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Effect of different culture media on growth and sporulation of *Alternaria dauci* causing carrot leaf blight

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Abstract
Alternaria leaf blight is most common and devastating disease in carrot plant caused by deuteromycotina fungi, *Alternaria dauci*. A total of 11 solid culture media were tested to assess their effect on cultural characters and sporulation of *A. dauci* and results revealed that mean colony diameter was significantly highest on PDA (90.00 mm), followed by Potato carrot agar (75.67 mm), Oat meal agar (70.67 mm), carrot root agar (62.41 mm), Carrot leaf extract agar (54.00 mm), Richard's agar (51.67mm) and Czapek's dox agar (50.67mm), Radish root extract agar (45.50mm), Ashby's agar (44.67mm) and V8 juice agar (39.33mm). The colony growth produced mostly circular and with cottony or cushiony to carpet like mycelial mat. Colony colour varied from white, grayish-white, and olivaceous green to black. At sporulation Oat meal agar and Potato dextrose agar exhibited excellent (++++) sporulation. Whereas, it was poor (+) on Radish leaf extract agar and Richard's agar. Potato dextrose agar was reported as most suitable culture medium for growth and sporulation of *Alternaria* spp.

Keywords: *Alternaria*, *Alternaria dauci*, culture media, carrot, carrot leaf blight, *Alternaria* spp

Introduction
Leaf blight of carrot (*Dacus carota* L.) is a threat to the profitable cultivation of carrot. The disease causes reduction in quantity and quality of carrot drastically. The characteristic symptoms induced by *A. dauci*, in carrot are: small lesions commonly on margins and tips of carrot leaflets, initial symptoms appear on older leaves as irregularly-shaped, minute, dark brown-to-black spots, with yellow border on edge of the leaflets. Small, brown, water soaked spots appear on leaves and petiole and lesions often surrounded by a well-defined yellow halo. Lesions on petioles are brown and irregular, increase in number and size, get coalesced exhibiting blighted (burned) appearance and eventually, such leaflets may shrivel and die. Infection by *Alternaria dauci* is favoured by cool to moderate temperatures and prolonged leaf wetness. Infection can occur in 8 to 12 hours at temperatures of 16-25ºC, the fungus sporulates readily on dead necrotic tissues and the spores germinate readily in water droplets and dew (Delahaut and Stevenson, 2004; Gugino *et al*, 2004; Chand and Singh, 2011; Bharat *et al*, 2012; Tulek and Dolar, 2015) [4, 6, 3, 12].

Alternaira dauci requires several specific compounds for their growth. In *in vitro* study, fungus is isolated as pure culture in specific media for studies on growth, nutrition, physiology and management of the fungus. A wide range of media can favor the isolation of the *A. dauci* fungus which supports the radial growth, dry weight growth and sporulation of the fungus. However the nutrient requirements for good growth of the fungus do not confirm the nutrient requirements for good sporulation. Various media compositions also influence the different colony morphology of *A. dauci*. In plants, carbohydrates are available in simple as well as in complex form and fungi convert the complex forms into simple water soluble sugars of low molecular weight before utilization. It has been shown that different fungi respond differently with a particular compound and the fungi exhibit marked variation in the utilization of different carbohydrate sources. A critical and comprehensive knowledge of nutritional patterns and factors influencing the growth of fungi is a prerequisite for any study leading to the understanding of host-pathogen relationship. Not much attention has been given on the culture and growth media parameters of the pathogen. Hence, thorough knowledge on the influence of various culture media on growth of the fungus as well as sporulation and colony characteristics of the fungus isolated from leaf blight infected carrot leaves is needed to be developed for suitable management strategies of the disease and may help in taxonomical and physiological study of the fungus.

Materials and Methods

Media preparation

A total of 11 solid culture media (Composition-Appendix-I) were evaluated for their effects on cultural characteristics and sporulation of *A. dauci*. The culture media *viz.,* Carrot leaf and root extract agar, Radish leaf and root extract agar (each extract @ 20%) and PDA were constituted and prepared locally and rest of the six media used were synthetic/ready-made, formulated by Hi-media.

The general preparation of medium was same in all the cases. In case of solid media preparation, agar was melted in 1000 ml distilled water. Then the other ingredients were dissolved in 1000 ml of distilled water. In case of natural and semi-synthetic media, the extract was made by the boiling the carrot leaves, radish, radish leaves, peeled-sliced potato, carrot respectively in 1000 ml of distilled water and then the extract was filtered, add agar and dextrose. All of the test culture media were autoclaved at 15lbs for 20 min. Upon cooling (40^0c), these were aseptically and separately poured (each @ 20 ml/plate) in sterilized glass Petri plates (90mm dia.) and allowed to solidify at room temperature. Upon solidification of the media in Petri plates, these were aseptically inoculated with a pure culture disc (5mm) obtained from a week old actively growing pure culture of *A. dauci* and incubated at 27 ± 2^0C, in BOD incubator. A triplicate set of each test culture medium per replication was maintained.

Incorporation of the fungus culture

The pure culture of the fungus was obtained by culturing the fungus on potato dextrose agar medium and making the fresh culture from "hyphal tip" selected from the periphery of actively growing colony under aseptic conditions. Pure culture was maintained by routine subculturing after 14 days. Mycelial blocks were cut out of 10 days old fungal colony near the margin by means of sterilized cork borer of 5 mm diameter. These blocks were transferred to the center of the petri plates All these were done under perfect aseptic condition inside an inoculation chamber which was sterilized previously by spraying formaldehyde solution (4%) and ultra violet (U. V.) radiation.

Fungus growth measurement technique

For solid media, linear growth of the fungus was determined directly by measuring the diameter of the colonies in the same axis after 7 days of inoculation. Linear growth of the colony was measured with the help of fine transparent plastic scale in millimeter.

Observation on characteristics of growth

Color of colony and substrate, margin of colony, topography of mycelium were observed by naked eye. For measuring sporulation on different media, a single block of 5 mm diameter was cut out from the fungal colony near the margin by sterilized cork borer and was transferred to 5 ml sterile distilled water in a test tube, where it was mixed thoroughly to make a uniform spore suspension. One small drop of spore suspension was taken on a slide and average spore count of three microscopic fields was recorded under low power (10X) objective of the microscope.

Statistical Analysis

The experiments were done under controlled laboratory conditions, and the data were analyzed following completely randomized design (CRD).

Results and discussion

A total of 11 solid culture media were tested to assess their effect on cultural characters and sporulation of *A. dauci* and results obtained thereof are presented in Table 1. The results revealed that better growth and variable sporulation of *A. dauci*. Mean colony diameter recorded on test culture media was ranged from 39.33 mm (V_8 juice agar) to 90.00 (PDA) mm. However, it was significantly highest on PDA (90.00 mm), followed by Potato carrot agar (75.67 mm), Oat meal agar (70.67 mm),carrot root agar (62.41 mm), Carrot leaf extract agar (54.00 mm), Richard's agar (51.67mm) and Czapek's dox agar (50.67), However, comparatively minimum growth was obtained on Radish root extract agar (45.50mm), Ashby's agar (44.67mm) and V8 juice agar (39.33mm). The colony growth produced on test culture media was mostly circular and with cottony or cushiony to carpet like mycelial mat. Colony colour varied from white, grayish-white, and olivaceous green to black.

Table 1: The sporulation of the spore suspension was estimated by using the following notations.

No. of spore per microscopic field	Designation
0	-(nil)
1-10	+ (poor)
11-20	++ (moderate)
21-30	+++ (good)
31-40	++++ (excellent)

The test culture media exhibited a wide range of sporulation. However, Oat meal agar and Potato dextrose agar exhibited excellent (++++) sporulation. It was good (+++) on V_8 juice agar, Potato carrot agar and Carrot root extract agar; fair (++) on Czapek**'**s dox agar, Ashby's agar and Radish root extract agar; whereas, it was poor (+) on Radish leaf extract agar and Richard's agar.

Potato dextrose agar was reported as most suitable culture medium for growth and sporulation of *Alternaria* spp, earlier by several workers (Yadav and Khan, 2008; Hubballi *et al.*, 2010; Apet *et al.*, 2014; Shabana *et al*., 2015; Koley and Mahapatra 2015) [13, 7, 1, 5, 11, 9]. Other culture media reported suitable for better growth and good to excellent sporulation of *A. dauci* in present study were Potato carrot agar, Oat meal agar and Carrot root extract agar. These findings are in consonance with earlier reports of many workers (Khan *et al.*, 2007; Ramjegathesh and Ebeneazar, 2012; Gaikwad *et al.*, 2014; Koley and Mahapatra, 2015) [8, 10, 5, 9],

Table 2: Effect of various culture media on cultural characteristics and sporulation of *A. dauci,* causing leaf blight in carrot

T. No	Treatments	Colony Diam.* (mm)	Cultural characters	Sporulation
T_1	Oat meal agar	70.67	Circular, cottony mycelia mat, whitish-gray coloured.	++++
T_2	V8 juice agar	39.33	Circular, cottony mycelial mat, grayish – white coloured.	+++
T_3	Potato carrot agar	75.67	Circular, cottony mycelial mat, whitish-black coloured.	+++
T_4	Czapek's dox agar	50.67	Circular, cushiony mycelial mat, grayish-white coloured.	++
T_5	Richard's agar	51.67	Circular, cushiony mycelial mat, grayish – black.	+
T_6	Potato dextrose agar	90.00	Circular, carpet like mycelial mat, olivaceous– black with white ring.	++++

T_7	Ashby's agar	44.67	Circular, cottony like mycelial mat, grayish-white.	++
T_8	Carrot leaf extract agar	54.00	Circular, cottony like mycelial mat, grayish-white.	++
T_9	Carrot root extract agar	62.41	Circular, cottony like mycelial mat, grayish-white.	+++
T_{10}	Radish leaf extract agar	45.50	Circular, cottony like mycelial mat, grayish-white.	+
T_{11}	Radish root extract agar	49.67	Circular, cottony like mycelial mat, grayish-white	++
	S.E. ±	0.59	---	----
	C.D. (P=0.01)	1.74	---	----

*: Mean of three replications, Dia.: Diameter Sporulation: ++++: Excellent, +++: Good, ++: Fair, +: Poor, -: No

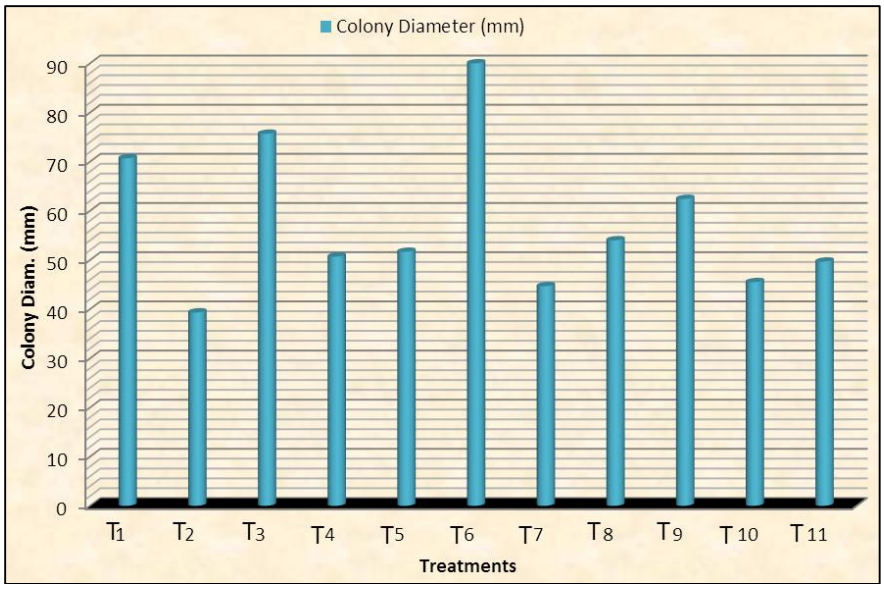

Fig 1: Effect of various culture media on radial growth of *A. dauci*, infecting carrot (graph)

Table 3: Effect of various culture media on radial growth of *A. dauci*, infecting carrot

T. No	Treatments	Tr. No	Treatment
T_1	Oat meal agar	T_7	Ashby's agar
T_2	V_8 juice agar	T_8	Carrot leaf extract (20%) agar
T_3	Potato carrot agar	T_9	Carrot root extract (20%) agar
T_4	Czapek's dox agar	T_{10}	Radish leaf extract (20%) agar
T_5	Richard's agar	T_{11}	Radish root extract (20%) agar
T_6	Potato dextrose agar	--	----------

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Appendix-I
Composition of various culture media used for *in vitro* studies

1	Oat meal agar	Oat meal: 60.0 g
		Agar agar: 12.5 g
		Distilled water:1000 ml
2	V_8 juice agar	V8 juice: 200 ml
		$CaCo_{3:}$ 3.0 g
		Agar agar: 15.0 g
		Distilled water:1000 ml
3	Potato Carrot agar	Peeled potato: 100g
		Carrot: 100 g
		Dextrose: 20 g
		Agar agar: 20g
		Distilled water: 1000 ml
4	Czapek's dox agar	Sucrose: 30 g
		Sodium nitrate: 2.0 g
		Dipotassium phosphate: 1.0 g
		Magnesium sulphate: 0.5 g
		Potassium chloride: 0.5 g
		Ferrous sulphate: 0.01 g
		Agar agar: 15 g
		Distilled water: 1000 ml
5	Richard's agar	Sucrose: 50 g
		Potassium nitrate: 10 g
		Potassium dihydrogen phosphate: 0.5g
		Magnesium sulphate: 2.5 g
		Ferric chloride: 0.02 g
		Agar agar: 15.0 g
		Distilled water: 1000 ml
6	Ashby's agar	Mannitol: 20.0 g
		Dipotassium phosphate: 0.2 g
		Magnesium sulphate: 0.2 g
		Sodium chloride: 0.2 g
		Calcium carbonate: 5.0 g
		Agar agar:15.0 g
		Distilled water:1000 ml
7	Potato dextrose agar	Peeled potato: 200g Dextrose: 20 g
		Agar agar: 20g
		Distilled water: 1000 ml
8	Carrot leaf extract agar	Healthy carrot leaves: 200g
		Dextrose: 20 g
		Agar agar: 20g
		Distilled water: 1000 ml
9	Carrot root extract agar	Healthy carrot: 200g
		Dextrose: 20 g
		Agar agar: 20g
		Distilled water: 1000 ml
10	Radish leaf extract agar	Healthy radish leaves: 200g
		Dextrose: 20 g
		Agar agar: 20g
		Distilled water: 1000 ml
11	Radish root extract agar	Healthy radish: 200g
		Dextrose: 20 g
		Agar agar: 20g
		Distilled water: 1000 ml