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In-vitro evaluation of different culture media for the growth of *Alterneria cucumerina* var. *cyamopsidis* caused Alternaria leaf spot of clusterbean

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Abstract
A laboratory study was conducted to study the effect of seven solid culture media, *viz*., Potato Dextrose agar, Czapek's-Dox agar, Richard's agar, Corn meal agar, Sabouraud's agar, Guar leaf extract and Oat meal agar on the growth rate of test pathogen *Alternaria cucumerina* var. *cyamopsidis* caused alternaria leaf spot of clusterbean. The maximum mycelium growth was recorded in potato dextrose agar media at 3, 5 and 7 Day after inoculation respectively and it was significantly superior over other tested media while the minimum growth was recorded on corn meal agar at 3, 5 and 7 days respectively.

Keywords: Culture media, *Alternaria cucumerina* var. *cyamopsidis*, mycelium growth

Introduction
The growth of microorganisms on an artificial medium is influenced by several physical and chemical factors. A nutrient material prepared for the growth of microorganisms in a laboratory is called culture medium and the nutrient composition of a culture medium plays a major role in microbial growth (Toratora *et al.* 1995) [5].
In laboratory, fungi are isolated on specific and non-specific culture medium for cultivation, preservation, microscopic examination as well as biochemical and physiological characterization (Northolt and Bullerman (1982) [4]; Kuhn and Ghannoum (2003) [3]; Kumara and Rawal (2008) [2]. In this study, *Alternaria cucumerina* var. *cyamopsidis*, which is a major pathogen for foliar disease of cluster bean in Northern India (Rangaswami and Rao, 1957). A wide range of media are used for isolation of different groups of fungi that influence the vegetative growth, colony morphology, pigmentation and sporulation depending upon the composition of specific culture medium, pH, temperature, light, water availability and surrounding atmospheric gas mixture (Northolt and Bullerman 1982) [4]. However, The experiments were performed in the laboratory condition to find out the effect of media composition on vegetative and reproductive growth of *Alternaria cucumerina* var. *cyamopsidis* on different culture media.

Materials and methods
The different solid culture media were evaluated for obtaining maximum mycelial growth of the *A. cucumerina* var. *cyamopsidis***.** The experiment was laid out in complete randomized design with replicated thrice times. Seven solid culture media *viz*., Potato Dextrose agar, Czapek's-Dox agar, Richard's agar, Corn meal agar, Sabouraud's agar, Guar leaf extract and Oat meal agar used to compare the growth rate of test pathogen**.**
The Culture medium were prepared by the standardized method and autoclaved at 121.6 °C, 15 psi pressure for twenty minutes 20 ml of each medium were poured in 90 mm Petri dish. After autoclaving streptomycin was added to prevent bacterial growth. Each Petri plate was inoculated separately with uniform mycelia culture bits (5 mm) cut with the help of cork borer from young (5 days) vigorously growing culture were placed on the middle of the each pre poured medium and incubated at 25±1 ^{0}C (Dela Paz *et al*., 2006). The fungal linear growth was measured in mm after 7 days of inoculation (Koley and Mahapatra, 2015).

The colour of the colony was observed by naked eye. For measuring the sporulation on different media The diameter of the growth of the fungus was measured after 3, 5, and 7 days after inoculation.

Table 1: Different culture media used for the growth of *A. cucumerina* var. cyamopsidis and their composition

S. No.	Name of Media	Ingredients	Quantity
1	Czapek's Dox Agar	Sucrose	30.0g
		Sodium nitrate	2.0g
		Dipotassium hydrogen phosphate	4.0g
		Magnesium sulphate	0.01g
		Agar-agar	15.0 g
		Distilled water	1000 ml
2.	Potato Dextrose Agar	Peeled and sliced potato	200.0 g
		Dextrose	20.0g
		Agar-agar	20.0g
		Distilled water	1000 ml
3.	Guar leaf extract	Grated guar leaf	200.0g
		Agar-agar	20.0g
		Dextrose	20.0gm
		Distilled water	1000.0ml
4.	Oat Meal Agar	Oat meal	200.00 g
		Glucose	20.00 g
		Agar-agar	20.0g
		Distilled water	1000 ml
5.	Corn Meal Agar	Corn meal	200.0g
		Glucose	20.0g
		Agar-agar	20.0g
		Distilled water	1000 ml
6.	Richard Agar	Potassium nitrate	10 g
		Potassium dihydrogen phosphate	5 g
		Magsnesium sulphate	2.5 g
		Ferric chloride	0.02 g
		Sucrose	50 g
		Distilled water	1000 ml
7.	Sabouraud's agar	Glucose	40 g
		Peptone	10 g
		Agar agar	20 g
		Distilled water	1000

Results

The fungal growth is mainly depended on the nutritional availability in culture media. In order to find out the most effective culture media for the growth of *Alterneria cucumerina* var. *cyamopsidis*. These studies have indicted that PDA oat meal agar gave good growth and better mycelial growth were recorded at 3, 5 and 7 days after inoculation. (table-2) At 7DAI, the maximum mycelium was recorded in potato dextrose agar (83.67 mm) medium followed by Richards agar (67.33 mm), oat meal agar (61.67 mm), czapex's dox agar (57.33 mm), guar leaf extract (57.00 mm) and sabourauds's agar (55.67 mm), while the minimum growth recorded in corn meal agar (49.67 mm) medium. The maximum mycelium growth was found on potato dextrose agar media at 3, 5 and 7DAI after inoculation and it was significantly superior over other tested media while the minimum growth was recorded on corn meal agar media at 3, 5 and 7 days.

Table 2: Effect of media culture media on the growth of *Alternaria cucumerina* var. *cyamopsidis*

S.no	Medium	Mean radial growth (mm)* at 2 days interval		
		3DAI	5DAI	7DAI
1.	Richards agar	28.67	52.67	67.33
2.	Czapex dox agar	19.33	35.33	57.33
3.	Corn meal agar	18.33	33.00	49.67
4.	Potato dextrose agar	36.67	65.67	83.67
5.	Sabouraud's agar	22.33	42.33	55.67
6.	Guar leaf extract	24.00	38.67	57.00
7.	Oat meal agar	31.00	46.00	61.67
SEm(±)		0.484	0.403	0.759
C.D. at 5%		1.482	1.235	2.323

Discussion

Berry (1960) [1] and Siddaramaiah (1981) [7] also reported that the fungus grows nicely on PDA medium. He further observed that isolates of *Alternaria cucumerina* var. *cyamopsidis* were found to differ markedly in cultural characteristics and virulence. The isolates differ in appearance (colony character) and growth rate too, good growth of Alternaria cucumerina var. cyamopsidis and seven different species of Alternaria on PDA and Oat meal agar (Pawar and Patel 1957 [8], Rangaswami and Rao 1957 and Rangaswami and Sambandan 1960 respectively). Also reported that PDA medium was good for the growth of *Alternaria cucumerina* var. *cyamopsidis.*

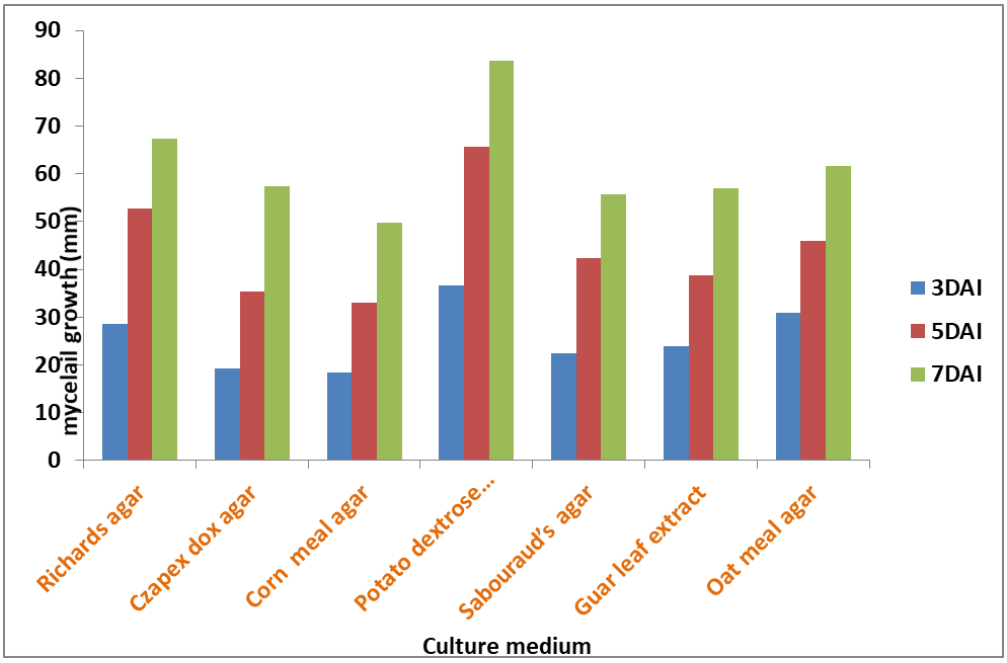

Fig 1: Mycelial growth of *Alternaria cucumerina* var. *cyamopsidis* on different culture media at 3, 5 and 7 DAI

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