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Assessment of different culture media on the growth and sporulation of *Alternaria alternata* causing leaf blight disease of sunflower

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Abstract
Alternaria blight is the most common and devastating disease caused by *Alternaria alternata* in sunflower. This fungus grows well in Yeast Peptone Agar and Carrot + Potato agar medium under *in vitro* conditions. The growth of the fungus was tested using ten different media. Carrot + Potato agar medium appeared to be better than other media for the growth of *A. alternata*. The growth characteristics of the fungus such as colour of the colony and sporulation were also different in different culture media. The colony of the fungus was dark brown in colour. Maximum sporulation of the test fungus was found on Richards agar (RA) Walksman agar (WA), Carrot medium and Carrot+potato agar medium whereas no sporulation was seen on Czapeck's dox agar medium. Thus the present work will be useful for further investigation on the physiology of the fungus and management of the disease.

Keywords: Sunflower, *Alternaria* leaf blight, *Alternaria alternata*, culture medium, Growth, sporulation

Introduction
Sunflower is one of the major oil seed crops grown in India. Its production can be limited by blight disease caused by *A. alternata*, which decreases seed germination and seedling survival (Udayashankar *et al.*, 2011) [9]. It causes severe leaf and stem spotting resulting in premature defoliation and stem breakage. High humidity and moderate to warm temperatures favor *Altemaria* leaf blight. Yield losses of 20 to 80%, with oil losses of 20 to 30%, have been reported from tropical and subtropical sunflower production regions. (Howard and Gent, 2007) [1]. In India, the losses due to *Alternaria* blight range upward to 80% (Shankergoud *et al.*, 2006) [7].

The production and productivity of sunflower in terms of seeds and seedling is highly affected by a number of phytopathogenic fungal diseases. *A. alternata* is economically important pathogens widely distributed throughout the world and cause devastating disease on field crops. *Alternaria* leaf blight is a common disease in all over India. The disease appears year after year in mild to severe form since the pathogen is seed- borne in nature. In early stages of infection, the water soaked spots appear on leaf blade which later turn greyish to dark brown with concentric zonation, demarcated with light brown lines inside the spot on the under surface. The lesions are light to greyish brown. Higher yield losses (43-78%) were recorded when leaves were infected at seedling stage than at old stage (Sharma, 1984).

The present study is focused on the growth and colony characteristics of *A.alternata* using different growth medium for the knowledge of nutritional pattern and factors influencing the growth of the this pathogen.

Materials and Methods
Different common synthetic, semi synthetic and natural media in solid form were used to culture the fungus. The purified culture of the fungus was inoculated into 11 different culture media *viz*., Asthana and Hawker's agar (AHA), Oat meal agar (OMA), Glucose peptone agar (GPA), Richards agar (RA), Sunflower leaf extract medium (SLEM), Walksman agar (WA), Potato dextrose agar medium (PDA), Yeast Peptone agar medium (YA), Carrot medium (CA), Czapeck's Dox agar (CDA), Carrot+potato (CPA) agar medium.

The culture media were prepared by the standardized method and autoclaved at 121 °C at 15 psi pressure for 20 minutes.

After autoclaving penicillin (200 mg/l) was added to prevent the bacteria growth. Uniform quantities (20 ml) of each medium were poured into 90 mm Petri plate. Each Petri plate was inoculated separately with uniform mycelia culture bits (5 mm) cut with the help of cork borer from young fresh growing culture, were placed on the centre of each Petri plate and incubated at 25±2 °C. The experiment was performed in triplicate form.

The fungal linear growth was measured in mm after 7 days of inoculation (Koley and Mahapatra, 2015) [2]. The colour of the colony was observed by naked eye. For measuring the sporulation on different media, a single block of 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 (10 x) objective of the microscope.

Table 1: Different media synthetic, semi synthetic and natural media and their ingredients.

S. No	Media	Ingredients
1	AHA	a) Potassium Nitrate - 3.50g
		b) Potassium monobasic phosphate - 1.75g
		c) Magnesium sulphate - 0.75g
		d) Glucose - 5g
		e)Agar - 20g
		f)Distilled water - 1 liter
2	OMA	a) Oats - 40g
		b) Agar - 20g
		c) Distilled water - 1 liter
3	GPA	a) Glucose - 10g
		b) Bacto peptone - 2g
		c) Di potassium phosphate - 1g
		d) Agar - 20g
		e) Distilled water - 1 liter
4	RA	a) Potassium Nitrate - 10 g
		b) Potassium monobasic phosphate - 1g
		c) Magnesium Nitrate - 2.5g
		d) Ferric Chloride - 0.02g
		e) Sucrose - 50g
		f) Agar - 20g
		g) Distilled water - 1 liter
5	SLEM	a) Sunflower - 100g
		b) Agar - 20g
		c) Distilled water - 1 liter
6	WA	a) Potassium monobasic phosphate - 1g
		b) Magnesium sulphate - 0.5g
		c) Bacto peptone - 5g
		d) Agar - 20g
		e) Distilled water - 1 liter
7	PDA	a) Potato - 250g
		b) Dextrose - 20g
		c) Agar - 20g
		d) Distilled water - 1 liter
8	YPA	a) Yeast extract - 5.0 g
		b) Peptone - 3.0 g
		c) Mannitol - 25.0 g
		d) Agar - 12.0 g
		e) Distilled water - 1 liter
9	CM	a) Carrot- 250g
		b) Agar - 20g
		c) Distilled water - 1 liter
10	CDA	a) Sodium Nitrate - 2g
		b) Dipottassium Chloride - 1g
		c) Ferrus sulphate – 0.01g
		d) Magnesium sulphate – 0.5g
		e) Sucrose – 30g
		f) Agar – 20g
		g) Distilled water – 1 liter
11	CPA	a) sucrose 30 g
		b) sodium nitrate
		c) Di potassium hydrogen phosphate 4 g
		d) Magnesium sulphate 0.001 g
		e) Agar - 20 g

Results and Discussion

Radial growth of the pathogen the culture of the plant pathogen on or in the suitable medium is the first step of pathological research. In present research work the table 1 showed that eleven culture media were evaluated to find out the most effective medium for the growth of *A. alternata* in *in vitro* conditions. The data reveals that Carrot + Potato Agar medium and Yeast extract medium (90.0 mm) was significantly superior over other tested media after 7 days of inoculation. It was followed by Carrot Agar (89.90 mm), Walksman Agar (76.60 mm), Richards agar (74.60 mm), Potato dextrose agar medium (73.00 mm), Sunflower leaf extract medium (64.90), Czapeck's agar (49.76 mm), Asthana and Hawker's agar (47.00 mm), Glucose peptone agar medium (46.66 mm), Oat meal agar (45.90 mm), poor growth was observed in Czapeck's dextrose agar medium.

Colour of the culture

Variation in the colour of colony and topography of mycelium showed the important information which may helpful in taxonomic identification of *A.alternata.* Among all media tested for evaluation, the colour of culture was slightly different from each other. Colony is transparent white at the centre with regular margin on Asthana and Hawker's agar, Light greyish colony, smooth growth with regular margin on Oat meal Agar. Blackish white mycelia growth on Glucose Peptone Agar, Submerged mycelium, colony is dirty white in colour with compact growth on Richards Agar, Whitish black mycelia growth on Sunflower leaf extract and Carrot and Potato Agar, Dirty white colour, regular margin with compact growth on Walksman agar, Colony is dull white with fluffy growth at the centre and regular margin on Potato Dextrose Agar, Aerial profuse mycelium, with broad concentric ring on Yeast Peptone Agar and Colony is transparent, white at centre and margin is wavy, prominent with dull white in colour on Czapeck's agar. Excellent sporulation (more than 30 spores / microscopic field) of the fungus was observed on Richards agar, Walksman agar, Carrot medium and Carrot+potato agar medium whereas good sporulation (21-30 spores / microscopic field) was observed on Sunflower leaf extract medium, Potato dextrose agar medium, Yeast Peptone Agar medium. Moderate sporulation (11-20 spores / microscopic field) was observed on Asthana and Hawker's agar, Oat meal agar and Glucose peptone agar medium. Sporulation of the fungus could not be observed in case of Czapeck's dextrose agar medium. Meena and Ratnoo (2013) [3] observed that the excellent growth and sporulation was found on Yeast agar medium and Carrot+potato agar medium which was followed by Carrot Agar medium *in vitro* conditions. Many workers have found PDA as best and Brown's medium and Asthana and Hawker's media very poor for *Alternaria* spp. for growth and sporulation in laboratory studies (Singh *et al.*, 2001; Pandey *et al.*, 2006; Waghunde and Patil, 2010) [8, 6, 10].

Total eleven culture media were evaluated for the growth of *A. alternata* under *in vitro* conditions in which maximum mycelial growth was found in Yeast Peptone Agar medium, Carrot Agar medium and Carrot+potato agar medium, while least growth was observed in Czapeck's agar. The present finding is conformity with the reports of earlier study by Pria *et al.*, (1997), Mishra and Mishra (2012) [11] and Munde *et al.*, (2013) [5].

Table 2: Efficacy of different media on the growth of *A.alternata* Causing leaf blight disease of sunflower

S. No	Culture medium	Color of colony	Radial growth (mm)	sporulation
1	Asthana and Hawker's agar (AHA)	Colony is transparent white at the centre with regular margin	47.00^{d}	++
2	Oat meal agar (OMA)	Light grayish colony, smooth growth with regular margin	45.90^{d}	++
3	Glucose peptone agar medium (GPA)	Blackish white mycelia growth	46.67^{d}	++
4	Richards agar (RA)	Submerged mycelium, colony is dirty white in colour with compact growth	74.60^{b}	++++
5	Sunflower leaf extract medium (SLEM)	Whitish black mycellial growth	64.90^{c}	+++
6	Walksman agar (WA)	Dirty white colour, regular margin with compact growth	76.80^{b}	++++
7	Potato dextrose agar medium (PDA)	Colony is dull white with fluffy growth at the centre and regular margin.	73.00^{b}	+++
8	Yeast Peptone agar medium	Aerial profuse mycelium, with broad concentric ring	90.00^{a}	+++
9	Carrot agar medium	Whitish mycelia growth	89.90^{a}	++++
10	Czapeck's Dox agar (CDA)	Colony is transparent, white at centre and margin is wavy, prominent with dull white in colour	49.76^{d}	-
11	Carrot+potato agar medium (CPA)	Whitish mycelia growth	90.00^{a}	++++

- (Nil), + (Poor), ++ (Moderate), +++ (Good), ++++ (Excellent)

The values in parenthesis are angular transform value

References

1. Howard FS, Gent DH. Sunflower Alternaria *leaf* spot. High plains IPM Guide, a cooperative effort of the University of Wyoming, University of Nebraska, Colorado State University and Montana State University, 2007.
2. Koley S, Mahapatra S. Evaluation of culture media for growth characteristics of *Alternaria solani causing* early blight of Tomato. J. Plant Pathol Microbiol. 2015; 9(1):1-5.
3. Meena PK, Ratnoo RS. Effect of growth and sporulation on different solid media and toxin production by *Alternaria* spp. causing leaf spot on cotton. International J Plant Protect. 2013; 6(1):293-295.
4. Mishra PT, Mishra V. Effect of media, temperature, and pH on the growth of *Alternaria alternata* causing leaf spot of cotton. Annals Plant Protect. Sci. 2012; 20:246-247.
5. Munde VG, Diwakar MP, Thombre BB, Dey U. Cultural and morphological characters of Alternaria solani on different media. Bioinfolet. 2013; 10:984-986.
6. Pandey BN, Srivastava SP, Srivastava RK. Studies on effect of various culture media on growth, sporulation and morphological variations of *Alternaria alternata* (Fr.) Keissler. Flora and Fauna. 2006; 12(2):247-248.
7. Shankergoud I, Shadakshari YG, Parameshwarappa KG,

Chandranath HT, Katti P, Mesta RK *et al*. Sunflower and Castor Research in Karnataka - An overview, University of Agricultural Sciences, Dharwad, India, 2006, 1-41.
8. Singh V, Kumar P, Sinha AP. Influence of different media, pH and temperature on growth and sporulation of *Alternaria alternata* (Fr.) Keissler, causing Alternaria blight of chickpea. Legume Res. 2001; 24(4):238-242.
9. Udayashankar AC, Nayaka S, Chandra Niranjana S, Rand Prakash HS. Comparative efficacy of strobilurin fungicides against leaf spot and blight disease of sunflower. Journal of Mycology and Plant Pathology. 2011; 41:11-19.
10. Waghunde RR, Patil RK. Physiological studies of Alternaria fruit rot (*Alternaria alternata)* of Aonla. J. Pl. Disease Sci. 2010; 5(1):73-75.
11. Mishra PK, Rama Rao CA, Siva Prasad S. Economic evaluation of farm pond in a micro-watershed in semiarid alfisol Deccan Plateau. Indian Journal of Soil Conservation. 1998; 26(1):59-60.