural on PDA
1.2 Culture on SEA
1.3 Cultural on SNA
1.4 Culture on WA

Plate-2: Morphological characteristics observed on the different cultural media

2.1 Micro-conidia
2.2 Micro-conidia borne on phialides
2.3 Sporodochia formed on Carnation leaf Agar media
2.3 Macro-conidia formed on Carnation leaf Agar medium

Influence of cultural media on growth of *Fusarium moniliforme*
Table-1 Effect of different media on colony color and sporulation

<table>
<thead>
<tr>
<th>Sl.No.</th>
<th>Medium</th>
<th>Colony diameter (mm) upto 8th day</th>
<th>Growth rate (mm/ day) growth</th>
<th>Mycelial growth</th>
<th>Colony colour</th>
<th>Sporulation (spores/ ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Potato Dextrose Agar (PDA)</td>
<td>82.33</td>
<td>25.72</td>
<td>Dense, cottony and fluffy</td>
<td>Whitish to pinkish</td>
<td>4x10^5 (Good sporulation)</td>
</tr>
<tr>
<td>2</td>
<td>Soil Extract Agar (SEA)</td>
<td>79.67</td>
<td>19.65</td>
<td>Moderately dense and cottony</td>
<td>Whitish to pinkish</td>
<td>5.7x10^5 (Good sporulation)</td>
</tr>
<tr>
<td>3</td>
<td>SpeziellerNahrstoffarmer Agar (SNA)</td>
<td>47.98</td>
<td>16.55</td>
<td>Moderate and not fluffy</td>
<td>White</td>
<td>6 x10^5 (Medium sporulation)</td>
</tr>
<tr>
<td>4</td>
<td>Water Agar (WA)</td>
<td>35</td>
<td>6.5</td>
<td>Poor growth and not fluffy</td>
<td>White</td>
<td>1.0x10^6 (Poor sporulation)</td>
</tr>
</tbody>
</table>

The effect of different cultural conditions on the growth and pigment production by *Fusarium moniliforme* causing bakanae disease has been reported in a number of publications Ilija et al. (2009), Pradeep et al. (2013); Pannu et al. (2013) and Yadav et al. (2014). From the present findings too, conclusion can be drawn that cultural characteristics, morphological features, sporulation of *Fusarium moniliforme in vitro* are affected by the nutrient component of the media.

References


