Journal of Pharmacognosy and Phytochemistry
Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
www.phytojournal.com
JPP 2021; 10(1): 1046-1049
Received: 03-11-2020
Accepted: 14-12-2020

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Nutritional studies on the isolates of *Colletotrichum capsici* causing fruit rot in chilli

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Abstract
Experiment was conducted *in vitro* to study the ability of different isolates to utilize carbon and nitrogen as essential element from different carbon and nitrogen sources, as its utilization depends on enzyme system. Infected chilli samples were collected from different regions of Vidarbha and Marathwada (Maharashtra), Bidar district (Karnataka) and Guntur district (Andra Pradesh) for isolation of *Colletrotrichum capsici*. Ten isolates of C. *capsici* isolated from collected samples. Eight different solid media *viz*., Asthana and Hawker's, Richard's, Czapek's dox agar, oat meal agar, carrot agar, yeast extract-dextrose agar, nutrient agar and potato dextrose agar medium consisting various nutrients were tested and the observations were recorded on 3rd and 7th day of incubation. Statistical significance was obtained among the media in supporting the growth of isolates of *C. capsici*. The results revealed that significant differences among the different monosaccharides and disaccharides utilized by the isolates of *C. capsici*. All the sources of nitrogen are not equally efficient for supporting the growth of the fungi as they are specific in the utilization of 'N' sources. Among the different nitrogen sources, urea was found to support the maximum growth of eight isolates of *C. capsici.*

Keywords: *Colletotrichum capsici*, different solid media, carbon sources, nitrogen sources, chilli

Introduction
Chilli (*Capsicum annuum* L.) belongs to the family Solanaceae is one of the important spice cum vegetable crop in India. The Chilli anthracnose pathogen *C. capsici* infects diverse host with a high degree of pathogenic variability (Akhtar and Singh, 2007) [1]. The symptom appears on fruits initially small circular spots appeared on the skin of the fruit. The spots were sunken and light grey coloured with black margin, fruiting bodies *viz*., acervuli were produced on the infected area. The anthracnose is one of serious diseases on chili to cause the yield loss and to reduce the quantity of marketable fruits. The fungus derive food and energy from the substrate upon which they grow in nature, so as to culture the fungus within the laboratory, there's no universal substrate or artificial medium upon which all the fungi can grow and reproduce. Therefore studies were conducted in several media to identify suitable medium, carbon source and nitrogen source for the growth of *C. capsici*.

Materials and Methods
Collection of disease samples
The disease samples of fruit rot of chilli (plant parts) were collected from different geographical areas of Vidarbha and Marathwada region of Maharashtra (M.S.). Some isolates of *Colletotrichum* causing diseases in chilli were collected from Bidar district of Karnataka and Guntur district of Andhra Pradesh.

Isolation and maintenance of cultures
The samples showing characteristic symptoms of fruit rot, dieback and anthracnose were collected from different localities and cut along with healthy tissues. The infected bits were washed with sterilized water and surface sterilized in 0.1 per cent mercuric chloride solution for one minute in the Petriplates and subsequently three changes of water was given to remove the traces of mercuric chloride. The bits were dried around the flame of spirit lamp, then transferred to solidified sterile potato dextrose agar (PDA) in Petriplates and were incubated at room temperature (27 ± 2 ºC) for seven days. All the operations were carried aseptically. The fungus growth of *Colletotrichum capsici* was then transferred on PDA slants. The cultures thus obtained were further purified by single spore isolation technique.

Nutritional Studies
To study the effect of different solid media on mycelial growth of *Colletotrichum capsici*,

eight solid media *viz*., Asthana and Hawker's, Richard's, Czapek's dox agar, oat meal agar, carrot agar, yeast extract-dextrose agar, nutrient agar and potato dextrose agar medium were used. These media were sterilized in autoclave at 1.05 kg cm^{-2} pressure for 15 min. Autoclaved media were poured in previously sterilized plates (20 ml $plate^{-1}$) and on solidified media the isolates were inoculated separately by using 6 mm mycelial disc of 7 days old culture. For each isolate three replications were maintained. Plates were incubated at room temperature (27 ± 2ºC). The radial mycelial growth in mm was measured after 3rd and 7th day of inoculation. The measurements were made in three marked directions at right angles to each other, passing through center and average colony diameter was worked out.

Effect of different carbon sources

Richard's medium was used as basic medium for assessment of best carbon source. Sucrose containing media was substituted by different carbon sources *viz*., fructose, lactose, mannitol, xylose on the basis of molecular weight. These modified media were autoclaved and poured in sterilized plates at 45ºC. After solidification of media 6 mm disc of each isolate of *Colletotrichum capsici* was kept at center on media separately. For each isolate three replications were made. The inoculated plates incubated at room temperature up to 7 days. Radial mycelial growth in mm at 3rd and 7th days of inoculation was measured.

Effect of different nitrogen sources

Potassium nitrate a nitrogen source of Richard's medium was substituted by sodium nitrate, ammonium sulphate, ammonium nitrate and urea on the basis of molecular weight. These modified media were inoculated with *Colletotrichum capsici* and incubated at room temperature for 7 days. Radial mycelial growth in mm at 3rd and 7th days of inoculation was measured.

Results and Discussion

Collection, isolation, purification and identification of pathogen

Fruit rot infected plant parts were collected from different geographical areas of Vidarbha and Marathwada region of Maharashtra and some samples were collected from Bidar (Karnataka) and Guntur (Andhra Pradesh). The usual tissue isolation technique was followed to isolate the pathogen from infected plant parts showing fruit rot, anthracnose symptoms. Potato dextrose agar was used as basal medium for isolation of the fungus. The pure culture was obtained using single spore method. The culture thus obtained was identified as *Colletotrichum capsici* on the basis of pathogenic ability and morphological characters as per the CMI publications. Purified cultures of the fungus were maintained on PDA slants for further studies and abbreviated as Cc.

Nutritional studies

Effect of different nutrient media

Eight different media consisting various nutrients were tested and the observations were recorded on 3rd and 7th day of incubation (Table 1). Statistical significance was obtained among the media in supporting the growth of isolates of *C. capsici*. There were significant differences between the growths of fungi on different nutrient media. Isolate Cc_1 preferred PDA (42.70 mm) followed by carrot agar (38.04 mm), whereas isolate Cc_8 preferred carrot agar (41.80 mm) followed by Richards (36.64 mm) for maximum growth when observed at 3rd day. Isolate Cc_5 showed 82.42 mm growth on PDA followed by oat meal (74.00 mm) and carrot agar (74.00 mm) and in isolate Cc_7 PDA supported maximum growth i.e. 81.94 mm followed by carrot agar (74.00 mm) when examined at 7th day of incubation. Isolate Cc_1 (79.18 mm), Cc_4 (78.83 mm), Cc_8 (79.00 mm), Cc_9 (78.39 mm) and Cc_{10} (74.61 mm) exhibited maximum growth on PDA followed by Czapek's agar in Cc_1, Ashthana and Hawker's in Cc_4 and carrot agar in Cc_8, Cc_9 and Cc_{10} at 7th day of incubation. Isolate Cc_2 (78.48 mm), Cc_3 (78.78 mm) and Cc_6 (76.61 mm) exerted maximum mean colony diameter on carrot agar followed by Richard's agar in Cc_3, and PDA in Cc_2 and Cc_6 at 7th day of incubation. Yeast extract – dextrose agar and nutrient agar were found to be least preferred media for supporting the growth of all ten isolates at 7th DAI, as the radial diameter was ranged between 54.53–67.84 mm and 60.33–64.31 mm respectively. Hence, the isolates indicated the variation as they showed the ability to utilize nutrients at different proportion through their metabolic activities. Thus rate of magnitude of growth on these media clearly establish the facts that all ten isolates differed in their abilities to utilize nutrients present in medium. Ekbote *et al.* (1997) [2] investigated both synthetic and non synthetic media for the growth and sporulation of *C. gloeosporioides* and these results correlates the present investigation. The maximum radial growth (90.00 mm) was recorded in Richard's, Browns and PDA followed by Czapek's dox agar (89.60 mm). Least radial mycelial growth was recorded in Asthana's and Hawker's medium 'A'. Similar results were noted while studying with *C. capsici*. Present results are also on the similar line of literature published by Khirbhat *et al.* (2004) [3] in *C. capsici* and reported that all the isolates grew well on oat meal agar followed by Richard's agar. In terms of variability isolate CC-6 distinctly different because of its slow rate of growth and there was no significant difference in the radial growth among the isolates CC-1, CC-2, CC-3, CC-4, CC-5 and CC-7. Venkataravanappa and Nargund, (2007) [4] reported variation in growth of *C. gloeosporioides*. The maximum radial growth was found on PDA (83.98mm) and least (77.40mm) on host leaf extract media. Synthetic media Ashthana and Hawker's agar supported maximum i.e. 81.80 mm and minimum mean colony diameter was recorded on Czapek's agar. Akhtar *et al.* (2018) [5] tested five different media, 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. Asalkar *et al*.,(2019) [6] reported that, among all the solid media maximum mycelial growth with excellent sporulation rating was obtained on Richard's agar medium (90 mm) within six days and was significantly superior to all the other media tested. This was followed by Potato dextrose agar (88.83 mm) and Corn meal agar (88.40mm) under in vitro conditions.

Table 1: Effect of different media on growth (mm) of *Colletotrichum capsici* isolates

Isolate	Cc_1		Cc_2		Cc_3		Cc_4		Cc_5		Cc_6		Cc_7		Cc_8		Cc_9		Cc_{10}	
Media	3 DAI	7 DAI	3 DAI	7 DAI	3 DAI	7 DAI	3 DAI	7 DAI	3 DAI	7 DAI	3 DAI	7 DAI	3 DAI	7 DAI	3 DAI	7 DAI	3 DAI	7 DAI	3 DAI	7 DAI
Ashthana and Hawker's	31.67	69.18	28.39	70.68	31.03	72.27	29.47	69.61	30.96	65.03	30.39	69.12	33.14	64.00	35.78	69.00	21.81	61.42	34.76	63.00
Richard's	34.77	71.34	35.55	74.13	38.22	78.16	32.42	66.87	31.67	69.03	35.83	71.23	37.03	72.53	36.64	69.00	29.06	59.37	38.22	66.67
Czapek's dox agar	34.97	72.40	34.17	68.83	37.50	73.72	32.39	65.05	29.83	69.28	31.79	72.03	31.03	69.00	35.29	62.76	30.89	59.56	35.52	70.62
Oat meal agar	35.64	62.00	34.92	72.19	33.25	74.00	33.83	65.64	32.94	74.00	31.27	73.14	31.63	67.87	36.61	74.00	27.50	63.00	34.17	71.61
Carrot agar	38.04	71.89	37.05	78.48	38.94	78.78	32.72	68.16	38.20	74.00	32.27	76.61	35.39	74.00	41.80	76.22	34.50	68.76	38.28	72.61
Yeast extract-dextrose agar	26.32	57.70	28.66	65.22	27.61	64.33	29.27	58.75	28.75	62.20	29.05	67.84	23.94	54.53	30.98	63.43	34.67	63.78	33.90	67.72
Nutrient agar	33.12	61.95	35.78	62.29	38.50	64.31	37.59	61.61	31.42	62.55	18.36	60.33	28.06	61.70	31.28	63.72	33.09	63.28	31.81	62.28
PDA	42.70	79.18	35.79	74.66	37.20	74.28	36.25	78.83	35.83	82.42	41.39	75.44	38.48	81.94	35.00	79.00	32.48	78.39	37.64	74.61
F test	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.
SE (m) ±	0.60	2.61	0.79	0.78	1.31	0.44	1.99	0.91	0.84	0.57	1.28	0.37	2.02	1.24	0.53	0.29	1.46	0.68	0.53	0.65
CD (P = 0.01)	2.51	10.79	3.25	3.22	5.45	1.83	8.23	3.76	3.48	2.39	5.32	1.53	8.36	5.13	2.20	1.20	6.05	2.84	2.21	2.68

DAI: Days After Incubation

Effect of different carbon sources

The growth of the fungus (*C. capsici*) on various carbon sources was studied by growing the fungus on Richard's medium, which was used as basal medium. The results revealed that significant differences among the different monosaccharides and disaccharides utilized by the isolates of *C. capsici* (Table 2). Sucrose was found to support the maximum growth in six isolates *viz*., Cc_1, Cc_2, Cc_3, Cc_5, Cc_6 and Cc_7 and it was between the range of 60.73 to 69.89 mm, whereas lactose was preferred by four isolates *viz*., Cc_4, Cc_8, Cc_9 and Cc_{10} and growth was ranged between 66.28 to 70.28 mm at 7th DAI. Xylose supported minimum radial mycelial growth in 8 isolates, whereas Cc_1 and Cc_{10} exhibited minimum growth on mannitol. Other sources of carbon indicate the variation among the isolates. After seventh day of incubation fructose was preferred for their growth and multiplication by all the ten isolates as a next best source. It indicates that every isolate had an ability to utilize energy from varied types of carbohydrates. Though the large preference was not achieved by the isolates but it is an indication that diversity exists among the isolates. The results of the present studies are in the line of research published by Sandhya and Murthy, (2004) [7] against *C. gloeosporioides* and recorded maximum growth in Richard's (88.4 mm), Brown's (87.6 mm) and potato dextrose agar medium (86.2 mm) and minimum growth was recorded on corn meal agar medium (31.6 mm). Wasantha and Rawal, (2008) [8] observed the pathogen under study varied in its ability to utilize different carbon and nitrogen sources. Fructose was found to be the best source of carbon for the growth and sporulation of most of the isolates. Kushwaha, (2015) [9] tested the different carbon sources, the pathogen preferred monosaccharide and oligosaccharides for nutrition. Maximum growth was recorded on maltose followed by glucose, fructose and sucrose. Disaccharides were good but polysaccharides not good sources. Gawai and Shinde, (2017) [10] mentioned that, among carbon sources glucose, lactose and sucrose were found to the best for growth of *Colletotrichum capsici*.

Table 2: Effect of different carbon sources on growth (mm) of *Colletotrichum capsici* isolates

Isolate	Cc_1		Cc_2		Cc_3		Cc_4		Cc_5		Cc_6		Cc_7		Cc_8		Cc_9		Cc_{10}	
Carbon source	**3 DAI**	**7 DAI**	**3 DAI**	**7 DAI**	**3 DAI**	**7 DAI**	**3 DAI**	**7 DAI**	**3 DAI**	**7 DAI**	**3 DAI**	**7 DAI**	**3 DAI**	**7 DAI**	**3 DAI**	**7 DAI**	**3 DAI**	**7 DAI**	**3 DAI**	**7 DAI**
Fructose	22.42	63.72	22.82	56.06	25.33	65.39	22.28	64.34	19.81	59.42	26.50	62.67	27.11	63.67	29.67	63.50	33.83	67.78	30.89	63.78
Lactose	22.76	63.17	23.90	58.09	29.56	68.61	24.46	67.70	24.00	55.22	24.00	59.50	23.72	55.67	32.56	66.28	34.39	70.28	33.39	69.78
Mannitol	21.59	58.89	22.53	59.17	26.39	65.57	22.94	63.03	21.67	57.48	21.17	56.61	24.44	56.39	28.11	60.89	31.28	64.50	28.61	55.28
Xylose	22.20	59.68	12.03	28.83	14.97	34.83	18.24	54.26	17.50	47.56	19.67	52.20	19.00	43.11	29.78	56.39	26.50	63.54	24.61	58.56
Sucrose	26.84	67.14	25.34	60.73	30.39	69.89	22.72	64.72	24.28	64.22	29.33	65.56	32.06	66.00	29.33	59.22	32.44	64.89	33.83	64.50
F test	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.
SE (m) ±	0.92	0.30	0.20	0.34	0.35	0.52	0.90	0.63	0.39	0.32	0.83	0.91	0.74	0.49	0.29	0.27	0.31	0.29	0.41	0.31
CD (P = 0.01)	4.15	1.36	0.89	1.55	1.59	2.35	4.06	2.85	1.77	1.47	3.75	4.09	3.32	2.23	1.33	1.22	1.41	1.31	1.86	1.40

DAI: Days After Incubation

Effect of different nitrogen sources

The growth of *C. capsici* isolates on various nitrogen sources was studied by growing the fungus on basal Richard's medium. All the sources of nitrogen are not equally efficient for supporting the growth of the fungi as they are specific in the utilization of 'N' sources (Table 3). Among the different nitrogen sources, urea was found to support the maximum growth of eight isolates of *C. capsici viz*., Cc_1, Cc_3, Cc_4, Cc_6, Cc_7, Cc_8, Cc_9 and Cc_{10} and it was in the range of 54.67 mm to 68.67 mm, whereas potassium nitrate found to support the maximum growth of two isolates *viz*., Cc_2 and Cc_5 with 54.11 mm and 63.94 mm respectively at 7th DAI, while ammonium sulphate supported minimum colony growth in all the isolates. 'N' source in the form of urea supported 54.67, 67.00, 68.67, 61.03, 61.06, 59.01, 61.61 and 64.86 mm growth of Cc_1, Cc_3, Cc_4, Cc_6, Cc_7, Cc_8, Cc_9 and Cc_{10} respectively, whereas potassium nitrate supported 54.11 and 63.94 mm growth of Cc_2 and Cc_5. The similar trend in growth was observed at 3rd DAI. These observations indicated the variation among the utilization of 'N' from different sources. Nitrogen is also an

important source for the growth and sporulation. Ammonia assimilation reaction resulting in the formation of glutamic acid and glutamine depend on the concentration of ammonia available in the cells. Utilization of 'N' from different sources by a specific isolates relates to the variation among the isolates. The utilization might be due to presence of particular enzymic activities in the isolates. These findings confirm work of Palarpawar, (1987) [11] studied the response of *C. curcuma* and *C. capsici* to different culture media and stated that KNO_3 – glucose supported good mycelial growth and sporulation of *C. capsici*. Similarly Naik *et al*., (1988) [12] reported the wide range of response of *C. gloeosporioides* to different carbon, nitrogen and sulphur sources. The present results confirm the work carried out by Khirbhat *et al*., (2004) [3] in *C. capsici*. Wasantha and Rawal, (2008) [8] reported that, among the nitrogen sources tested, aspartic acid supported the maximum growth of isolates followed by potassium nitrate and proline. In contrast to this, isolates sporulated better in media containing potassium nitrate, ammonium nitrate or sodium nitrate as the sole nitrogen source. Kushwaha, (2015) [9] reported that among the nitrogen sources, the pathogen preferred potassium nitrate followed by sodium nitrate and asparagines. Lead nitrate and ammonium chloride supported least growth. Gawai and Shinde, (2017) [10] found that, potassium nitrate, casein and calcium nitrate were found to be good for the growth of *Colletotrichum capsici.* Jawalgekar and Mandge, (2018) [13] tested 11 nitrogen sources, the pathogen produced maximum mycelial growth in peptone and followed by Ammonium phosphate, Ammonium oxalate, casein, sodium nitrate and in potassium nitrate shows least growth.

Table 3: Effect of different nitrogen sources on growth (mm) of *Colletotrichum capsici* isolates

Isolate	Cc_1		Cc_2		Cc_3		Cc_4		Cc_5		Cc_6		Cc_7		Cc_8		Cc_9		Cc_{10}	
Nitrogen source	**3 DAI**	**7 DAI**	**3 DAI**	**7 DAI**	**3 DAI**	**7 DAI**	**3 DAI**	**7 DAI**	**3 DAI**	**7 DAI**	**3 DAI**	**7 DAI**	**3 DAI**	**7 DAI**	**3 DAI**	**7 DAI**	**3 DAI**	**7 DAI**	**3 DAI**	**7 DAI**
Sodium nitrate	27.28	52.64	28.72	48.20	31.28	57.39	27.83	51.98	31.39	52.00	16.78	30.46	30.44	48.61	32.67	53.09	34.11	58.50	30.39	55.44
Ammonium sulphate	21.48	47.07	24.98	32.23	22.33	33.70	16.28	19.00	22.28	26.61	5.44	11.89	15.08	25.56	25.83	36.63	24.11	31.26	18.18	22.26
Ammonium nitrate	21.81	49.57	26.48	46.94	32.11	55.00	36.06	60.22	26.72	38.72	24.06	49.48	31.17	55.83	30.50	49.94	34.11	60.61	27.38	57.48
Urea	29.31	54.67	27.56	50.59	33.83	67.00	38.44	68.67	28.78	58.50	34.78	61.03	35.94	61.06	36.28	59.01	38.39	61.61	35.77	64.86
Potassium nitrate	22.44	52.21	29.39	54.11	31.56	65.98	35.44	64.22	32.17	63.94	25.00	57.07	30.72	59.11	32.06	55.89	36.91	60.44	32.22	59.83
F test	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.
SE (m) ±	0.40	0.56	0.41	0.56	0.59	0.31	0.50	0.42	0.22	0.38	0.43	1.40	0.33	0.32	0.46	0.40	0.87	0.25	0.56	0.42
CD (P = 0.01)	1.81	2.56	1.86	2.51	2.68	1.40	2.26	1.90	0.99	1.72	1.92	6.30	1.50	1.28	2.07	1.80	3.92	1.14	2.52	1.88

DAI: Days after Incubation

Conclusions

The pathogen *Colletotrichum* species was found to be associated with fruit rot of chilli in all the disease fruits collected from the chilli growing areas of Maharashtra, Karnataka and Guntoor and varied in morphological characters. Among the different nutrient media PDA supported the maximum growth in seven isolates *viz*., Cc_1, Cc_4, Cc_5, Cc_7, Cc_8, Cc_9 and Cc_{10} whereas, carrot agar supported maximum growth in three isolates *viz*., Cc_2, Cc_3 and Cc_6. Though sucrose as carbon source gave maximum growth in Cc_1, Cc_2, Cc_3, Cc_5, Cc_6 and Cc_7, while lactose was preferred by Cc_4, Cc_8, Cc_9 and Cc_{10}. Preference towards 'N' from urea was shown by Cc_1, Cc_3, Cc_4, Cc_6, Cc_7, Cc_8, Cc_9 and Cc_{10}, while Cc_2 and Cc_5 preferred potassium nitrate.

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