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Influence of media, temperature and pH on growth of *Colletotrichum capsici* (Syd.) causing anthracnose disease of chilli

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Abstract
Colletotrichum capsici, the incitant of anthracnose disease of chilli, is a serious disease of chill crop in causing major economic loss to chilli production worldwide, especially in tropical and sub-tropical countries. An attempt was made to identify best supporting medium as well as different temperatures and pH which may support luxuriant growth of the fungus. Five different media tested, among them, Potato Dextrose Agar medium (PDA) was found very suitable for mycelia growth of *Colletotrichum capsici* resulting full plate growth (9 cm) within 9 days followed by Yeast Extract Potato Dextrose Agar medium, Chilli Extract Agar medium, Wheat grain Extract Agar medium and Malt Extract Agar medium. The growth of *Colletotrichum capsici* was found to be more vigrous at the temperature 30^0C where full plate growth of the mycelium (9cm) was found within 9 days followed by 25^0C, 35^0C and 20^0C. Among the different pH maximum radial growth (9cm) was recorded at pH7 within 9 days followed by 8, 6, 5 and 4.

Keywords: *Colletotrichum capsici,* anthracnose, medium, pH, temperature

Introduction
Chilli (*Capsicum annuum* L.) is a very important vegetable as well spice crop and is being grown in almost all parts of tropical and subtropical regions of the world (Pickersgill, 1997) [2]. An attempt was made to identify best supporting medium as well as different temperatures and pH which may support luxuriant growth of the fungus. Five types of culture media viz. Potato dextrose agar medium (PDA), Malt extract agar medium, Yeast extract potato dextrose agar medium, Chilli extract agar medium and Wheat extract agar medium were used for evaluation of mycelial growth of *Colletotrichum capsici.* Suitability of temperature was observed for mycelial growth of pathogen *Colletotrichum capsici* at different temperature, viz. 20°C, 25°C, 30°C, 35°C and 40°C in BOD incubators. In best suitable temperature and media, suitability of pH was observed for mycelial growth of pathogen at different pH, viz. pH-4, pH-5, pH-6, pH-7 and pH-8 in BOD incubators.

Materials and Methods
Isolation of pathogen
Small bits of infected tissue (2-3 mm size) were cut at the juncture of diseased and healthy portion with the help of disinfected blade after surface sterilizing the sample with alcohol. These bits were surface sterilized in 0.1 per cent mercuric chloride ($HgCl_2$) solution for about 20 seconds followed by three times washing with sterilized distilled water in petriplates, under aseptic conditions using laminar air flow chamber to remove the traces of mercuric chloride. After blot drying with sterilized filter paper, these bits were transferred to potato dextrose agar (PDA) medium in sterilized petriplates. Three such bits were placed in each petriplate and incubated in BOD icubator for three days at 27±10C. The culture thus obtained was subjected to purification.

The isolated fungal pathogen was grouped based on colony morphology, colony colour, mycelial growth, shape and size of spores, spore septation and other characteristic features which was observed under binocular microscope. Identifications were made after comparing the microscopic and morphological features of the pathogen *Colletotrichum capsici* with the available standard literature for establishing their identity (Smith and Black, 1990) [4]. Subsequently the pure culture thus obtained was maintained on PDA slants (Choi *et al*., 1999) [1]. The slants were incubated at 27±10C in Biological Oxygen Demand (BOD) incubator. The cultures were revived after every month and maintained throughout the course of studies on PDA in sealed culture tubes at 5°C in the refrigerator.

Preparation of culture media

Five types of culture media viz. Potato dextrose agar medium (PDA), Malt extract agar medium, Yeast extract potato dextrose agar medium, Chilli extract agar medium and Wheat extract agar medium were used for evaluation of mycelial growth of *Colletotrichum capsici.* 15-20 ml. of medium was poured in sterilized petriplate. These plates were inoculated with 5 mm. mycelia bit of 7 days old culture *Colletotrichum capsici* separately. Suitability of media was observed for mycelial growth of pathogen *Colletotrichum capsici.* Three replications of each treatment were maintained.

Effect of temperature

Suitability of temperature was observed for mycelial growth of pathogen *Colletotrichum capsici.* 15-20 ml. of medium was poured in sterilized petriplate. These plates were inoculated with 5 mm. mycelia bit of 7 days old culture *Colletotrichum capsici* separately. These inoculated petriplate were incubated at different temperature, viz. 20°C, 25°C, 30°C, 35°C and 40°C in BOD incubators. The observations on radial growth were recorded with the help of metric scale and the data were analyzed statistically using completely randomized design. Three replications of each treatment were maintained.

Effect of pH

To study the effect of pH on the growth of the test fungus, best suitable media and temperature was selected. The basal medium was adjusted at pH-4, pH-5, pH-6, pH-7 and pH-8 levels for further study, by using NaOH (0.1N) and HCl (0.1N) solution. Sorenson buffer solution was added before autoclaving to keep the pH constant. The procedure of inoculation, incubation and recording of data were the same as described for media and temperature.

Results and Discussion

Suitability of different culture media

In this investigation, five different culture media viz. Potato Dextrose Agar medium, Yeast Extract Potato Dextrose Agar medium, Malt Extract Agar medium, Wheat grain Extract Agar medium and Chilli Extract Agar medium were evaluated for radial growth of *Colletotrichum capsici*.

The data recorded is presented in Table 1.1 which indicates the growth of *Colletotrichum capsici* was fast in Potato Dextrose Agar medium (PDA) resulting full plate growth (9 cm) within 9 days followed by Yeast Extract Potato Dextrose Agar medium (YEPDA), Chilli Extract Agar medium (CEA), Wheat grain Extract Agar medium (WEA) and Malt Extract Agar medium (MEA) which results full plate growth were recorded in 10, 11, 12, and 13 days respectively. Comparatively, Malt Extract Agar medium was found least suitable for mycelial growth of *Colletotrichum capsici*.

The result is confirmative with findings of Roy *et al.,* (2012) [3]. They have investigated the growth of *Colletotrichum capsici* in four different culture media viz. Potato Dextrose Agar (PDA), Czapek Dox Agar (CDA), Sabouraud Dextrose Agar (SDA) and Peptone Yeast Extract Dextrose Agar (PYDA). Among the different media tested, PDA was found supported the maximum growth for the pathogen significantly compared to all other media.

Table 1: Evaluation of different culture media for radial growth of *Colletotrichum capsici*.

No. of days	Radial growth (cm) in different media				
	Malt Extract Agar medium	Wheat grain Extract Agar medium	Chilli Extract Agar medium	Yeast Extract Potato Dextrose Agar medium	Potato Dextrose Agar medium
2	1.53	1.66	1.86	2.23	2.53
3	1.8	1.93	2.13	2.53	3.1
4	2.53	2.13	2.93	3.06	3.96
5	2.83	2.93	3.56	3.76	5.13
6	3.06	3.5	4.4	5.03	6.4
7	3.53	4.36	5.43	6.13	7.8
8	4.4	5.43	6.56	7.2	8.73
9	5.03	6.53	7.73	8.36	9.0
10	6.16	7.63	8.56	9.0	
11	7.2	8.5	9.0		
12	8.36	9.0			
13	9.0				
C.V.			0.771		
C.D. (P0.05)			0.084		

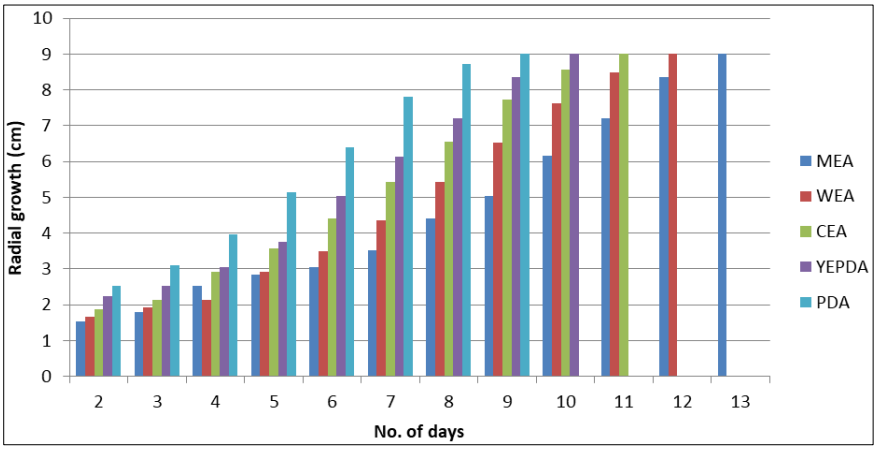

Fig 1: Effect of culture media on radial growth of *Colletotrichum capsici*.

Mycelial growth at different temperature

After selecting Potato Dextrose Agar medium as best suited medium for fast growth of *Colletotrichum capsici*; the suitability of temperature for appropriate growth of the pathogen was analyzed at different temperature. *Colletotrichum capsici* was allowed to grow at five temperature viz. 20^0C, 25^0C, 30^0C, 35^0C and 40^0C for observation of radial growth of mycelia.

The effect of different temperature viz. 20^0C, 25^0C, 30^0C, 35^0C and 40^0C are shown in Table 1.2. The maximum radial growth (9cm) was recorded at 30^0C within 9 days followed by 25^0C, 35^0C, 20^0C and no growth of the *Colletotrichum capsici* was found at 40^0C.

These results are confirmative with findings of Tripathi *et al*., (2016) [5]. They observed that growth of *Colletotrichum capsici* was most significant at 28^0C.

Table 2: Effect of temperature on radial growth of *Colletotrichum capsici.*

No. of days	Radial growth (cm) at different temperature				
	20^0C	**25^0C**	**30^0C**	**35^0C**	**40^0C**
2	1.4	2.1	2.6	2.0	0
3	2.7	3.1	3.5	3.03	0
4	3.2	4.1	4.6	3.8	0
5	3.75	4.7	5.4	4.3	0
6	4.8	5.6	6.5	5.2	0
7	5.5	6.7	7.6	6.1	0
8	6.6	7.7	8.5	7.13	0
9	7.06	8.13	9.0	7.96	0
C.V.			0.695		
C.D. (P0.05)			0.081		

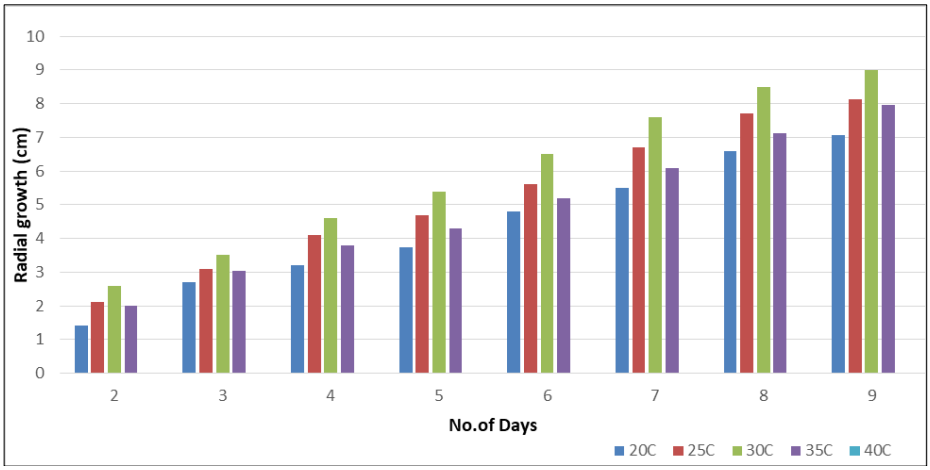

Fig 2: Effect of temperature on radial growth of *Colletotrichum capsici.*

Mycelial growth at different pH

After selecting Potato Dextrose Agar medium as best suited medium and 30^0C as best temperature for fast growth of *Colletotrichum capsici*; the suitability of pH for appropriate growth of the pathogen was analyzed at different pH. *Colletotrichum capsici* was allowed to grow at five pH viz. 4, 5, 6, 7 and 8 for observation of radial growth of mycelia.

The effect of different pH viz. 4, 5, 6, and 7 are shown in Table 1.3. The maximum radial growth (9cm) was recorded at pH7 within 9 days followed by 8, 6, 5 and least growth of the *Colletotrichum capsici* was found at pH4.

Table 3: Effect of pH on radial growth of *Colletotrichum capsici.*

No. of days	Radial growth (cm) at different Ph				
	4	**5**	**6**	**7**	**8**
2	1.73	2.1	2.7	2.96	2.8
3	2.3	3.1	3.86	4.26	4.06
4	3.13	4.0	5.06	5.43	5.26
5	4.03	5.4	5.93	6.33	6.13
6	5.5	6.0	6.56	6.93	6.76
7	6.03	6.7	7.23	7.66	7.33
8	6.7	7.16	7.73	8.66	7.86
9	7.23	7.9	8.13	9.00	8.6
C.V.			1.047		
C.D. (P0.05)			0.158		

These results are confirmative with findings of Tripathi *et al*., (2016) [5]. They observed that growth of *Colletotrichum capsici* was most significant at pH 6.

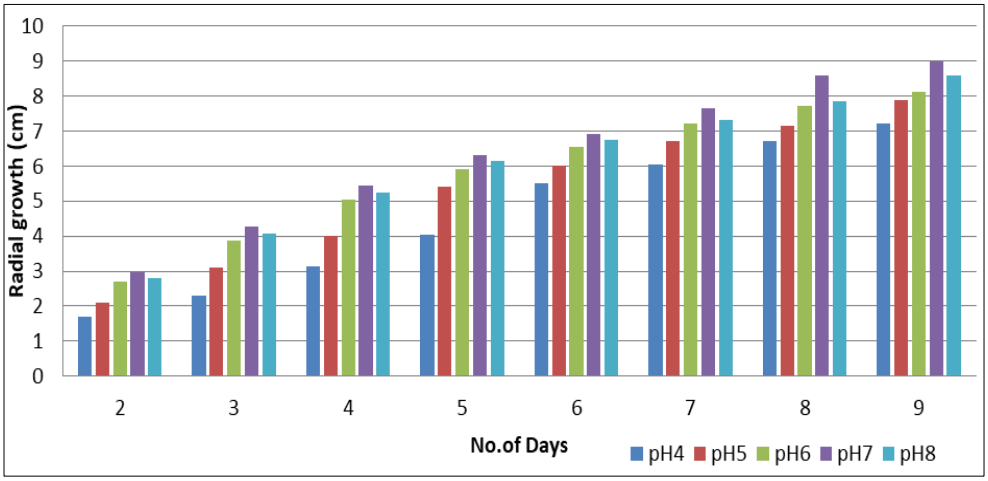

Fig 3: Effect of pH on radial growth of *Colletotrichum capsici.*

Plate 1: Mycelial growth of *C. capsici* on different culture media.

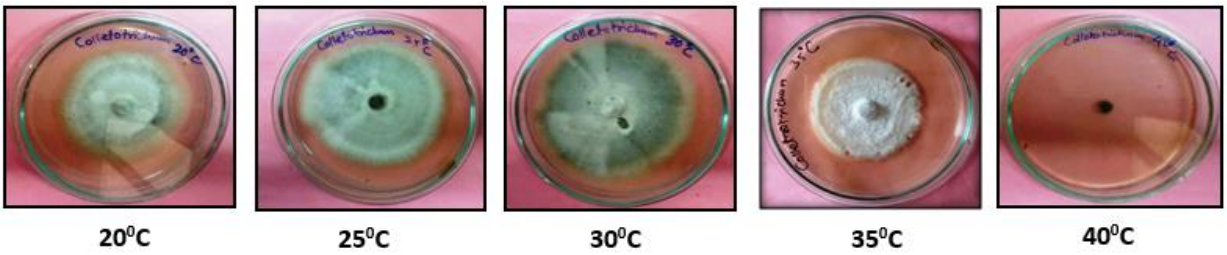

Plate 2: Mycelial growth of *C. capsici* at different temperature.

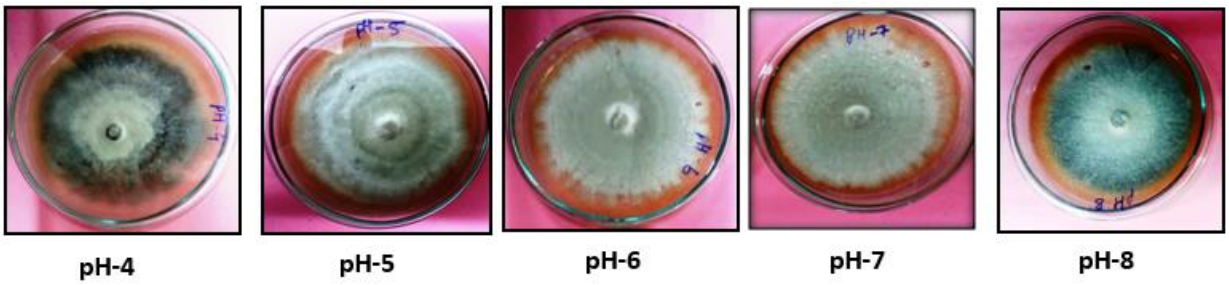

Plate 3: Mycelial growth of *C. capsici* at different pH

Summary and Conclusion

The present experiment was conducted to figure out the suitability of culture medium, pH and temperature for the mycelial growth of the pathogen *Colletotrichum capsici* and to study its morphological character. The silent features of experimental findings are summarized here under:

Potato Dextrose Agar medium (PDA) was found very suitable for mycelia growth of *Colletotrichum capsici* resulting full plate growth (9 cm) within 9 days followed by Yeast Extract Potato Dextrose Agar medium, Chilli Extract Agar medium, Wheat grain Extract Agar medium and Malt Extract Agar medium. The growth of *Colletotrichum capsici* was found to be more vigrous at the temperature 30^{0}C where full plate growth of the mycelium (9cm) was found within 9 days followed by 25^{0}C, 35^{0}C and 20^{0}C. Among the different pH maximum radial growth (9cm) was recorded at pH7 within 9 days followed by 8, 6, 5 and 4. pH4 was found least suitable for the growth of *Colletotrichum capsici*.

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