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Study on effect of different solid and liquid media on growth and sporulation of *Alternaria lini*

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Abstract
Alternaria lini caused linseed blight is a highly destructive pathogen. The linseed plant's aerial parts, including its leaves, stem, and pods, are all susceptible to the disease that afflicts it. The purpose of this investigation is to determine the impact that solid and liquid media have on the rate of growth, the number of spores produced, and the amount of mycelial dry weight. Result depicted that maximum mycelial growth observed in PDA (89.33 mm) followed by Potato+ $CaCO_3$ (86.67 mm), Czapek dox agar (78.00 mm). Minimum mycelial growth observed in Rose Bengal agar (48.00 mm). Colony color varied from dark gray, gray and light gray. Colony characters fluffy, cottony, smooth texture with circular or irregular growth pattern and thickness of mycelium thick, thin and very thin. Sporulation was found excellent in PDA medium. In case of broth media maximum mycelium dry weight was obtained in Potato dextrose broth (236.67 mg) followed by Potato + $CaCO_3$ broth (133.33 mg), Asthana and Hawker's broth (123.33 mg), Czapek dox broth (120.00) and Ashby broth (106.67). Minimum mycelial dry weight was obtained in Rose Bengal broth (4.33 mg) followed by Richard's broth (8.67 mg). According to the findings, the best medium for the growth and sporulation of *Alternaria lini* was Potato dextrose Agar, followed by Potato +$CaCO_3$ and Czapek dox agar. Furthermore, the best broth medium for the production of biomass by *Alternaria lini* was Potato dextrose broth, followed by Asthana and Hawker's broth.

Keywords: *Alternaria lini*, media, mycelial growth, sporulation

Introduction
Alternaria lini causes Alternaria blight of linseed. According to Singh *et al*. (2009) [8], *Alternaria lini* Dey is the primary biotic stress limiting crop yield in hot and humid environments. Dey (1933) [2] was the first to identify this disease. Later, Siddiqui (1963) [7] reported the occurrence of Alternaria blight on linseed cultures at IARI, New Delhi, and other locations throughout the nation. Sangwan *et al*. (2005) [6] investigated the fungal diseases of linseed in India, which included rust, powdery mildew, Alternaria blight, wilt, and seed rot. According to Chauhan and Srivastava (1975) [1], Alternaria blight reduces linseed yield by between 28 and 60 percent. Different media compositions also affect the morphologies of *A. lini* colonies. Fungi can use both the simple and complex forms of carbohydrates found in plants, with the latter being converted by the former into the simpler, water-soluble sugars of low molecular weight. It has been shown that various fungi have widely varying responses to a given compound and vastly dissimilar carbohydrate source utilization. Research into the host-pathogen relationship must begin with a deep and critical knowledge of the nutritional habits of fungi and the factors that affect their growth. Little thought has been given to the pathogen's culture and growth medium parameters. Therefore, it is important to study the taxonomic and physiological properties of the fungus isolated from blight-infected linseed leaves, as well as the effect of different culture media on the growth of the fungus, in order to develop effective disease management strategies.

Materials and Methods
Effect of different media
The experiment was done to see how different culture media like PDA, Richard's medium, Asthana and Hawkers, Czapex's dox agar, Ashby's agar, Potato+$CaCo_3$, and Rose Bengal medium affected the growth and sporulation of *Alternaria lini*. 20 ml of each medium was poured into a petri dish, and a 5 mm mycelial disc from a 10-day-old culture of *Alternaria lini* was placed in the middle of the medium. There were three copies of each medium that were

kept at 27±2 °C for incubation. After 7 days or when full growth was seen in any of the petri dishes that were inoculated, observations of mycelial growth were made. By looking, different colony traits and pigmentation were also noted on different culture media. After 10 days of being inoculated, sporulation was seen.

Table 1: Composition of different media used in present investigation

S. No.	Media	Ingredient	Content(g)
1	PDA (Potato Dextrose Agar)	Dextrose Agar- Agar Peeled potato Distilled water	20 20 200 1000 ml
2	Richard's medium	Potassium dihydrogen phosphate Potassium nitrate Magnesium sulphate Ferric chloride Sucrose Agar-Agar Distilled water	5 10 2.50 0.02 50 20 1000 ml
3	Asthana and Hawker's medium	Potassium dihydrogen phosphate Potassium nitrate Magnesium sulphate Glucose Agar-Agar Distilled water	1.75 3.50 0.75 5 20 1000 ml
4	Czapex's Dox agar medium	Dipotassium phosphate Sodium nitrate Magnesium sulphate Ferrous sulphate Sucrose Agar-Agar Distilled water	1 2 0.5 0.01 30 15 1000 ml
5	Ashby's agar medium	Dipotassium phosphate Sodium chloride Magnesium sulphate Calcium carbonate Mannitol Agar- Agar Water	0.2 0.2 0.2 5 20 15 1000 ml
6	Potato+$CaCo_3$ agar medium	Potatoes $CaCo_3$ Agar Distilled Water	20g 3g 20g 1000 ml
7	Rose Bengal agar medium	Potassium dihydrogen phosphate Peptone Magnesium sulphate Rose Bengal Glucose Agar-Agar Distilled water	1 5 0.5 0.05 10 15 1000 ml

Effect of different broth media

The purpose of this study was to determine which broth media is optimal for the biomass production of *Alternaria lini*. The biomass production of *A. lini* was tested across seven different broth media. The only difference between solid media and broth is the addition of agar to the solid media recipe. The potato dextrose broth, Richard's broth, Asthana and Hawkers broth, Czapex's dox broth, Ashby's broth, Potato+$CaCo_3$ broth, and Rose Bengal broth are all examples of different types of broth media. A hundred millilitres of a special liquid medium (Broth) were placed in a clean beaker. The flasks were placed in an autoclave for 20 minutes at 15 lb psi (121° C), after which they were allowed to cool. Each flask was inoculated with a single 5-millimeter disc of *A. lini* culture that had been growing for ten days. For each treatment, three replicates were created and incubated at 27.2 °C. After 10 days of inoculation, the flasks were collected. Whatman No.1 filter paper was used to refine the culture. The filter papers were dried in a hot air oven at 50 °C until they weighed the same before being used. We washed the mycelial mat on the filter paper with distilled water and dried it in a hot air oven at 50 degrees Celsius. A digital electronic balance was used to determine the weight of the filter paper containing the mycelium mat.

Result and Discussion

Effect of different media on growth, cultural characteristics and sporulation of *Alternaria lini*

The effects of different culture media on the growth of *A. lini* mycelium, its morphological characteristics, and its ability to produce spores have been presented on Table 1. Result depicted that maximum mycelial growth observed in PDA (89.33 mm) followed by Potato+ $CaCO_3$ (86.67 mm), Czapek dox agar (78.00 mm). Moderate mycelial growth observed in Richard's agar (75.00 mm) followed by Asthana and Hawker's (73.67 mm) and Ashby agar (63.00 mm). Minimum

mycelial growth observed in Rose Bengal agar (48.00 mm). Whereas colony color ranged from dark gray in PDA, Czapek dox agar and Ashby agar, gray color in Richard's agar and Potato+ $CaCO_3$, light gray in Rose Bengal agar, Asthana and Hawker's. Colony characters observed fluffy texture, circular and irregular growth pattern, with thick and thin mycelial appearance in PDA, Asthana and Hawker's, Potato+ $CaCO_3$. Cottony texture, irregular and circular growth pattern with thin and very thin mycelium appearance in Ashby agar, Rose Bengal agar and Czapek dox agar. Smooth texture, irregular growth pattern with thick mycelium appearance in Richard's agar. Pigmentation ranged from dark black in PDA, black in Ashby agar, grayish brown in Potato+$CaCO_3$, Asthana and Hawker's, brownish black in Czapek dox agar and light brown in Richard's agar and Rose Bengal agar. Sporulation was found excellent in PDA, good in Czapek dox agar and Potato + $CaCO_3$, moderate in, Richard's agar, Asthana and Hawker's, and Ashby agar, poor sporulation observed in Rose Bengal agar. The results indicated that Potato dextrose Agar was the optimal medium for the growth and sporulation of *Alternaria lini*, followed by Potato +$CaCO_3$ and Czapek dox agar. Earlier reports on the cultural characteristics of *Alternaria lini* in various media support the findings of the present study. According to Pandey *et al*. (2006) [4] and Gupta *et al*. (2013) [3], the optimal medium for growth and sporulation of *Alternaria lini* was PDA, followed by Oat meal and Richard's media. According to Ram *et al*. (2007) [5], PDA is the optimal medium for the growth and sporulation of *Alternaria lini*.

Table 2: Effect of different media on growth, cultural characteristics and sporulation of *Alternaria lini*

Media	Radial growth (mm)*	Colony colour	Growth pattern	Colony texture	Thickness of mycelium	Pigmentation	Sporulation**
Potato dextrose agar	89.33	Dark gray	Circular	Fluffy	Thick	Dark black	++++
Richard's agar	75.00	Gray	Irregular	Smooth	Very thin	Light brown	++
Czapex's dox agar	78.00	Dark gray	Circular	Cottony	Thin	Brownish black	+++
Asthana & Hawker's	73.67	Light gray	Circular	Fluffy	Thin	Grayish brown	++
Ashby agar	63.00	Dark gray	Irregular	Cottony	Very Thin	Black	++
Potato + $CaCO_3$	86.67	Gray	Irregular	Fluffy	Thick	Grayish brown	+++
Rose Bengal	48.00	Light gray	Irregular	Cottony	Thin	Light brown	+
CD at 1%	2.76						
SE(m)±	0.90						
CV	2.12						

*Mean of three replications **Sporulation (+ = poor, ++ = moderate, +++ = good, ++++ = excellent

Effect of different broth media on mycelial dry weight (biomass) production of *Alternaria lini*

After 10 days of incubation, the mycelial dry weight of *Alternaria lini* showed significant differences across all broth media, as shown in (Table 3), which presents the results of the current study. Mycelial dry weight was highest in Potato dextrose broth (236.67 mg), then in Asthana and Hawker's (123.33 mg), Czapek dox (120.00 mg), and Ashby (106.67 mg). Rose Bengal broth yielded the least mycelial dry weight (4.33 mg), followed by Richard's broth (8.67 mg). The study's findings showed that, Potato dextrose broth yielded maximum biomass of *Alternaria lini*. The findings are consistent with previous research on the subject. The highest mycelial dry weight of *Alternaria alternata* was obtained by Somappa *et al.* (2013) [9] in Potato Dextrose Broth, followed closely by Czapek Dox Broth.

Table 3: Mycelium dry weight of *Alternaria lini* on different broth media

S. No.	Broth media	Mycelium dry weight(mg)*
1.	Potato Dextrose Broth	236.67
2.	Richard's Broth	8.67
3.	Czapek dox Broth	120.00
4.	Asthana & Hawker's Broth	123.33
5.	Ashby Broth	106.67
6.	Potato + $CaCO_3$ Broth	133.33
7.	Rose Bengal Broth	4.33
	CD at 1%	7.74
	SE(m)±	2.53
	CV	4.18

*Mean of three replications

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