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Cultural and physiological studies of *Colletotrichum lindemuthianum* (Sacc. and Magn.) causing anthracnose of field bean

HB Arpitha, M Mahesh, P Venkataravana, Ambruthavarshini, Dr. Devaraja and YH Preeti

Abstract
To understand the growth and sporulation of the pathogen, the physiological and cultural characteristics of *Colletotrichum lindemuthianum* were investigated on six distinct solid media at various pH and temperature levels. The colony growth was significantly superior on Richard's agar medium with mean colony diameter of 90.00 mm which showed on par with Potato dextrose agar (88.00 mm). Varied degrees of colony colour were observed with brown to white colour having smooth to serrated margin. Among the various degrees of temperature evaluated, with a mean colony diameter of 82.63 mm, it was discovered that 30 °C was the optimal temperature for the pathogen's growth, followed by 25 °C with colony diameter of 71.44 mm. Of the six distinct media, the one with the least amount of fungal growth was observed at 15 °C and the average colony diameter being 6.72 mm. Out of the six tested media Potato dextrose agar and Richard's agar media were determined to be the most conducive to *C. lindemuthianum* growth having an average colony diameter of 63.18 mm and 61.34 mm respectively. All the pH levels tested supported growth of the pathogen in different media. The colony diameter of the fungus measured at pH 7 was 78.76 mm, indicating its mean maximum growth. This was succeeded by pH 8 and pH 6, where the colony diameter measured 69.78 mm and 62.27 mm, respectively. Richard's agar and potato dextrose agar were shown to be the most effective among the studied media, with mean colony diameters of 74.01 mm and 75.07 mm, respectively, across all pH regimes.

Keywords: *Colletotrichum*, cultural studies, physiological studies and anthracnose

1. Introduction
Field bean is an important herbaceous versatile legume crop raised for pulse, vegetable and forage purposes. It is used for human consumption as an excellent protein source and vegetable in the form of immature pods, immature and mature seeds. It is an essential source of food source to people of all income categories, especially to the poor farmers as a source of dietary protein (Wortmann *et al.*, 1998) [12]. India is the world's top producer of field beans, with 0.085 million hectares under cultivation, 0.030 million tons production and a productivity of 236 kg/ha. With an area of 0.38 lakh hectares, production of 0.29 lakh tons and productivity of 649 kg/ha, Karnataka state alone accounts for about 90% of India's field bean production (Anon., 2019) [2]. Despite the state's expanding acreage dedicated to this crop, productivity is noticeably poor. (Rekha and Mallapur, 2007) [11].
Anthracnose caused by *Colletotrichum lindemuthianum* (Sacc. and Magnus) Briosi and Cavara is an important disease that affects field bean throughout the world and severe in tropical and subtropical regions (Corrales *et al.*, 1995) [3]. It is one of the important seed borne diseases of field bean (Amin *et al*., 2014) [1]. It affects all the plant parts *viz*., leaves, pods and seeds. The disease has been identified throughout the bean-producing regions of country where the growth season is characterized by cool and humid weather. The onset of a brick-red to purplish-red discoloration along the veins on the lower surface of the leaves is the first indication of an infection. As the disease progress similar symptoms will manifest on the upper surface of leaves. Infection of pods directly damages the seeds and results in yield loss (Manjunath *et al.* 2012) [10].
C. lindemuthianum produce hyaline, single celled, oblong, sickle shaped conidia with oil globules, sometimes with blunt end. The fungus drives its food from the substrates upon which it is grown in laboratory. As *C. lindemuthianum* is a slow growing pathogen and is mostly grown on Potato Dextrose Agar medium, thus, a comparison study was undertaken to

determine the best medium among the tested media for rapid mycelial growth of the fungus. The fungal growth is greatly influenced by the substrate present in culture media, temperature and pH of the growth media. All these factors *viz.*, growth media, pH and temperature play important role in growth, sporulation and other activities of fungi. Therefore, present studies on the influence of cultural and physiological factors on growth and sporulation of *C. lindemuthianum* was carried out.

2. Materials and Methods

2.1 Collection of diseased samples of field bean

Field bean leaves and pods displaying the typical symptoms of field bean anthracnose were collected and used from local fields and subjected for isolation of the pathogen.

2.2 Isolation, identification and purification of anthracnose causing pathogen

Following the microscopic confirmation of *Colletotrichum* spores in the specimens, by using conventional tissue isolation technique, the causal organism was isolated by using the plant sections displaying the typical symptoms of anthracnose disease. (Dhingra and Sinclair, 2012) [6]. Initial observation of fungus was done by observing morphological characters. The culture obtained was purified by the single spore isolation method and subsequently the resultant pure culture was maintained on PDA slants at 25±1 °C in an incubator. Identification of the pathogen causing anthracnose of field bean was carried out by observing cultural and morphological taxonomic characters like mycelial characteristics, conidia and the fruiting body under the microscope and after that, their identities were determined by comparison with the standard literature that was available. The pathogen accountable for anthracnose of field bean was identified by closely examining the cultural and morphological taxonomic features such as mycelial characteristics, conidia and the fruiting body under a microscope and cross-referencing the results with the existing standard literature.

2.3 Cultural studies

The cultural characteristics of fungus were examined using six distinct solid media *viz.*, Czapek's Dox agar medium, Richard's agar medium, Potato dextrose agar medium, Potato sucrose agar medium, Yeast extract agar medium and Oat meal agar medium. Petri plates containing various cultural media were inoculated with 5 mm disc of the pathogen. Each set of experiment was replicated thrice and incubated at 28±1 °C. When the highest growth was achieved in any one of the tested media, the observations were made. Records were kept on cultural traits like colony diameter, growth rate, colour, kind of margin, and sporulation.

2.4 Physiological studies

2.4.1 Effect of temperature and pH on growth of pathogen

In the six distinct solid mediums described above, development of the fungus was examined at various pH levels (5, 6, 7, 8, 9, and 10) and temperatures (15, 20, 25, 30, and 35 °C). The hydrogen ion (pH) concentration of media was determined by using pH meter. Adjustment of pH was done by using 0.1 N alkali (Sodium hydroxide) and 0.1 N acid (Hydrochloric acid) and autoclaved at 121.6 °C for 15 minutes. 15mL of each medium with different temperature and pH levels was independently added to each Petri plate, and left to solidify. These plates were inoculated with 5 mm discs of the pathogen and incubated at 28±1 °C. Observation was taken when the growth of any culture covered the entire Petri plate. Three replications of each treatment were carried out in the experiment by utilizing Completely Randomized Design (CRD). By taking the colony width into account, the optimal pH for growth of the fungus was ascertained.

3. Results and Discussion

3.1 Cultural studies of *C. lindemuthianum*

Below are the findings from the use of six distinct solid medium to analyse the pathogen's cultural characteristics. (Table 1, Plate 1 and Fig 1). The fungal colony growth was significantly faster on Richard's agar medium with average colony diameter of 90.00 mm succeeded by Potato dextrose agar medium (88.00 mm) which was on par with Richard's agar medium statistically. However, moderate growth was observed on Yeast extract agar with average colony diameter of 84.57 mm. The colony growth of 75.17 mm was identified on Oat meal agar. Colony growth on Czapek's Dox agar and Potato sucrose agar media was on par with each other with mean colony diameter of 69.00 and 67.13 mm, respectively. However, the least growth was recorded on Potato sucrose agar having mean colony diameter of 67.13 mm.

Variations in the pathogen's colony characteristics, including colour, texture, development rate, and kind, were noted throughout the investigation on various solid media (Table 1). The colour of the colonies ranged from white to brown. Light yellow to white colony was observed on Richard's agar and completely white colour colony was observed on Potato dextrose agar, Oat meal agar and Czapek's Dox agar. In Yeast extract agar and Potato sucrose agar the colony colour was light brown at centre surrounded by white mycelia. Sparse colony texture was observed on Richard's agar. White cottony fluffy growth was observed in Potato dextrose agar and Czapek's Dox agar. Slightly fluffy colony was observed on Yeast extract agar and Oat meal agar whereas, irregular white cottony with fluffy growth was recorded on Potato sucrose agar medium. Growth rate was good and fast on Richard's agar, Potato dextrose agar and Yeast extract agar. However, it was moderate in Potato sucrose agar, Oat meal agar and Czapek's Dox agar. Type of margin varied from smooth to serrated. Completely serrated margin was observed on Richard's agar and Czapek's Dox agar media. The margin was slightly serrated on Yeast extract agar. Smooth margin was identified on Potato dextrose agar and Oat meal agar and Potato sucrose agar media. Sporulation of the pathogen was recorded in Richard's agar, Potato dextrose agar and Potato sucrose agar media. However, sporulation was not observed in Oat meal agar and Yeast extract agar media.

It is likely that the nitrogen supply in the media caused the variation in the pathogen's development and sporulation. Some media supported the growth very well but others fail to support good growth, development and sporulation. The results are supported by the findings of Kulkarni (2019) [8], Dev *et al.* (2017) [5] who reported excellent sporulation and highest radial growth of pathogen on Richard's medium and Oat meal agar, which showed on par with and Potato dextrose agar medium.

3.2 Physiological studies of *C. lindemuthianum*

3.2.1 Effect of different temperature levels on growth of *C. lindemuthianum* on different solid media

Temperature is the most important factor influencing the growth and metabolism of *C. lindemuthianum.* Different levels of temperature *viz.,* 15 °C, 20 °C, 25 °C, 30 °C and 35 °C on the growth of *C. lindemuthianum* were studied.

Differences in the pathogen's growth among the different temperature levels on different solid media was found statistically significant (Table 2, Plate 2 and Fig 2). Among the different temperature levels evaluated, 30 °C was revealed to be the best temperature for growth of the pathogen and the subsequent best was 25 °C. All the temperature levels tested supported the growth and development on different solid media. However, the growth of the pathogen was very less at 15 °C in all the tested media. At 30 °C the growth was supported very well by all the media used. The complete growth of 90.00 mm diameter was observed in Richard's agar and PDA media followed by Czapek dox gar (88.15 mm) media. The least growth was documented in Oat meal agar medium (68.40 mm). Similarly at 25 °C the highest possible growth was noticed on Richard's agar (89.80 mm) followed by PDA media (88.13 mm). The least amount of increase in growth was seen in Yeast extract agar media (58.40 mm). The growth of the pathogen at 20 °C and 35 °C varied significantly in all tested media. At 35 °C the highest growth was observed in PDA (73.10mm) and the least growth was observed on Oat meal agar with 58.20 mm of colony diameter. At 20 °C maximum growth was observed in PDA (58.80 mm) and the least growth was observed on Czapek's Dox agar medium (41.10 mm). At 15 °C, pathogen's growth was very meagre. At 15 °C temperature, the growth was supported by Potato sucrose agar, Czapek's Dox agar, Yeast extract agar, PDA, and Oat meal agar media with colony diameter of 11.15 mm, 9.82 mm, 8.30 mm 5.90 mm and 5.15 mm respectively. Fungal growth was completely suppressed at this temperature on Richard's agar medium.

The results retrieved on the effect of varied temperature levels on various solid media revealed that the growth and morphological characters of the fungus varied with the varying temperature and different growth media. Fungus can survive under wide range of temperature regimes but minimum, optimum and maximum temperature are required for their growth. Findings of the study depicted that the temperature of 30 °C was the most effective temperature for the growth in all the tested media. Similar results were reported from the findings of Hailmi *et al.* (2017) [7], Kumara and Rawal (2008) [9] which revealed that *C. gloeosporioides* isolates varied in its ability to grow under different temperature levels. However, the range of 20 °C to 30 °C was best for the growth on PDA medium.

3.2.2 Effect of hydrogen ion concentration (pH) on the growth of *C. lindemuthianum* on different solid media

The data related to the study conducted on six distinct media to ascertain the pH requirements of *C. lindemuthianum* for its growth is given in the Table 3, Plate 3 and Fig 3. Fungal growth was supported at different pH regimes and was varied significantly in different media. The hydrogen ion (pH) concentration of 6, 7 and 8 found to be good for all the media. The pH of 7 was found to be the best out of all pH levels tested, for growth and morphological characters of the pathogen. The mean maximum growth of the pathogen was recorded at pH 7 on both Richard's agar and PDA (90.00 mm) succeeded by Yeast extract agar (83.30 mm). The minimal pathogen growth was identified on Potato sucrose agar (65.30 mm). At pH 8 also the maximal growth was encouraged by Richard's agar (85.60 mm) and the minimal growth of 60.00 mm colony diameter was documented on Potato sucrose agar. Significant variation in growth of the pathogen was observed at pH 6 and 9 in different solid media. At pH 6 the pathogen growth was superior on Richard's agar with colony diameter of 71.13 mm and in Yeast extract agar the growth was very less with colony diameter of 51.00 mm. At pH 9 also the maximum fungal growth was supported by Richard's agar with colony diameter of 75.17 mm. The least growth was supported by Potato dextrose agar with colony diameter of 51.10 mm. At pH 5 Richard's agar supported the highest growth of the pathogen with 70.83 mm and the Oat meal agar medium provided the least growth having colony diameter of 30.83 mm. At pH 10 the growth was superior in Potato sucrose agar (58.00 mm).

The results of this study within the various pH ranges on six distinct media tested, depicted that the mean maximum fungal growth was documented at pH 7 having colony diameter of 78.76 mm succeeded by pH 8 and pH6 with mean colony diameter of 69.78 mm and 62.27 mm respectively. The fungal growth was moderate at pH 9 and 5 with mean colony diameter of 60.76 mm and 55.64 mm respectively. The growth of the fungus was least supported at pH 10 with mean colony diameter of 51.94 mm.

Among the different media tested at different pH levels, Potato dextrose agar and Richard's agar media were found to be best at all the pH regimes with mean colony diameter of 75.07 mm and 74.01 mm respectively and were comparable to one another followed by Czapek's Dox agar media (72.71 mm). The fungus showed signs of moderate development in Potato sucrose agar and Yeast extract agar with mean colony diameter of 61.22 mm and 59.55 mm respectively. The minimal growth was found on Oat meal agar (56.18 mm). All the pH levels supported the growth of pathogen in different media however, Richard's agar and Potato dextrose agar media found to be the best at all pH levels, and pH of 7 was discovered to be optimal for the pathogen's proliferation.

The hydrogen ion concentration influences the growth of fungi. Every organism has its own maximum, optimum and minimum pH levels for its growth and development. The growth of the pathogen requires specific pH levels and the growth at different levels varied because of the varied levels of hydrogen ion concentration present in the media. The present study is in support with the findings, who recorded maximum radial growth (9 cm) of *C. capsici* at pH 7 followed by 8, 6, 5 and 4. The results are also in consistent with the conclusions of Hailmi *et al.* (2017) [7], Deshmukh *et al*. (2012) [13] and Kumara and Rawal. (2008) [9].

Table 1: Cultural characteristics of *Colletotrichum lindemuthianum*

Sl. No.	Media	Colony diameter (mm)	Colony colour	Colony texture	Rate of growth	Type of margin	Sporulation
1	Richard's agar	90.00 (69.08)	Light yellow to white	Sparse	Good growth	Serrated margin	+
2	Potato dextrose agar	88.00 (64.02)	White	Cottony fluffy growth	Good growth	Smooth margin	+
3	Yeast extract agar	84.57 (63.09)	Light brown at centre surrounded by white mycelia	Slightly fluffy	Good growth	Slightly serrated	-
4	Oat meal agar	75.17 (57.06)	White	Slightly fluffy	Moderate growth	Smooth	-
5	Czapek's Dox agar	69.00 (51.28)	White	Cottony fluffy growth	Moderate growth	Serrated margin	-
6	Potato sucrose agar	67.13 (52.15)	Brown at centre surrounded by white mycelia	Irregular cottony with fluffy growth	Moderate growth	Smooth	+
	S. Em±	0.70					
	CD @1%	2.17					

***** Figures in the parenthesis are arc sine transformation values

Note: (-) Non-sporulated Up to 50 mm: Poor growth

(+) sporulated 50 to 80 mm: Moderate growth

80 to 90 mm: Good growth

Table 2: Effect of different temperature levels on growth of *C. lindemuthianum* on different solid media

Sl. No	Media	Colony diameter (mm)					
		15 °C	20 °C	25 °C	30 °C	35 °C	Mean
1	RA	0.00 (0.00)	55.50 (45.31)	89.80 (64.09)	90.00 (69.08)	71.42 (56.87)	61.34
2	PDA	5.90 (14.06)	58.80 (50.06)	88.13 (69.85)	90.00 (69.56)	73.10 (58.75)	63.18
3	CDA	9.82 (18.26)	41.10 (39.29)	63.13 (50.95)	88.15 (64.03)	70.80 (56.83)	54.60
4	YEA	8.30 (16.74)	56.21 (46.38)	58.40 (46.95)	85.15 (64.82)	65.23 (51.92)	54.66
5	PSA	11.15 (19.51)	45.43 (39.54)	68.20 (51.24)	74.40 (57.06)	66.07 (51.12)	53.05
6	OMA	5.15 (13.76)	55.50 (45.28)	61.00 (50.82)	68.40 (51.26)	58.20 (45.47)	48.62
Mean		6.72	52.09	71.44	82.63	67.47	
Factors		CD @1%			S.Em±		
Media (A)		0.64			0.22		
Temperature (B)		0.58			0.20		
(AX B)		1.44			0.51		

Table 3: Effect of different pH levels on the growth of *C. lindemuthianum* on different solid media

Sl. No	Media	Colony diameter (mm)						
		5	6	7	8	9	10	Mean
1	RA	70.83 (56.84)	71.13 (56.86)	90.00 (64.10)	85.60 (63.84)	75.17 (57.11)	51.30 (45.07)	74.01
2	PDA	60.90 (50.82)	66.23 (51.13)	90.00 (71.56)	75.30 (57.12)	51.10 (45.06)	50.80 (45.04)	75.07
3	CDA	60.67 (50.81)	65.00 (51.06)	71.00 (56.84)	65.00 (51.07)	62.30 (49.17)	51.00 (45.05)	72.71
4	YEA	48.33 (39.71)	51.00 (45.05)	83.30 (63.66)	63.33 (50.95)	61.30 (50.83)	50.08 (45.05)	59.55
5	PSA	62.33 (50.90)	63.00 (50.93)	65.30 (51.07)	60.00 (49.03)	58.70 (45.48	58.00 (45.45)	61.22
6	OMA	30.83 (33.57)	57.27 (45.41)	73.00 (57.10)	69.50 (53.13)	56.0 (45.34)	50.50 (43.30)	56.18
	Mean	55.64	62.27	78.76	69.78	60.76	51.94	
Factors		CD @1%			S.Em±			
Media (A)		1.240			0.443			
pH (B)		1.246			0.441			
(AX B)		1.548			1.08			

***** Figures in the parenthesis are arc sine transformation values.

Note: RA: Richard's agar, PDA: Potato dextrose agar**,** CDA: Czapek's Dox agar, OMA: Oat meal agar, YEA: Yeast extract agar, PSA: Potato sucrose agar

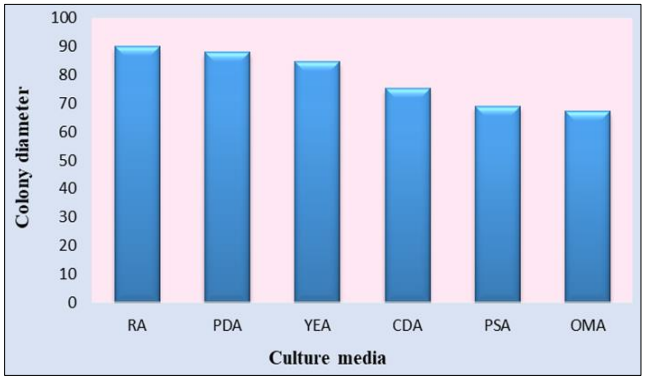


Fig 1: Colony growth of *C. lindemuthianum* on different solid media

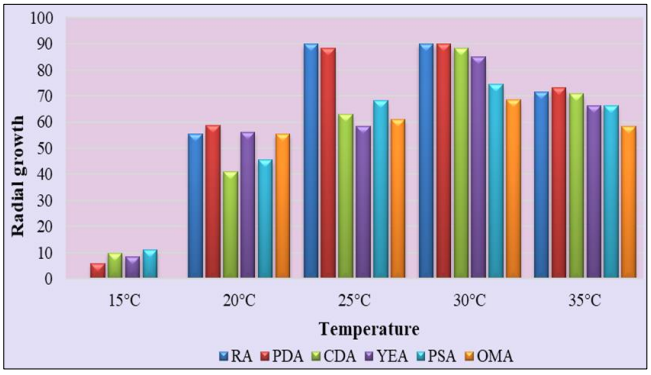


Fig 2: Effect of different temperature levels on growth of *C. lindemuthianum* on different solid media

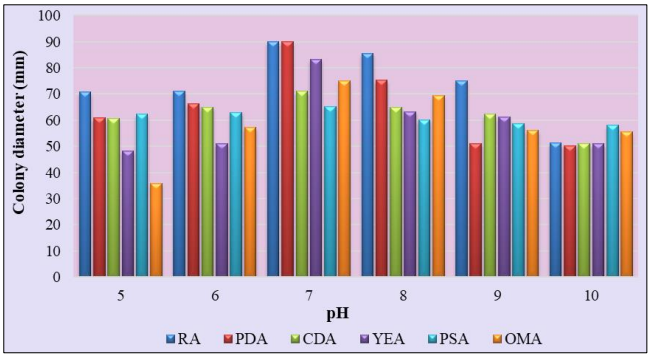
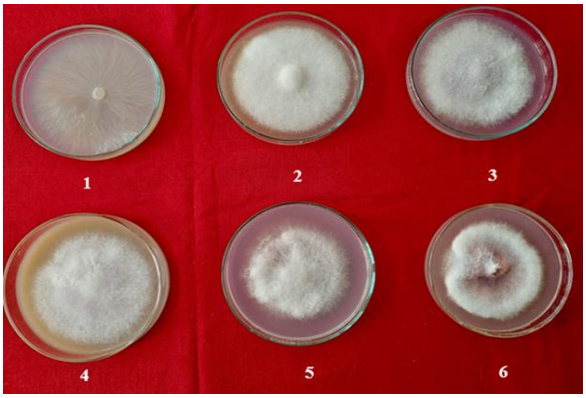


Fig 3: Effect of different pH levels on growth of *C. lindemuthianum* on different solid media



1. Richard's agar
2. Potato dextrose agar
3. Yeast extract agar
4. Czapek's Dox agar
5. Potato sucrose agar
6. Oat meal agar

Plate 1: Effect of different solid media on growth of *C. lindemuthianum*

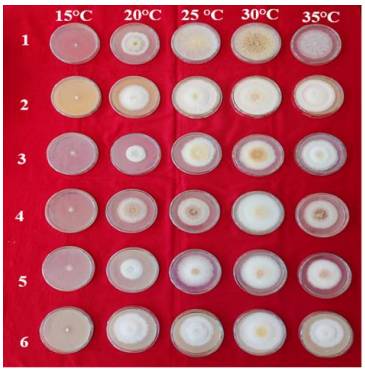


Plate 2: Effect of different temperature levels

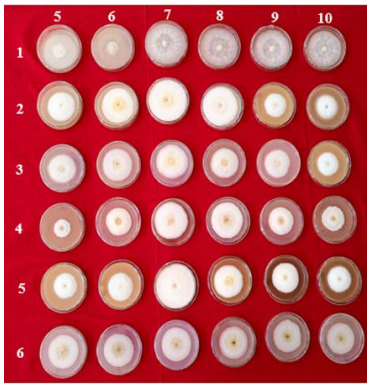


Plate 3: Effect of different pH levels

1. Richard's agar 3. Czapek's Dox agar 5. Potato sucrose agar
2. Potato dextrose agar 4. Yeast extract agar 6. Oat meal agar

4. Conclusion

The current study on cultural and physiological studies of *C. lindemuthianum* causing anthracnose of field bean indicated that the growth and sporulation of the fungus was significantly superior and faster on Richard's agar medium followed by Potato dextrose agar. Varied degrees of colony colour were observed with brown to white colour having smooth to serrated margin. Among the different temperature levels evaluated, 30 °C was found to be the best temperature for growth of the pathogen followed by 25 °C. The pH of 7 was identified to be the best out of all pH levels tested, for growth and morphological characters of the pathogen. However, the hydrogen ion (pH) concentration of 6, 7 and 8 were found to be good for all the media.

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