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Cultural characterization of *Colletotrichum graminicola* causing anthracnose of sorghum

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Abstract
Sorghum (*Sorghum bicolour* (L.) Moench) is a major staple food crop and the fifth most important cereal crop in the world. However, its production in India is adversely affected by different biotic and abiotic constraints and among them sorghum anthracnose caused by *Colletotrichum Gramin cola* is one of the most damaging diseases. It is also responsible for major economic loss of sorghum production worldwide, especially in tropical and sub-tropical countries. In the present study, an *in vitro* experiment was conducted to account for the influence of nutritional and physiological factors on vegetative growth of the pathogen. Among the six different media tested, Potato Dextrose Agar (PDA) and Oatmeal Agar (OMA) were found best suitable for mycelial growth of the pathogen. The optimum temperatures for *C. graminicola* were 30 °C and 25 °C. *C. graminicola* grows best at pH-6.5 followed by pH-7. The present finding can assist in understanding the growth nature of the pathogen as well as adoption of better management practices.

Keywords: Sorghum, *Colletotrichum graminicola*, media, temperature, pH

Introduction
Sorghum (*Sorghum bicolour* (L.) Moench) is a major staple food crop in the semi-arid and tropical regions of the world. It is carbohydrate-rich, multi-purpose crop playing a significant part in providing food security and is extensively produced in Africa, China, the United States, Mexico, and India (Khaton *et al*., 2016) [7]. In Asia, India is the main producer of sorghum despite the crop being mostly cultivated by small and marginal farmers in the stress-prone semi-arid regions (Chapke and Tonapi, 2019) [3]. The production of sorghum in India is adversely affected by different biotic and abiotic constraints. Among the biotic stress are several fungal diseases namely anthracnose, zonate leaf spot, Gray and rough leaf spot, leaf blight, rust, smut and grain mild, of which, anthracnose caused by *Colletotrichum graminicola* (Ces.) Wilson is the one of the most destructive foliar fungal diseases of sorghum damaging leaves, stalks, peduncles and panicles (Harris and Fisher, 1973) [5]. Sorghum anthracnose can reduce the crop yield (>50%) in susceptible cultivars and in areas with alternate dry and humid weather conditions combined with moderate to high temperatures (Prom *et al.*, 2018) [13]. Sorghum anthracnose occurs in four phases including root rot at seedling stage, leaf and sheath phase, stalk rot and grain mild (Little *et al*., 2018) [9]. The symptoms initially appear as circular and/or elliptical spots which elongate and coalesce covering the entire leaf with the presence of few or many fungal fruiting bodies known as acervuline within leaf lesions that proceed to stalk rot, peduncle breakage and grain deterioration (Chala *et al.*, 2010) [2]. *C. graminicola* can infect all tissues of sorghum and thereby, can devastate the whole crop in absence of efficient disease management practices (Mengistu *et al.*, 2018) [10]. The environmental temperature and pH play an important role in the growth and differentiation of microorganisms. Therefore, the studies of cultural, and morphological characters of the pathogen are of immense importance in understanding the nature and adaptability of the pathogen in different environmental and nutritional conditions.

Considering the importance associated with the anthracnose disease of sorghum, the objective of this investigation was to identify best supporting medium as well as different temperatures and pH which may optimize abundant growth of the fungus. Thereby, the present studies will assist in understanding of pathogenicity of the pathogen as well as adoption of better management practices.

Materials and Methods

Isolation and culturing of test pathogen

Leaf samples with typical anthracnose symptoms were collected from sorghum fields at the livestock research centre, G. B. Pant University of Agriculture and Technology, Pant Nagar (Uttarakhand). The pathogen was isolated from the leaves of sorghum showing typical anthracnose symptoms by standard tissue isolation method. Two-to-three-millimetre (mm) size bits of infected tissue were cut at the junction of diseased and healthy portion with the help of disinfected blade. These bits were surface sterilized in 0.1 per cent sodium hypochlorite solution for about 1 minute followed by three washing with sterilized distilled water aseptically using laminar air flow chamber. After blot drying with sterilized filter paper, these bits were placed on potato dextrose agar (PDA) medium in sterilized Petri plates and incubated in BOD incubator for three days at 27±2 °C and further purified by single spore isolation method on PDA medium. Pure cultures were preserved in PDA slants at 4°C, for further use.

Effect of different solid media

Pant Nagar isolate of *C. graminicola* was used for the study of cultural characters on six non-synthetic/semi-synthetic media, viz. potato dextrose agar (PDA), oatmeal agar (OMA), corn meal agar (CMA), V8 agar and two synthetic solid media that includes Richard's agar and Capek Dox agar. Twenty ml of each medium was poured aseptically into 90 mm diameter Petri plates. Five mm discs of the *C. graminicola* were collected from actively growing culture using a cork borer and a single disc was placed at the centre of Petri plates and incubated at 27±2 °C. Cultural characteristics such as the colony colour, growth pattern and colony diameter were recorded with the help of metric scale on the tenth day after inoculation.

Effect of different temperature regimes

The suitability of different temperature regimes on the mycelium growth of the test fungus was studied. Twenty ml of fresh PDA medium was poured aseptically into 90 mm diameter Petri plates. After solidification, five mm mycelium disc of the *C. graminicola* was placed at the centre of Petri plate. Each set of experiment were replicated thrice and incubated at 10, 15, 20, 25, 30, 35 and 40 °C temperatures. The observations on radial growth were recorded on the tenth day after inoculation.

Effect of different pH levels

The effect of different pH on the growth of the test fungus was studied using PDA medium. The basal medium was adjusted at pH-4, pH-5, pH-6, pH-6.5, pH-7 and pH-8 levels, by using NaOH (0.1 N) and HCl (0.1 N) solution. The procedure of inoculation, incubation and recording of data were the same as described for media and temperature. Descriptive statistics and ANOVA were calculated using R software 'vR-4.2.2'.

Results and Discussion

In the present study, the results of ANOVA indicated highly significant (p<0.01) differences among the different solid media, temperature regime and pH evaluated for the growth of *C. graminicola* (Table 1).

Table 1: Descriptive statistic and ANOVA for the growth of *C. graminicola* on different solid medium, temperature regime and pH

	Media	Temperature	pH
Mean	63.8±0.5 mm	42±1.0 mm	62.7±0.6 mm
Maximum	73.9	60	70.5
Minimum	52.6	15.6	46.3
MSS	2.06	8.28	2.63
CD (0.01)	1.7	3.2	2.3

Effects of different solid media on growth characteristics

The *C. graminicola* culture showed distinctive morphological characteristics on the different media. Colonies colour varied from grey to white and growth pattern varied from raised to flat, compact to sparse mycelium with regular to irregular margins. In the media, varied pigmentation was observed from light yellow, creamy and white to no pigmentation (Fig.1). These results were in accordance with Pande *et al.* (1991) [12] and Latha *et al.* (2003) [8]. Pande *et al.* (1991) [12] reported that colonies of *C. graminicola* isolates on PDA ranged from white to a grey to dark brown with common light tan and colonies were felty to woolly, tufted and compact with well-defined to faint or no zonation. Latha *et al.* (2003) [8] observed that on OMA medium, colony growth was raised or submerged and woolly to felty with white-grey colour. Maximum mycelial growth of *C. graminicola* was recorded on PDA medium (73.9 mm), which was observed to be on par with OMA (72.0 mm) and minimum radial growth (52.6 mm) was observed on V8 agar medium (Table 2; Fig. 2a). Whereas Jamil and Nicholson (1989) [6] observed maximum mycelium growth on OMA medium.

Effect of different temperature regimes on mycelial growth

Further, the study showed that radial growth of *C. graminicola* was significantly influenced by temperature. Maximum radial growth of the pathogen was recorded at 30 °C (60.0 mm), followed by 25 °C (57.8 mm), 35 °C (44.7 mm) and 20 °C (34.6 mm) (Fig. 2b). Although the mycelial growth was restricted, a considerable mycelial growth was recorded at temperature 40 °C (29.0 mm), 15 °C (26.0 mm) and 10 °C (15.6 mm) indicating that the fungus can strife in varied temperature regimes. The present finding was in congruent with Yang *et al.* (2000) [15]. They observed that *C. graminicola* grew and sporulated in a wide range of temperature regimes, with optimal temperature at 27 to 30 °C. Similarly, Thomas *et al.* (2008) [14] reported that fungal growth was observed at temperatures from 10 to 30 °C with maximum growth rate occurring at 25 °C. For *Colletotrichum gloeosporioides*, the causal agent to anthracnose of mango the minimum, optimum and maximum temperatures were 20 °C, 30 °C and 40 °C, respectively (Ansari *et al.*, 2018) [1].

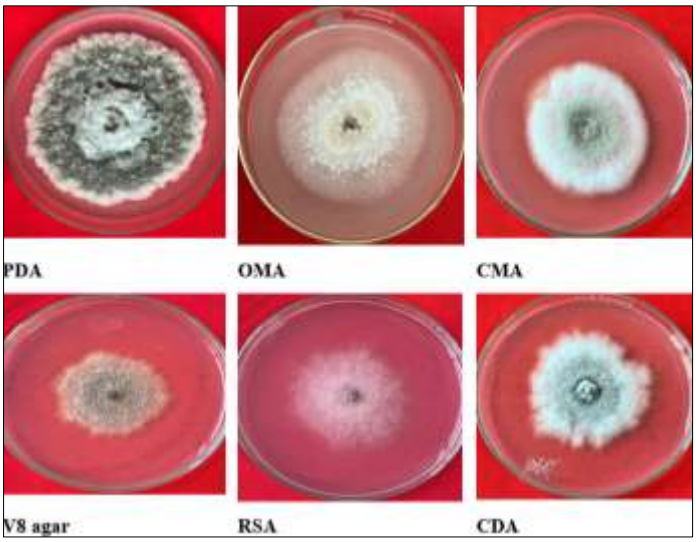

Fig 1: Cultural characteristic of *C. graminicola* on different solid media

Table 2: Effect of different solid media on the growth of *C. graminicola*

Medium	Mycelium colony			
	Mean radial growth (mm)	Colour	Growth pattern	Pigmentation
Potato dextrose agar	73.9	Olive grey centre and white edge	Raised, compact with regular margin	Light yellow
Oat meal agar	72.0	white centre and lighter towards the edge	Raised, compact with regular margin	white
Corn meal agar	66.33	Greyish white	Flat and sparse with regular margin	creamy
V8 agar	52.6	Grey	Flat and sparse with irregular margin	creamy
Richard's synthetic agar	58.8	white	Flat and sparse with irregular margin	No pigmentation
Capek Dox agar	59.3	Greyish centre and white towards the edge	Flat and compact and irregular margin	Creamy

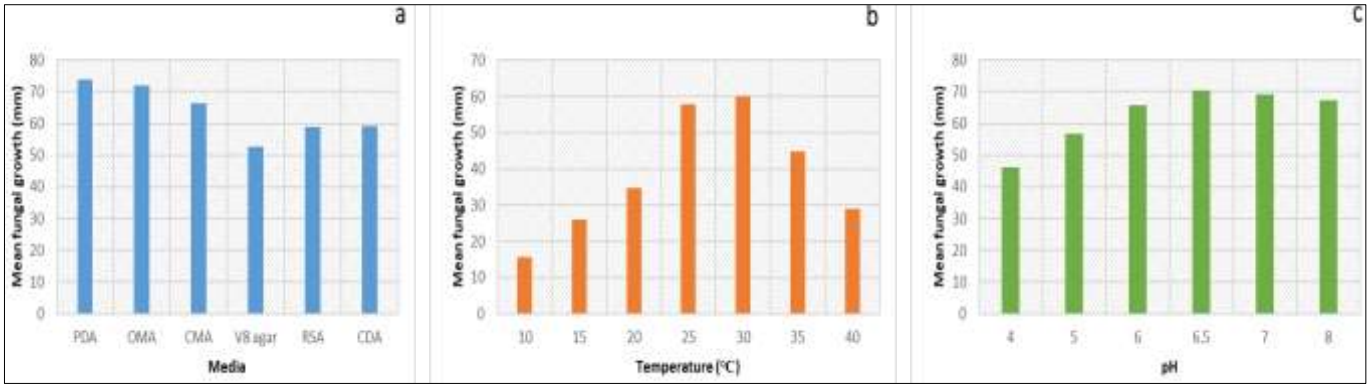

Fig 2: Effect of different solid media on the colony growth of *C. graminicola* (a), effect of different temperature regimes on the growth of *C. graminicola* (b), effect of different pH level on the growth of *C. graminicola* (c)

Effect of different pH levels on mycelial growth

The mycelium radial growth of *C. graminicola* varied with the changing pH on culture medium. Maximum mycelium radial growth of 70.5 mm was recorded at pH-6.5 followed by 69.3 mm at pH 7. The least radial growth was observed at pH 4 with 46.3 mm in diameter. The data further reveal that there was a slightly decreasing trend in mycelium growth at pH below 6.5 and above 7 (Fig. 2c). These data indicate that the pH of the medium significantly influence the mycelium growth of *C. graminicola.* In their study, Nitzan and Tsror (2003) [11] reported that the optimal pH level for growth of all isolates of *C. coccids* isolates from different VCGs was pH-6 and pH-7. Similarly, Ansari *et al.* (2018) [1] reported that *C. gloeosporioides*, grows best at pH-5 followed by pH-6. For *Colletotrichum causatum*, the causal agent of anthracnose of strawberry the mycelial growth, sporulation and germination of all strains reach an optimum at pH-5, 5.5, 6 and 6.5 (Es-Soufi *et al.*, 2018) [4].

Conclusion

During the course of their development, fungi generally require different nutritional and physiological conditions. The growth of *Colletotrichum graminicola* varied in different growth medium, temperature and pH levels studied. PDA medium was found most suitable followed by OMA whereas V8 agar was found least suitable for mycelial growth of this pathogen. *C. graminicola* grew well at 30 ℃ and 25 ℃ temperatures. Reasonable mycelium growth was supported even at 40 ℃. Among the different pH, optimum radial growth was recorded at pH-6.5 followed by pH-7.

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Authors' contributions: Yogendra Singh conceived the study and supervised the research. B K Namriboi performed all the experiments, data collection, analysis and the first draft of the manuscript was written. All authors commented, read and approved the final manuscript.

Conflicts of interest: The authors report no conflicts of interest.

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