



Plant Archives

Journal homepage: <http://www.plantarchives.org>

DOI Url : <https://doi.org/10.51470/PLANTARCHIVES.2026.v26.no.1.236>

IN-VITRO EVALUATION OF CULTURE MEDIA FOR MYCELIAL GROWTH AND SPORULATION OF *ALTERNARIA CUCUMERINA* VAR. *CYAMOPSISIDIS* CAUSING ALTERNARIA BLIGHT OF CLUSTER BEAN

Yogesh Kumar^{1*}, Rajni Singh Sasode¹, Neelam Soni,¹ Anita Kumari Pandey,¹
Hari Shankar Kumawat² and Habil Dongre³

¹Department of Plant Pathology, College of Agriculture, RVSKVV, Gwalior - 474 001, Madhya Pradesh, India.

²Department of Plant Pathology, College of Agriculture, Agriculture University, Jodhpur - 342 304, Rajasthan, India.

³Department of Horticulture, College of Agriculture, RVSKVV, Gwalior - 474 001, Madhya Pradesh, India.

*Corresponding author E-mail : yogeshjakhhar1000@gmail.com

(Date of Receiving-07-02-2026; Date of Revision-28-03-2026; Date of Acceptance-12-04-2026)

ABSTRACT

A laboratory study was conducted to study the effect of ten culture media, viz., Potato dextrose agar, Czapek's dox agar, Richard's agar, Sabouraud's agar, Carrot agar, Potato carrot agar, Walksman agar, V₈ juice agar, Guar leaf extract agar and Oat meal agar on growth rate of test pathogen *Alternaria cucumerina* var. *cyamopsisidis* caused Alternaria blight of clusterbean. The maximum mycelium growth was recorded in Potato dextrose agar media at 3,5 and 7 days after inoculation and it was significantly superior over other test media while the minimum growth was recorded on V₈ juice agar at 3,5 and 7 days after inoculation and the excellent sporulation observed on Potato dextrose agar and Potato carrot agar while poor sporulation found on walksman agar and V₈ juice agar.

Key words : Culture media, Alternaria blight, *Alternaria cucumerina* var. *cyamopsisidis*, Days after inoculation.

Introduction

Cluster bean (*Cyamopsis tetragonoloba* (L.) Taub.), commonly referred to as guar, has emerged as a significant commercial crop in arid and semi-arid regions. Cluster bean is an annual leguminous crop predominantly grown under resource-constrained conditions of arid and semi-arid ecosystems (Kumar, 2005). It belongs to the family Leguminosae (Fabaceae) and is distinguished by its deep-rooted system, which enables it to tolerate prolonged drought and high temperature regimes (Kumar and Rodge, 2012). The endosperm of the cluster bean seed contains approximately 30-33% galactomannan gum. The identification of galactomannan gum in the endosperm in 1948 transformed this previously minor crop into a highly valuable industrial commodity. Cluster bean has been cultivated in India since ancient times. Although it considered to have originated in Africa (Vavilov, 1951), it was domesticated several centuries ago in the north-western region of the Indo-Pakistan subcontinent

(Hymowitz, 1972). In Madhya Pradesh alone, cluster bean is cultivated over an area of 31.4 lakh hectares, with a total production of 15.19 lakh tonnes and an average productivity of 750 kg per hectare (Anonymous, 2021). *Alternaria cucumerina* var. *cyamopsisidis* is a significant causal agent of foliar disease in cluster bean crops in Northern India (Rangaswami and Rao, 1957). Greater yield losses (43-78%) were observed when infection occurred at the seedling stage compared to the later growth stage (Sharma, 1983). A variety of culture media are employed for the isolation of diverse fungal groups influencing vegetative growth, colony morphology, pigmentation and sporulation, which vary according to the composition of the medium, pH, temperature, illumination, moisture availability and surrounding gaseous composition (Northolt and Bullerman, 1982). To assess the effect of medium composition on the vegetative and reproductive development of *Alternaria cucumerina* var. *cyamopsisidis* across different culture media, experimental

investigations were performed under controlled laboratory conditions. A culture media refers to the nutrient material formulated in the laboratory to support microbial growth. The composition of this medium plays a crucial role in determining the extent and rate of microbial development (Tortora *et al.*, 1995).

Materials and Methods

The experiment aimed to evaluate different solid culture media for the optimal conditions required for maximum mycelial growth of *Alternaria cucumerina*

Table 1 : Composition of different media.

S. no.	Name of media	Ingredients	Quantity
1	Potato dextrose agar	Peeled potato	200 g
		Dextrose	20 g
		Agar-agar	20 g
		Distilled water	1000 ml
2	Czapek's dox agar	Sucrose	30 g
		Sodium nitrate	2 g
		Agar-agar	15 g
		Water	1000 ml
3	Richard's agar	Potassium nitrate	10 g
		Monopotassium dihydrogen phosphate	5 g
		Magnesium sulphate	2.50 g
		Ferric chloride	0.020 g
		Sucrose	50 g
		Agar-agar	15 g
		Water	1000 ml
4	Sabouraud's agar	Peptone	10 g
		Dextrose	40 g
		Agar-agar	20 g
		Water	1000 ml
5	Carrot agar	Grated carrot	250 g
		Agar-agar	20 g
		Dextrose	20 g
		Water	1000 ml
6	Potato carrot agar	Grated potato	200 g
		Grated carrot	20 g
		Agar-agar	20 g
		Water	1000 ml

Table 1 continued...

Table 1 continued...

7	Walksman agar	Peptone	5 g
		Beef extract	5 g
		Sodium chloride	5 g
		Glucose	10 g
		Agar-agar	12.5 g
		Water	1000 ml
8	V ₈ juice agar	V ₈ juice	200 ml
		Calcium carbonate	3 g
		Agar-agar	15 g
		Water	800 ml
9	Guar leaf extract agar	Guar leaves extract	200 g
		Dextrose	20 g
		Agar-agar	20 g
		Water	1000 ml
10	Oat meal agar	Oat meal	40 g
		Agar-agar	20 g
		Water	1000 ml

var. cyamopsidis. It was conducted by using a completely randomized design with three replications. Ten culture media *viz.*, Potato dextrose agar, Czapek's dox agar, Richard's agar, Sabouraud's agar, Carrot agar media, Potato carrot agar, Walksman agar, V₈ juice agar, Guar Leaf Extract and Oat meal agar. For each type of media 20ml was poured into 90mm sterilized Petri-plates. After the media solidified, fungal isolates were individually inoculated into each media using 5mm mycelia discs obtained from a cork borer under sterilized conditions. The plates were then incubated at a temperature of 27±10°C.

The growth of fungal isolates was assessed by measuring the radial growth of the fungal colony (in mm) at 3, 5 and 7 days post-inoculation. This provided a comprehensive view of the growth rate on each type of media. The colony's colour was visible to the unaided eye. A single mm-diameter block was removed from the fungal colony close to the edge using a sterilized cork borer in order to measure the sporulation on various media. It was then placed in a test tube with 5ml of sterile distilled water and thoroughly mixed to create a homogenous spore suspension. A single tiny drop of spore suspension was placed on a slide and using the microscope's low power (10x) objective, the average number of spores in three microscopic field was noted.

Results

Fungal growth is primarily influenced by the nutritional

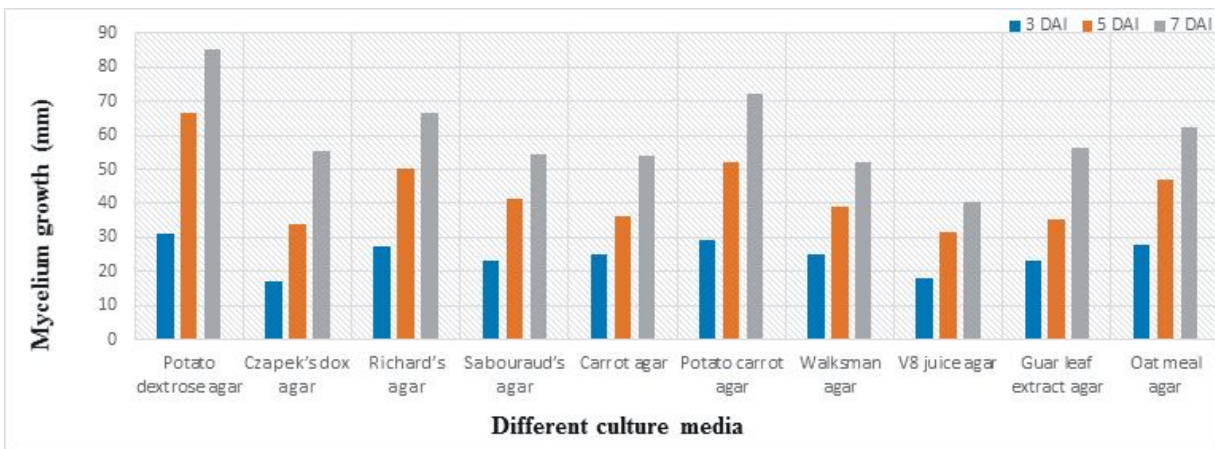


Fig. 1 : Mycelium growth of *Alternaria cucumerina* var. *cyamopsidis* on different media at 3, 5 and 7 DAI.

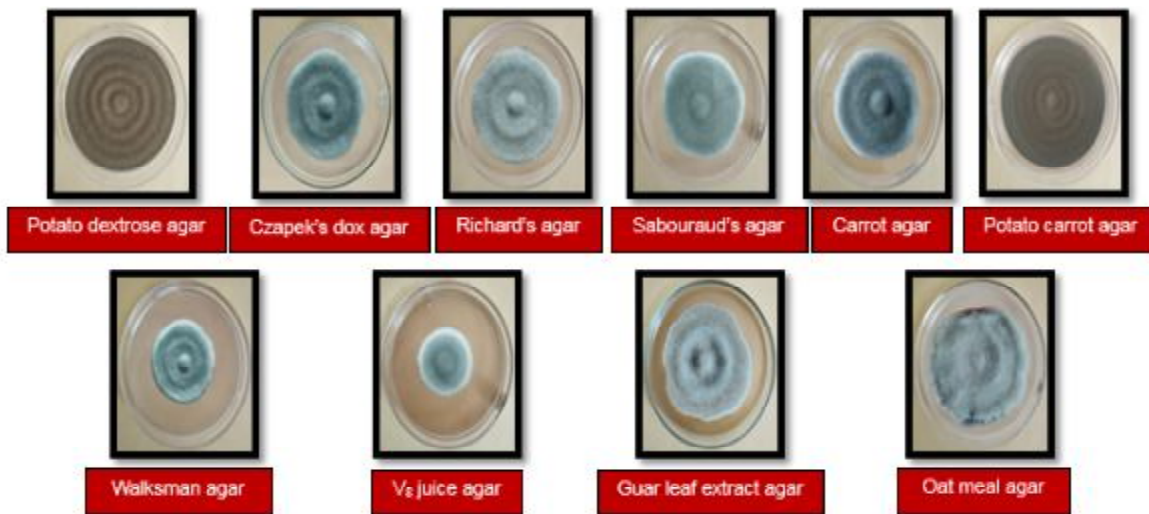


Fig. 2 : In-vitro evaluation of culture media against *Alternaria cucumerina* var. *cyamopsidis* at 7 DAI.

composition and availability of nutrients in the culture medium. To determine the most suitable culture medium for supporting the growth of *Alternaria cucumerina* var. *cyamopsidis*, ten different culture media were evaluated under laboratory conditions.

Mycelial growth

The results of these investigations revealed that Potato Dextrose Agar (PDA) and Potato Carrot Agar (PCA) supported superior mycelial proliferation, with appreciable growth recorded at 3, 5 and 7 days' post inoculation. Among the tested media, the maximum radial mycelial growth at 7 days after inoculation was observed on Potato Dextrose Agar, exhibiting an average colony diameter of 85.10 mm. This was followed by Potato Carrot Agar (72.21 mm), Richard's Agar (66.74 mm), Oat Meal Agar (62.17 mm), Guar Leaf Extract Agar (56.15 mm), Czapek's Dox Agar (55.33 mm), Sabouraud's Agar (54.59 mm), Carrot Agar (54.00 mm) and Walksman Agar (52.31 mm). The minimum mycelial growth was recorded on V₈ Juice Agar medium, which showed an average colony diameter of 40.35 mm. These findings

suggest that media enriched with potato-based nutrients are highly conducive for the vegetative growth and development of *Alternaria cucumerina* var. *cyamopsidis*, possibly due to their optimal carbon and nitrogen balance and readily assimilable nutrient content.

Colour and sporulation

Variation in the colour of colony and topography of mycelium showed the important information which may be helpful in taxonomic identification of *Alternaria cucumerina* var. *cyamopsidis*. Among all media tested for evaluation, the colour of culture was slightly different from each other. Colony is Brownish – black on Potato dextrose agar and Potato carrot agar. Grayish – white on Czapek's dox agar, Sabouraud's agar, Carrot agar and Walksman agar and whitish-gray on Richard's agar, V₈ juice agar, Guar leaf extract agar and Oat meal agar.

The test culture media exhibited a wide range of sporulation. However, Potato dextrose agar and Potato carrot agar exhibited excellent (++++); it was good (+++) on Czapek's dox agar, Richard's agar, Carrot agar, Guar

Table 2 : Effect of different media on the mycelium growth, colony colour and sporulation of *Alternaria cucumerina* var. *cymopsidis*.

S. no.	Medium	Mycelium growth (mm)			Mean of mycelium growth (mm)	Colony colour	Sporulation
		3DAI	5DAI	7DAI			
1	Potato dextrose agar	30.89	66.70	85.10	60.90	Brownish – black	++++
2	Czapek’s dox agar	17.11	34.12	55.33	35.52	grayish – white	+++
3	Richard’s agar	27.17	50.13	66.74	48.01	whitish-gray	+++
4	Sabouraud’s agar	23.33	41.31	54.59	39.74	grayish – white	++
5	Carrot agar	25.00	36.20	54.00	38.40	grayish – white	+++
6	Potato carrot agar	29.12	52.11	72.21	51.14	Brownish – black	++++
7	Walksman agar	25.20	39.13	52.31	38.88	grayish – white	+
8	V ₈ juice agar	18.15	31.50	40.35	30.00	whitish-gray	+
9	Guar leaf extract agar	23.21	35.10	56.15	38.15	whitish-gray	+++
10	Oat meal agar	28.00	47.10	62.17	45.75	whitish-gray	+++

Sporulation:- +++++: Excellent, +++: Good, ++: Fair and +: Poor.

leaf extract agar and Oat meal agar; fair (++) on Sabouraud’s agar; whereas, it was poor (+) on Walksman agar and V₈ juice agar.

Discussion

The superior growth on Potato dextrose agar (PDA) highlights the fungus’s preference for simple carbohydrates and starch, which provide easily accessible energy. The high-performance of Potato carrot and Richard’s agar further suggest a requirement for specific vitamins and mineral salts. Conversely, the poor results in V₈ juice agar indicate that its acidic profile or complex vegetable base inhibited optimal mycelial expansion.

Similarly, Anupriya *et al.* (2020) reported that most effective culture media for the growth of *A. cucumerina* was recorded PDA (85.67 mm) followed by Richards agar (67.33 mm), Oat meal agar (61.67 mm), Czapek’s dox agar (57.33 mm), Guar leaf extract (57.00 mm), Sabouraud’s agar (55.67 mm) and Corn meal agar (49.67 mm). Krishna *et al.* (2018) reported that the growth of *A. dauci* was best on PDA (90 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 (51.67 mm), Czapek’s dox agar (50.67 mm), Radish root extract agar (45.50 mm), Ashby’s agar (44.67 mm) and V₈ juice agar (39.33 mm). Goswami *et al.* (2022) reported that most effective culture media for the growth of *A. alternata* was recorded Richard’s agar (86.83 mm) followed by Czapek’s dox agar (85.5 mm), Oatmeal agar (75.5 mm), Potato Dextrose agar (75 mm), Tomato Fruit agar (74.43 mm), Rose Bengal agar (72 mm), Carrot agar (62.23 mm), Radish agar (57.1 mm), Chilli fruit agar (47 mm), Chilli leaf agar (43.13 mm) and Tomato leaf agar (32

mm). Mohapatra (1977) found that among various growth media, Potato Dextrose Broth (PDB) supported the maximum growth of *Alternaria sesame*, which infects sesame crops. This was followed by Richard’s, Czapek’s dox and oat meal broth. Abeer *et al.* (2014) reported that potato dextrose agar was the best medium for radial growth and sporulation of *Alternaria alternata* from *Avicennia marina*.

Conclusion

The present investigation revealed that the growth, morphology and sporulation of *Alternaria cucumerina* var. *cymopsidis* are greatly influenced by the composition of the culture medium. Among the ten media evaluated, Potato Dextrose Agar (PDA) proved to be the most suitable, supporting maximum mycelial growth and excellent sporulation, followed closely by Potato Carrot Agar (PCA). These media promoted rapid and consistent fungal growth at all observation intervals, likely due to their rich and balanced nutrient content. Moderate growth and sporulation were observed on Richard’s Agar, Oat Meal Agar and Czapek’s Dox Agar, whereas minimum growth occurred on V₈ Juice Agar, indicating its unsuitability. Variations in colony colour and morphology across different media further highlighted the role of nutrients in fungal development and identification. Overall, potato-based media were found to be highly effective and are recommended for culturing and studying *Alternaria cucumerina* var. *cymopsidis* under laboratory conditions.

Acknowledgments

I wholeheartedly extend my profound thanks to my Major Advisor, Dr. Rajni Singh Sasode, Scientist, COA,

Gwalior, RVSKVV, Gwalior, Madhya Pradesh, for his constant support, insightful guidance and committed involvement throughout the duration of this research work.

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