

# Evaluation of Culture Media for Growth Characteristics of *Alternaria solani*, Causing Early Blight of Tomato

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## Abstract

Early blight is most common and devastating disease in tomato plant caused by deuteromycotina fungi, *Alternaria solani*. This fungus grows well in potato dextrose agar and Richard's broth medium *in vitro*. The growth of the fungi were tested under culture in twelve different liquid and solid media and compared with each other. Potato dextrose agar and oat meal agar among solid media and Richard's broth and Sabouraud's broth among liquid media appeared to be better than other media for growth of tomato early blight causing fungi. The growth characteristics such as color of colony and substrate, margin of colony, topography of mycelium along with the sporulation of the test fungus were studied on these solid media. Fungus sporulation was best in oat meal agar media. Maximum growth of the fungus was observed at 8 days after inoculation with continuous increasing growth in the potato dextrose broth (PDB) medium, although growth rate was decreasing after the 2 days of inoculation. This study will be helpful for further investigations on the physiology of the fungus and management of the disease. This investigation may be useful for taxonomic study of the fungus.

**Keywords:** Early blight; *Alternaria solani*; Artificial culture; Media; Growth behavior

## Introduction

Early blight of tomato (*Solanum lycopersicum L.*) is a threat to the profitable cultivation of tomato. The disease causes reduction in quantity and quality of tomato fruits drastically. Symptoms of the disease are characterized by brown to dark brown colored necrotic spots [1]. Under humid condition, these spots progressed upwards and coalesced to produce the concentric zone on the leaves, appearing like bull's eye [2]. Lesions on the fruits are observed at the stem-end which is dark, leathery, and sunken with target board like appearance. Severe infection of the early blight fungus leads to defoliation, drying off of twigs and premature fruit drop causing 50 to 86% losses in fruit yield [3].

*A. solani* requires several specific compounds for their growth, although the fungus is cosmopolitan in nature. 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 *solani* 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. solani*. Morphological characterization is the classical approaches to distinguish fungal species that is one of the main requisite of fungal taxonomy [4,5].

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 early blight infected tomato 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

Some of the common synthetic, semi synthetic and natural media, in solid and liquid form, were used to culture the fungus. Purified culture of the fungus was inoculated into the 12 different solid agar and liquid broth media (without agar), namely host extract (HEA/HEB), potato dextrose (PDA/PDB), malt extract (MEA/MEB), oat meal (OMA/OMB), corn meal (CMA/CMB), glucose peptone (GPA/GPB), Richard's (RA/RB), Asthana and Hawker's (AHA/AHB), Waksman (WA/WB), Sabouraud's (SA/SB), Hansen's (HA/HB) and Czapek's Dox (CDA/CDB) media. These media contain various elements (in 1000 ml distilled water), e.g., HEA [healthy tomato leaves (green)-200 g, agar-20 g], PDA [peeled and sliced potato-200 g, dextrose-20 g, agar-20 g], MEA [malt extract-25 g, agar-20 g], OMA [rolled oats-40 g, agar-20 g], CMA [corn meal-60 g, agar-20 g], GPA [glucose-10 g, bacto-peptone-2 g, di-potassium phosphate ( $K_2HPO_4$ )-1 g, magnesium sulphate ( $MgSO_4 \cdot 7H_2O$ )-0.5 g, agar-20 g], RA [potassium nitrate ( $KNO_3$ )-10 g, potassium monobasic phosphate ( $KH_2PO_4$ )-5 g, magnesium sulphate ( $MgSO_4 \cdot 7H_2O$ )-2.5 g, ferric chloride ( $FeCl_3 \cdot 6H_2O$ )-0.02 g, sucrose ( $C_{12}H_{22}O_{11}$ )-50 g, agar-20 g], AHA [potassium nitrate ( $KNO_3$ )-3.50 g, potassium monobasic phosphate ( $KH_2PO_4$ )-1.75 g, magnesium sulphate ( $MgSO_4 \cdot 7H_2O$ )-0.75 g, glucose-5 g, agar-20 g], WA [potassium monobasic phosphate ( $KH_2PO_4$ )-1 g, magnesium

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sulphate ( $MgSO_4 \cdot 7H_2O$ )-0.5 g, glucose-10 g, bacto-peptone-5 g, agar-20 g, SA [maltose-40 g, bacto-peptone-10 g, agar-20 g], HA [potassium monobasic phosphate ( $KH_2PO_4$ )-0.3 g, magnesium sulphate ( $MgSO_4 \cdot 7H_2O$ )-0.2 g, maltose-5.9 g, bacto-peptone-1 g, agar-20 g] and CDA [sodium nitrate ( $NaNO_3$ )-2 g, di-potassium phosphate ( $K_2HPO_4$ )-1 g, potassium chloride (KCl)-0.5 g, ferrous sulphate ( $FeSO_4$ )-0.01 g, magnesium sulphate ( $MgSO_4 \cdot 7H_2O$ )-0.5 g, sucrose-30 g, agar-20 g]. The general preparation of medium was same in all the cases. In case of solid media preparation, agar was melted in 500 ml distilled water. Then the other ingredients were dissolved in 500 ml of distilled water in case of synthetic media (GPA, RA, AHA, WA, SA, HA and CDA). In case of natural (HEA) and semi-synthetic media (PDA, MEA, OMA, CMA), the extract was made by the boiling the tomato leaves, peeled-sliced potato, malt extract, rolled oat and corn meal respectively in 500 ml of distilled water and then the extract was filtered. The two solutions were mixed thoroughly and the volume was made up to 1000 ml by adding distilled water, antibiotic ampicillin (50 mg/L) was added and then autoclaved. In case of broth preparation for all the above mentioned media, same procedure was followed with the same ingredients without adding the agar. pH of the media was adjusted to 7.0. In case of solid media, 10 ml of media was poured into the 90 mm sterilized petri plate whereas 30 ml of the medium was poured into the 100 ml flasks in case of liquid media aseptically.

### 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 sub-culturing after 14 days. In case of both solid and liquid media, 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 either to the center of the petri plates or were put into the conical flasks as the case may be, depending upon whether the medium was solid or liquid by means of a sterilized inoculating needle. 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

In case of all 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. But in case of liquid media, after 7 days of incubation, the mycelial mats were harvested by filtering through Whatman filter paper No. 1. The initial weight of the filter papers was taken before using them.

The mycelial mat with filter paper was dried in a hot air oven at 60°C for 48 hours, after which it was taken out and kept inside the desiccators having calcium chloride inside to avoid absorption of moisture. The weighing and heating were continued until a constant weight was achieved.

### 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 (Table 1).

### Measurement of growth rate

In order to study the rate of growth of the fungus per day, conical flasks of 100 ml capacity were taken and 30 ml PDB medium was poured into each flask. The fungus was inoculated to potato dextrose broth medium in those conical flasks under aseptic conditions and incubated at  $27 \pm 1^\circ C$  for 15 days. Observations on dry weight growth of the fungus were recorded at every 2 days interval after inoculation up to 14 days (7 treatments) with 3 replications.

### Statistical Analysis

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

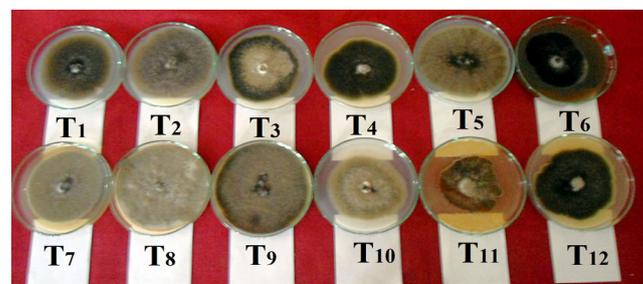
### Results

#### Radial growth on 12 different solid media

In order to study the radial growth of *A. solani*, the same was grown on 12 different solid culture media including synthetic, semi synthetic and natural media as discussed earlier and the data are presented in Figure 1 and Table 2. The data revealed that potato dextrose agar medium had significantly the highest growth (88.67 mm) of the fungus, followed by oat meal agar medium (87.83 mm), both being statistically at par with each other. Corn meal agar medium and Hansen's agar medium were the second group of the media with growth diameter of 84.00 mm and 81.00 mm, respectively, both being statistically at par with each other. The third group of culture media included Asthana and Hawker's agar medium, malt extract agar medium and Waksman agar medium producing growth of 78.00 mm, 75.67 mm and 74.67 mm, respectively and these three media were statistically at par with the each other. The rest other culture media supported had the growth of the

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

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



T1: Asthana and Hawker's Agar; T2: Corn Meal Agar; T3: Czapek's Dox Agar; T4: Glucose Peptone Agar; T5: Hansen's Agar; T6: Host Leaf Extract Agar; T7: Malt Extract Agar; T8: Oat Meal Agar; T9: Potato Dextrose Agar; T10: Richard's Agar; T11: Sabouraud's Agar; T12: Waksman Agar.

**Figure 1:** Growth of *A. solani* on different solid media.

Sl. No.	Treatments (Solid Media)	Mean Radial Growth (mm)
1	Asthana and Hawker's Agar	78
2	Corn Meal Agar	84
3	Czapek's Dox Agar	69
4	Glucose Peptone Agar	67
5	Hansen's Agar	81
6	Host Leaf Extract Agar	54.67
7	Malt Extract Agar	75.67
8	Oat Meal Agar	87.83
9	Potato Dextrose Agar	88.67
10	Richard's Agar	73
11	Sabouraud's Agar	58
12	Waksman Agar	74.67
	S.E. (m) ±	1.27
	CD (0.05)	3.7

Table 2: Growth of *A. solani* on different solid media.

fungus ranging from 54.67 mm to 73.00 mm. The host leaf extract agar medium supported least growth diameter of the fungus (54.67 mm).

### Dry weight growth on 12 different liquid media

The dry weight growth of *A. solani* was also studied on the same 12 media but without agar (Figure 2). Richard's broth medium produced significantly the highest dry weight growth (713.33 mg) of the fungus, followed by Sabouraud's broth (533.33 mg) (Table 3). The second group of liquid media supporting good dry weight growth of the fungus included malt extract broth (293.33 mg), potato dextrose broth (289.33 mg) and oat meal broth (281.33 mg), all these three media being statistically at par with each other. The other liquid media, used for the study, produced dry weight growth of the fungus ranging from 70.00 mg to 240.67 mg. Hansen's broth medium supported the least growth of the fungus.

### Growth characteristics on 12 different solid media

The growth characteristics like color of colony and substrate, margin of colony, topography of mycelium (Figure 1) along with the sporulation of the test fungus were also studied on the above solid media. The color of the colony of *A. solani* was having dark brown tinge in case of corn meal agar, host leaf extract agar and PDA medium whereas the same was light brown in case of Hansen's agar and Richard's agar medium, but dull reddish brown in Sabouraud's agar medium (Table 4). Oat meal agar medium imparted grey color to the colony, glucose peptone agar medium developed green color and Waksman agar resulted in dark color of the colony. Substrate colors of different media with growth of the fungus were light to light brown, light grayish to dark grayish and yellowish in different media. The margin of the colony also varied from regular and smooth to irregular and wavy in different media. Topography of the mycelium of the fungus in different media was sub-merged, merged and aerial. Excellent sporulation (more than 30 spores/microscopic field) of the fungus was observed on oat meal agar medium whereas good sporulation (21-30 spores/microscopic field) was observed on glucose peptone agar, host leaf extract agar, PDA and Sabouraud's agar medium. Moderate sporulation (11-20 spores/microscopic field) was observed on Czapek's dox agar, Hansen's agar and Waksman agar media but poor sporulation (1-10 spores/microscopic field) was observed on corn meal agar, malt extract agar and Richard's agar media. Sporulation of the fungus could not be observed in case of Asthana and Hawker's agar medium.

### Growth rate on potato dextrose broth medium

In order to study the rate of growth of the fungus per two days in

PDB medium, it was evident from the (Table 5) that the highest rate of dry weight growth (93.67 mg) was produced during the first 2 days after inoculation, but the rate of growth of the fungus reduced to 81 mg during the second 2 days interval *i.e.* 4 days after inoculation and this way, the growth rate went on reducing up to 34.33 mg during fourth 2 days *i.e.* 8 days after inoculation. The highest growth of 271.00 mg was observed at 8 days after inoculation with continuous increase in the dry weight, though the rate of growth was decreasing. The highest dry weight growth (271 mg) was reduced to 242 mg at 10 days after inoculation and this way it went on reducing up to 202 mg as observed at 14 days after inoculation *i.e.* during seventh 2 days interval.

### Discussion

Pathogen culture in the best suitable media is the first step of pathological research. For the growth study of *A. solani*, most of the earlier researchers used the PDA, RB, CDB and HEA media. Some researchers also used the SA medium for the growth study. But, the influence and comparison among 12 different media, including synthetic, semi-synthetic and natural, in solid as well as in liquid form on the growth of *Alternaria* fungus are the objectives of the study. Studies on growth of *A. solani* on different solid media showed that PDA medium supported the highest diameter growth, followed by that of oat meal agar medium. Corn meal agar medium and Hansen's agar medium were the 2<sup>nd</sup> group of media. However, the fungus was found to grow on all the culture media tested, but semi-synthetic solid media were more favourable for fungus growth. The present finding is in conformity with the reports of earlier [6-8]. It is concluded that PDA



T1: Potato Dextrose Broth; T2: Asthana and Hawker's Broth; T3: Corn Meal Broth; T4: Czapek's Dox Broth; T5: Glucose Peptone Broth; T6: Hansen's Broth; T7: Host Leaf Extract Broth; T8: Malt Extract Broth; T9: Oat Meal Broth; T10: Richard's Broth; T11: Sabouraud's Broth; T12: Waksman Broth.

Figure 2: Growth of *A. solani* on different liquid media.

Sl. No.	Treatments (Liquid Media)	Mean Dry Weight (mg)
1	Potato Dextrose Broth	289.33
2	Asthana and Hawker's Broth	76.67
3	Corn Meal Broth	164
4	Czapek's Dox Broth	240.67
5	Glucose Peptone Broth	189.33
6	Hansen's Broth	70
7	Host Leaf Extract Broth	71.33
8	Malt Extract Broth	293.33
9	Oat Meal Broth	281.33
10	Richard's Broth	713.33
11	Sabouraud's Broth	533.33
12	Waksman Broth	185.67
	S.E. (m) ±	6.67
	CD (0.05)	19.46

Table 3: Growth rate of *A. solani* on potato dextrose broth medium.

Sl. No.	Solid media	Colony characters				Sporulation
		Colony color	Substrate color	Margin of colony	Mycelium topography	
1	Asthana and Hawker's Agar	Olive green	Light color	Regular	Merged	–
2	Corn Meal Agar	Grayish brown to dark brown	Light brown	Irregular, smooth	Merged	+
3	Czapek's Dox Agar	Whitish colony with greenish border	Light color	Thin flat, smooth	Submerged	++
4	Glucose Peptone Agar	Green	Light grayish	Smooth wavy margin	Merged	+++
5	Hansen's Agar	Light brown	Light grayish	Irregular, smooth	Submerged	++
6	Host Leaf Extract Agar	Dark brown to black	Dark grayish	Smooth, irregular	Submerged	+++
7	Malt Extract Agar	Whitish grey	Grayish	Thin flat, regular	Submerged	+
8	Oat Meal Agar	Grey	Grayish	Smooth, irregular	Aerial	++++
9	Potato Dextrose Agar	Dark brown	Light brownish	Irregular	Aerial	+++
10	Richard's Agar	Light brown at center and white at margin	Light grayish	Smooth	Aerial	+
11	Sabouraud's Agar	Dull reddish brown	Yellowish	Irregular	Merged	+++
12	Waksman Agar	Dark green	Grayish	Irregular, smooth wavy	Merged	++

-(nil); + (poor); ++ (moderate); +++ (good); ++++ (excellent)

Table 4: Growth of *A. solani* on different liquid media.

Sl no.	Days after inoculation	Mean dry weight growth (mg)	Mean increase growth rate (mg/2day)
1	2	93.67	93.67
2	4	174.67	81
3	6	236.67	62
4	8	271	34.33
5	10	242	-29
6	12	225	-17
7	14	202	-18

Table 5: Colony characters and sporulation of *A. solani* on different solid media.

has the simple formulation and more nutrient contents, supporting the best mycelial growth of the fungus [9].

While studying, the growth of the fungus in the above mentioned same media without agar, *i.e.*, liquid/broth media, it was found that Richard's broth medium supported the highest dry weight growth of the fungus, followed by Sabouraud's broth. Though PDA medium had supported highest radial growth, PDB was observed to be producing lesser dry weight growth of the fungus, being the liquid medium of 2<sup>nd</sup> choice. Mahapatra [10] had reported maximum growth of *A. sesami* (infecting sesame) on PDB followed by Richard's, Czapek's dox and oat meal broth media whereas Somappa [11] had reported PDB to be the best medium supporting good growth of *A. solani* infecting tomato. The result of the present investigation, however, corroborates the findings of these former workers, though the report of PDB medium being the best liquid medium could not be confirmed. RA media contains all three major compound for fungus growth *i.e.* carbon, nitrogen, phosphate as well as there are presence of potassium, magnesium, sulphur elements in the media. These elements support the dry weight growth of the fungus, although complex formulation of the media does not allow the fast radial growth of *A. solani*. It also indicates apparently that diameter growth quality of the test fungus in the solid media does not always correlate to the dry weight growth quality in the liquid media.

Variation in the colour of colony and substrate, margin of colony and topography of mycelium on 12 different solid media adds the important information which may help in taxonomic identification of *A. solani*. In the study of sporulation of the fungus in different media, it was found that oat meal agar media showed best sporulation of the fungus, followed by glucose peptone agar, host leaf extract agar, potato dextrose agar and Sabouraud's agar media exhibiting good sporulation.

Czapek's dox agar, Hansen's agar and Waksman agar resulted in average sporulation whereas corn meal agar, malt extract agar and Richard's agar media showed poor sporulation. Zhu [12] had reported profuse sporulation of *A. Solani* in corn meal agar medium. But, in the present study, several others media, were reported, which were much more capable of influencing sporulation of *A. solani* in positive manner than corn meal agar media. PDA has the simple formulation which allows the best mycelial growth of the fungus [9], but it contains too much nutrients that leads to ultimate loss of sporulation [13]. Waggoner and Horsfall [14] reported that *A. solani* requires a carbon source (sugar) for higher sporulation, but high availability of sugar inhibits the conidia production. But, the OMA, has the lower sugar contents than PDA, which induces sporulation.

Study on growth rate of the fungus in PDB revealed that the highest growth rate was observed on the 2<sup>nd</sup> day after inoculation which went on reducing as observed at 2 days intervals. The highest growth of the fungus was observed at 8 days after inoculation with continuous growth increase in the PDB, though the rate of growth went on reducing. Kulkarni [15] reported maximum growth of *A. solani* infecting potato after 7<sup>th</sup> day of inoculation on PDA, which is confirmed by the present findings. Earlier workers like Padmanabhan and Narayanaswamy [16] and Desai [17] had reported maximum growth of *A. macrospora* (infecting cotton) on 14<sup>th</sup> and 12<sup>th</sup> days of incubation, respectively. Mahabaleswarappa [18] recorded maximum growth of *A. carthami* (infecting safflower) on the 12<sup>th</sup> day of inoculation whereas Sandhya [19] had reported maximum growth of *A. alternata* (infecting geranium) after 16 days of incubation in Czapek's dox medium, which were not in conformity with the present finding. The reduction of total dry weight of 271 mg (after 8 days) to 202 mg (after 14 days of inoculation) was

perhaps due to exhaustion of the nutrients of medium (30 ml), leading to lysis of the fungal cells during the lag phase.

This investigation reveals that potato dextrose agar medium influences the best radial growth, Richard's broth medium shows maximum dry weight growth whereas oat meal agar medium supports the excellent sporulation of *A. solani*. The study will be helpful to understand the growth and reproduction of the deuteromycotina fungi, *A. solani* and its management to combat the disease caused by it. Such study may add to the knowledge of the taxonomic behavior of the fungus. Moreover, use of diverse culture media will be helpful to study the growth parameters of the test fungus.

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