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Evaluation of Different Culture Media on the Growth, Sporulation and Mycelial Dry Weight of *Alternaria solani* causing Early Blight in Tomato

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ABSTRACT: Early blight caused by *Alternaria solani* is the most prevalent and destructive disease in tomato. Any study leading to an understanding of the host-pathogen connection must have a critical and complete grasp of dietary patterns and factors driving fungal growth. The pathogen's culture and growth media conditions have not received significant attention. Hence, 17 solid culture media (synthetic and non synthetic media) were tested to assess their influence on cultural characters of *A. solani,* and the results showed that mean colony diameter (9 cm) was significantly highest on Richard's agar medium, PDA and host based media at 12 DAI. The least growth of 1.72 cm was noticed in PDA+ $CaCO_3$, followed by water agar (4.95 cm) and V8 juice agar (5.00 cm). A medium mycelial growth range of 6.45 to 8.80 cm was noticed in the rest of tested culture media. On examination of data on sporulation of *A. solani,* water agar showed excellent sporulation with 56.67 spores per microscopic field. Whereas poor sporulation was observed in czapak's dox agar medium with an average of 5.22 spores per microscopic field, followed by a carrot dextrose agar medium with moderate sporulation of 14.00 spores per microscopic field. Out of 16 different liquid culture media, the maximum mycelium dry weight (333.68 mg) was observed in Sabourd's broth, followed by potato dextrose broth (331.82 mg), oat meal broth (328.81 mg) and tomato stem dextrose broth (328.41 mg) at 12 DAI. The least mycelium dry weight (108.59 mg) was noticed in Asthana and Hawkers's broth, followed by Czapak's dox broth (149.62 mg), PDA + $CaCO_3$ (270.26 mg) broth and carrot dextrose broth (279.04). Thus, the present work will be useful for further investigation into the physiology of the fungus and management of the disease.

Keywords: Early blight, *Alternaria solani*, culture medium, Growth, sporulation and mycelial dry weight.

INTRODUCTION

Tomato is the chief vegetable crop and is cultivated in the wide range of climates under open and protected conditions around the world. Prevalence of high humidity and warm temperature favours not only luxuriant growth but also development of various fungal, bacterial and viral diseases (Balanchard, 1992; Mark and Brooke, 2006). More than 200 diseases have been described to infect tomato across the globe (Atherton and Rudich, 1986). Early blight or fruit rot caused by *A. solani* (Ellis and Martin, 1882); Jones and Grout (1986), is the most threatening biological constraint among the fungal diseases (Jones *et al*., 1991; Abada *et al*., 2008; Gannibal *et al*., 2014; Landschoot *et al*., 2017).

A. solani belongs to the class Deuteromycetes, sub class Hyphomycetes, order Moniliales, family Dematiaceae, genus *Alternaria* and species *solani* (Jones and Grout, 1986). The causal organism is air borne as well as soil inhabiting and is responsible for early blight, collar rot and fruit rot of tomato (Datar and Mayee, 1981). Early blight affects seedlings, leaves, petioles, stems, branches, calyx, blossom and fruits (Pandey *et al*., 2002). Most prominent symptoms were observed on older leaves, stems and fruits producing dark velvety sunken spots of considerable size with concentric margins (Ramakrishnan *et al*., 1971). However, symptom production is reported to vary with the species of with considerable yield loss (50 to 86 %) and also affect marketing quality of fruits (Mathur and Shekhawat, 1986).

Fungal spores are frequently used as a clue to identify and categorise organisms. *A. solani* can persist in the soil for more than ten years on plant detritus and seeds at ideal conditions (Moore and Thomas, 1942; Basu, 1971; Rotem, 1998). Several solanaceous cultivated crops, such as potato, pepper, eggplant and other weed hosts, can also support *A. solani*. It was generally known that the tomato early blight fungus could live on infected seeds for several days. However, it is yet unknown if the *A. solani* seed-borne inoculum acts as a source of primary infection in the following season (Neergaard, 1945). *A. solani* spores germinate in vulnerable tissue or through wounds and create additional conidia, which are dispersed by wind, splashing rain and other means (Agrios, 2005). The disease can occur in a variety of climatic situations,

although it is more prevalent in locations with substantial dew deposition, heavy rainfall and high relative humidity. High humidity and relatively high temperatures (24-29°C) are more conducive to disease development during heavy rain (Peralta *et al*., 2005).

For *A. solani* to grow, it needs a variety of specific compounds. Fungus is isolated as a pure culture in precise and specialized media for studies on growth, nutrition, physiology and control of the fungus in an *in vitro* study. The isolation of the *A. solani* fungus, as well as its development and sporulation, can be aided by a variety of media. The nutrient requirements for optimal fungal growth, on the other hand, do not imply adequate nutrient supply for good sporulation. The diverse colony morphologies of *A. solani* are also influenced by different medium compositions. Carbohydrates are found in both simple and complex forms in plants, and fungi transform the complex forms into simple water soluble sugars with a low molecular weight before using them. It has been demonstrated that various fungus react differently to a given molecule, and that the fungi use different carbohydrate sources in different ways. As a result, detailed knowledge of the effects of various culture media on fungus growth, as well as sporulation and colony characteristics of the fungus isolated from early blight-infected tomato leaves, is required to develop effective disease management strategies and may aid in taxonomic and physiological research.

MATERIALS AND METHODS

Disease sample collection and isolation of pathogen: Early blight infected tomato leaves were collected in polythene bags or brown paper covers with proper labelling and brought to laboratory and isolated the pathogen by following standard procedure made by Prasad and Nayak (2004).

Isolation and purification of pathogen: Tomato leaf bits infected with early blight were surface sterilised for 30 seconds with sodium hypochlorite (1.0%) or mercuric chloride (0.1%), then washed thoroughly in three changes of sterile distilled water immediately and kept on clean tissue paper for two minutes before transfer to PDA medium containing 100 ppm streptomycin sulphate in Petri plates. The Petri dishes were incubated for three days at 25±2°C. The pathogen initial growth was sub-cultured in agar slants. The single spore method (Johnson and Booth, 1983) or hyphal tip technique (Pathak, 1972) were used to obtain pure culture of *A. solani*, which was preserved for further research.

Identification of the Pathogen: The pathogen culture was identified based on the characteristics of the colony, hyphae, conidiophore and conidia. Conidial dimensions (Length, breadth and beak length) were measured for 25 conidia under high power objective 40X microscopic field and compared with the dimensions for the tomato early blight pathogenic fungi reported by earlier workers.

The conidial dimensions were recorded with Olympus microscope with progress capture pro 2.5 version software (Labo America Inc. Ferment, California, USA). The uniqueness of the fungus was confirmed by comparing the ranges of dimensions derived by the following formula (John, 1970) with the standard dimensions for the pathogens published in literature.

$$\mu = \chi \pm t \ 0.05 \text{ (SE)}$$

$$SE = \sigma / \sqrt{\eta}$$

where σ = Standard deviation, μ = Population mean, χ = Sample mean, η = Number of spores observed, t = Table t value (P=0.05), SE = Standard error.

Pathogenicity test: Tomato seedlings of the Arka Vikas variety, susceptible to early blight were raised in earthen pots filled with sterilized soil. Before inoculation, 30 days old plants were predisposed to high humidity by spraying distilled water and covering them with a polythene bag for 24 hours. When the plants are 50 days old, the leaves are thoroughly cleaned with sterilized distilled water and spray inoculated with the spore (1×10^4 spores/ml concentrations) and mycelial suspension, prepared from a pure culture of *A. solani*. Control is also maintained by inoculating the plants with sterile distilled water in a similar manner. The inoculated plants were covered with polythene bags for 24 h and regular observations were made for the appearance and development of symptoms. After the symptom expression, the pathogen was re-isolated from diseased spots and confirmed their identity with original culture and Koch's postulates were proved.

Multiplication of pathogen: Pure culture of the pathogen was sub cultured by transferring the actively growing 5mm mycelial disc from old culture to fresh potato dextrose agar medium and incubated at 25±2°C for 10-15 days for *in vitro* growth of the fungus.

Maintenance of culture: The pathogen was maintained at 4°C in refrigerator and sub cultured intermittently at an interval of 30 days during the course of the study. Virulence of fungus was continued by passing over the host after every three months.

Effect of solid media on growth and sporulation of *A. solani*: The impact of various culture media on the cultural characteristics of *A. solani* was determined. Twenty ml of each molten medium was decanted into each of the 90 mm Petri plates. A Five mm disc of fungus culture was cut with the help of a sterilized cork borer from the margin of a 7 day old pure culture of *A. solani* grown on potato dextrose agar. One disc of the culture was placed in an inverted position in the centre of each Petri plate. The Petri plates were incubated at 25±2°C and the growth of the culture was measured in each medium separately. Three replications were maintained for each medium. Details of the composition of culture media are presented in Annexure I.

Effect of different liquid culture media on dry weight of *A. solani*: In a 250 ml sterile conical flask, 100 ml of different liquid media (broth) were added. The flasks were then sterilised in an autoclave at 15 lb psi (121°C) for 20 minutes and allowed to cool. In each flask, one 5 mm disc of a seven-day-old *A. solani* culture was inoculated. Three replications were maintained for each treatment and kept for incubation at 25±2°C. Flasks were harvested after the 12th day of

inoculation. The culture was filtered through Whatman No. 1 filter paper. Before filtering, the filter papers were dried to a constant weight by drying in a hot air oven at 50°C. The mycelial mat on the filter paper was thoroughly washed with distilled water and dried in a hot air oven at 50°C. The filter paper with mycelium mat was weighed on a digital electronic balance. The 16 liquid media (broth) were used during the investigation to determine the biomass production of *A. solani*. The composition and preparation procedure of broth are similar to solid media except for the addition of agar in solid media.

Statistical analysis: Completely randomized design (CRD) was used conduct the current laboratory experiments. The data obtained from all the experimentations were statistically tested by following the standard procedures made by Gomez and Gomez (1984), Panse and Sukhathme (1985); Sheoran, (2010).

RESULTS AND DISCUSSION

Isolation and identification of pathogen

Early blight infected tomato leaves were collected separately, surface sterilized and incubated on PDA medium at 27±1°C for three days for isolation of the causal organism. Pathogen pure culture was obtained by the single spore method or hyphal tip technique and was used for identification and for further investigations (Plate 1).

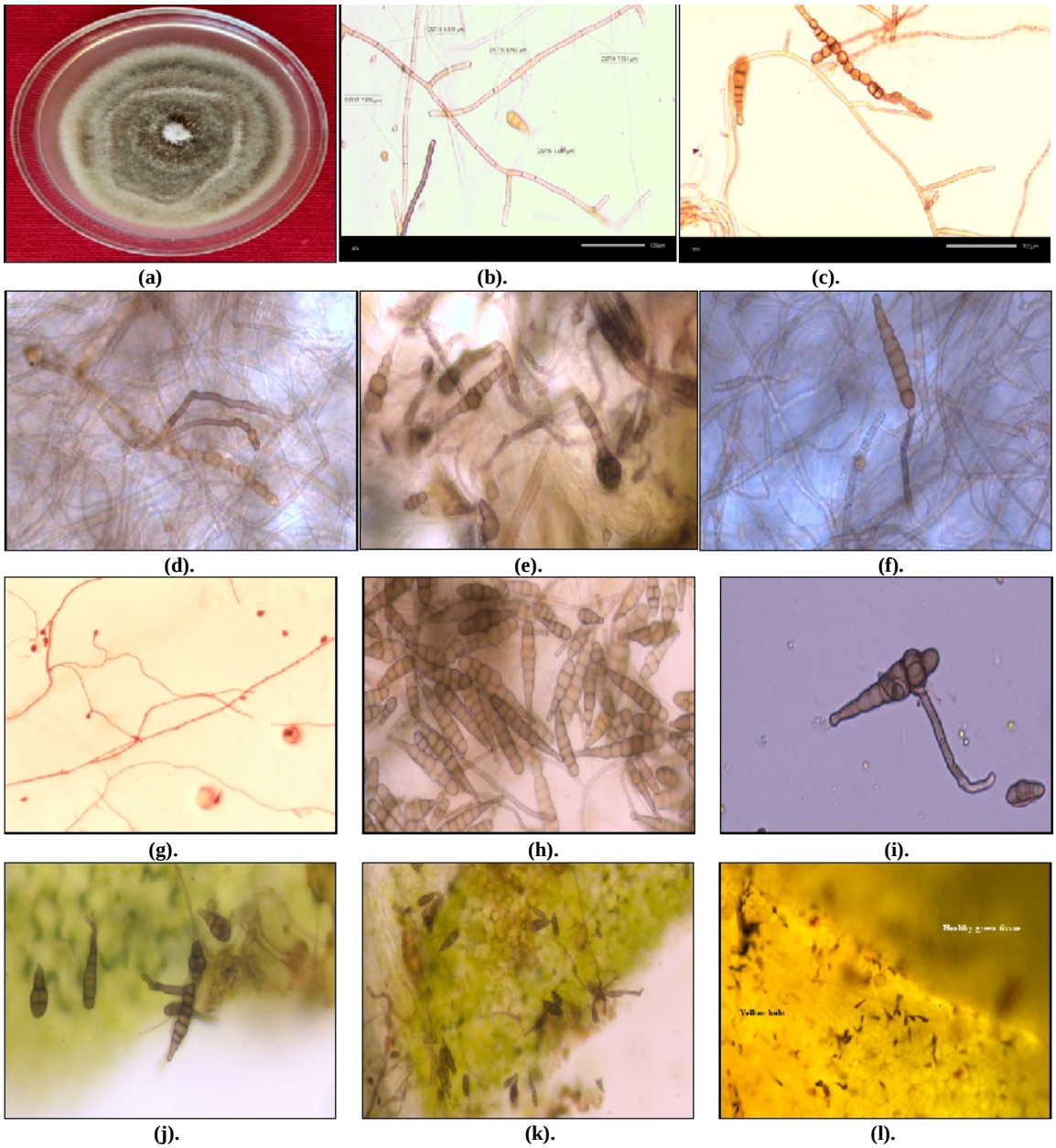


Plate 1. a. Pure culture of *A. solani;* b. Septate myceliu; c. Moniliform mycelium (As-38); d. Initiation of conidium; e. Terminal and intercalary chlymadospores (AS-37); f. Formation of solitary conidium in PDA; g. Formation of solitary conidium in WA; h. Conidia of *A. solani;* i. Germinating conidium; j. Germination of conidium on host; k. Germination and growth of pathogen on/in host; l. Presence of conidia on yellow halo.

Culture from samples that exhibited typical early blight symptoms had grayish white and brown, sparse cottony mycelium, which later turned dark brownish black. Colony was uniform fluffy with regular smooth and flat white and imparted brownish black colour to the medium. Conidia from this culture were muriform, light brown to dark brown, ellipsoidal, flexuous, with pale coloured beaks and produced singly, had 5-8 horizontal and 0-2 vertical septa (Plate 1). Conidial dimensions were measured and were subjected to statistical analysis (Table 1).

Table 1: Conidial dimensions of *A. solani.*

Characters	Population mean (µm)	Total (µm)	Population mean (µm)	Total µm)	Range (µm)
Length	148.20- 2.07 (3.50)	140.96	148.20+ 2.07 (3.50)	155.45	140.96 - 155.45
Breadth	20.98 – 2.07 (0.21)	20.55	20.98 + 2.07 (0.21)	21.41	20.55 - 21.41
Beak length	49.55- 2.07 (0.59)	48.33	49.55+ 2.07 (0.59)	50.77	48.33 - 50.77

To compare the conidial measurements with earlier reports they were statistically tested with the formula $\mu = \chi \pm t\ 0.05$ (SE) (John, 1970)

Standard error (SE) = $\sigma / \sqrt{\eta}$

where,

μ = Population mean

χ = Sample mean

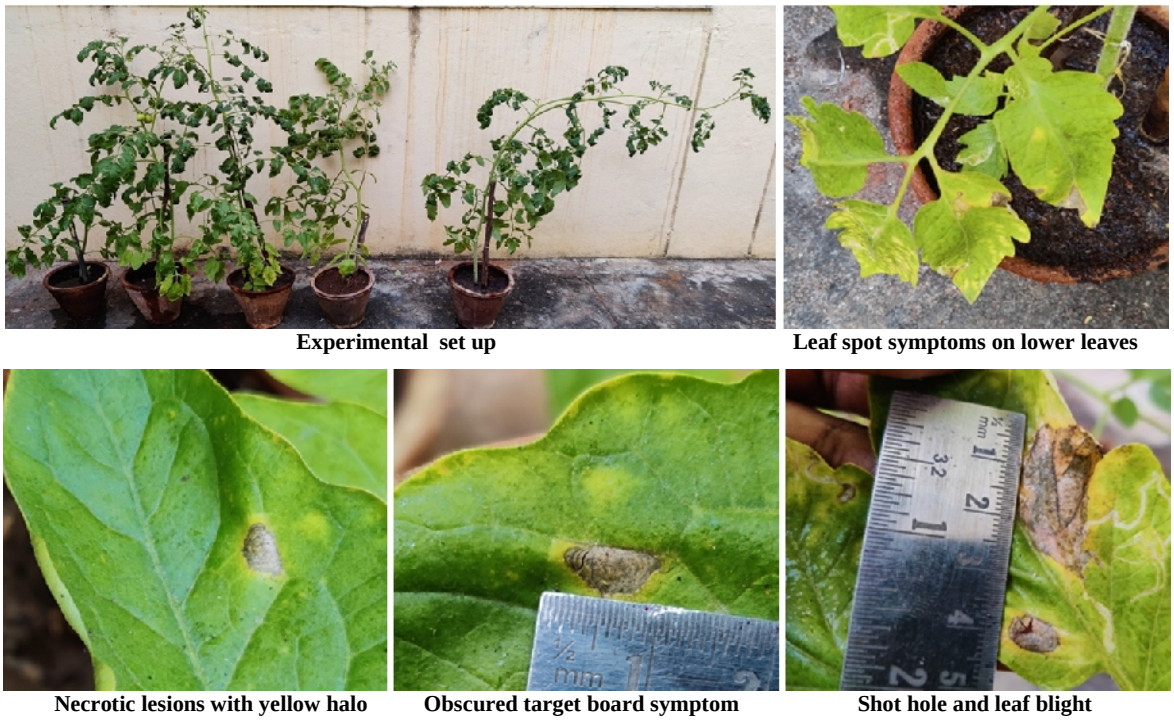
σ = Standard deviation

η = Number of spores observed (25)

t = Table t value (P=0.05)

The conidial dimensions of the culture isolated from early blight infected tomato leaves were in conformity with the standard descriptions (75-350 × 20-30 µm) made by Ellis and Martin (1882); Rao (1969). Varma *et al.* (2006); Yadav and Pathak (2011): Kumar *et al.*, (2016); Ambarish *et al.*, (2021) also confirmed the pathogen culture by comparing with these standard descriptions. Based on colony features and conidial dimensions, the culture was identified as *A. solani*.

Pathogenicity test with pure culture: The culture was tested for their pathogenicity by spray inoculation of spore suspensions with mycelial bits at 1×10^4 spores/ml concentration on leaves of healthy plants (*cv.* Arka vikas) maintained under isolated conditions in a polyhouse. Inoculation with *A. solani* spore suspension led to the production of small yellowish lesions (3-4 DAI), later turn to light brown followed by dark brown spots on the leaves after 6 to 8 DAI (average of 7.25 days), the spots gradually increased in size, became circular to oval with or without concentric rings, with or without clear chlorotic halo and attained 0.3-2.2 cm (average of 1.45 cm) in diameter by 18 DAI. In addition, disintegration of central necrotic area of few lesions and shot hole formation was noticed (Plate 2). Reisolated and purified culture from these artificially infected leaves was identical to the original culture. Disease symptoms were not observed on uninoculated plants. Similar infection patterns and symptoms were observed by Devi, (2014); Biswas (2016), who used the same kind of symptom for reisolation of the pathogen to confirm Koch's postulates.



Experimental set up | **Leaf spot symptoms on lower leaves**

Necrotic lesions with yellow halo | **Obscured target board symptom** | **Shot hole and leaf blight**

Plate 2: Pathogenicity of *A. solani* on tomato (*cv.* Arka Vikas).

Growth and sporulation of *A. solani* on different solid media: The data pertaining to the effect of different solid culture media on growth and sporulation of *A. solani* has been presented in Table 2. At three DAI, highest mean mycelial growth was noticed on Sabourd's agar medium (3.20 cm) and was statistically on par with the tomato stem dextrose agar medium with a mean mycelial growth of 2.98 cm. However, the least mycelial growth was noticed in the PDA+ $CaCO_3$ medium (0.65 cm), which was significantly on par with the water agar (0.88 cm). Except Asthana and Hawkers's medium (1.61 cm) and carrot dextrose agar medium (1.83 cm), the remaining tested culture media were more or less significantly on par with mean mycelial growth range of 2.20 to 2.93 cm.

Radial growth continued to be significantly higher in Sabourd's agar medium (7.00 cm) than in other culture medias at six DAI and which was statistically on par with the tomato stem dextrose agar medium (6.72 cm), oat meal agar medium (6.57 cm) and tomato leaf dextrose agar medium (6.45 cm). The least mean mycelial growth was noticed in PDA+ $CaCO_3$ medium (1.52 cm), followed by water agar (2.37 cm), Asthana and Hawkers's medium (3.50 cm) and carrot dextrose agar medium (3.85 cm) and V8 juice agar medium (4.78 cm). The growth of the pathogen in the rest of the culture media shows more or less similar mycelial growth of 5.62 to 6.23 cm and they were significantly on par with each other.

At nine days after inoculation, the PDA medium demonstrated high mean mycelial growth (8.57 cm), which was statistically comparable to the tomato stem dextrose agar medium (8.43 cm), tomato leaf dextrose agar medium (8.30 cm) and tomato leaf extract agar medium (8.12 cm). whereas, PDA + $CaCO_3$ medium recorded the least mean mycelial growth (1.57 cm), followed by water agar (3.00 cm), V8 juice agar medium (4.47 cm), Asthana and Hawkers's medium (4.60 cm), carrot dextrose agar medium (5.33 cm) and czapak's dox agar medium (6.97 cm). Other culture media tested were on par with each other in influencing the pathogen growth and documented a mean mycelial growth range of 7.55 to 7.95 cm.

Table 2: Effect of different culture media on growth and sporulation of *A. solani.*

Sr. No.	Medium	Colony diameter*					Sporulation (10X)	
		3DAI	6DAI	9DAI	12DAI	Mean	No. of spores per microscopic field*	Category
1.	Asthana and Hawkers's medium	1.61^{b}	3.50^{c}	4.60^{c}	6.55^{c}	4.07	23.67^{c}	Good
2.	Carrot dextrose agar medium	1.83^{b}	3.85^{c}	5.33^{d}	6.45^{c}	4.37	14.00^{b}	Moderate
3.	Conns agar medium	2.20^{c}	5.62^{e}	7.57^{f}	8.45^{e}	5.96	22.33^{c}	Good
4.	Czapak's Dox agar medium	2.40^{cd}	5.87^{efg}	6.97^{e}	8.15^{d}	5.85	05.22^{a}	Poor
5.	Malt extract agar medium	2.75^{efgh}	5.97^{efg}	7.55^{f}	8.07^{d}	6.09	24.11^{c}	Good
6.	Oat meal agar medium	2.87^{fgh}	6.57^{ghi}	7.63^{f}	8.12^{d}	6.30	42.44^{g}	Excellent
7.	PDA+ $CaCO_3$ medium	0.65^{a}	1.52^{a}	1.57^{a}	1.72^{a}	1.37	28.00^{cde}	Good
8.	Potato carrot agar medium	2.52^{de}	5.80^{ef}	7.95^{fg}	8.80^{f}	6.27	23.56^{c}	Good
9.	Potato Dextrose a gar	2.82^{fgh}	6.23^{efgh}	8.57^{h}	9.00^{g}	6.66	24.89^{c}	Good
10.	Richard's agar medium	2.60^{def}	5.87^{efg}	7.93^{fg}	9.00^{g}	6.35	26.22^{cd}	Good
11.	Sabourd's agar medium	3.20^{i}	7.00^{i}	7.70^{f}	8.45^{e}	6.59	32.89^{ef}	Excellent
12.	Tomato leaf extract agar medium	2.67^{defg}	6.17^{efgh}	8.12^{fgh}	9.00^{g}	6.49	42.89^{g}	Excellent
13.	Tomato leaf dextrose agar medium	2.93^{gh}	6.45^{fghi}	8.30^{gh}	9.00^{g}	6.67	38.44^{fg}	Excellent
14.	Tomato stem extract agar medium	2.47^{d}	5.83^{ef}	7.72^{f}	9.00^{g}	6.26	33.78^{ef}	Excellent
15.	Tomato stem dextrose agar medium	2.98^{hi}	6.72^{hi}	8.43^{gh}	9.00^{g}	6.78	32.22^{def}	Excellent
16.	V8 juice agar medium	2.53^{de}	4.78^{d}	4.47^{c}	5.00^{b}	4.20	31.33^{de}	Excellent
17.	Water agar medium	0.88^{a}	2.37^{b}	3.00^{b}	4.95^{b}	2.80	56.67^{h}	Excellent
SEm±		**0.06**	**0.16**	**0.13**	**0.04**	-	**1.56**	-
CD (P≤0.01)		**0.19**	**0.47**	**0.39**	**0.11**		**4.58**	

* Mean of three replications and Values in the column followed by common letters are non-significant at p = 0.01 as per DMRT; 0 spore: No sporulation, 1-10 spores per microscopic field: Poor sporulation, 11-20 spores per microscopic field: moderate sporulation, 21-30 spores per microscopic field: good sporulation, >30 spores per microscopic field: excellent sporulation.

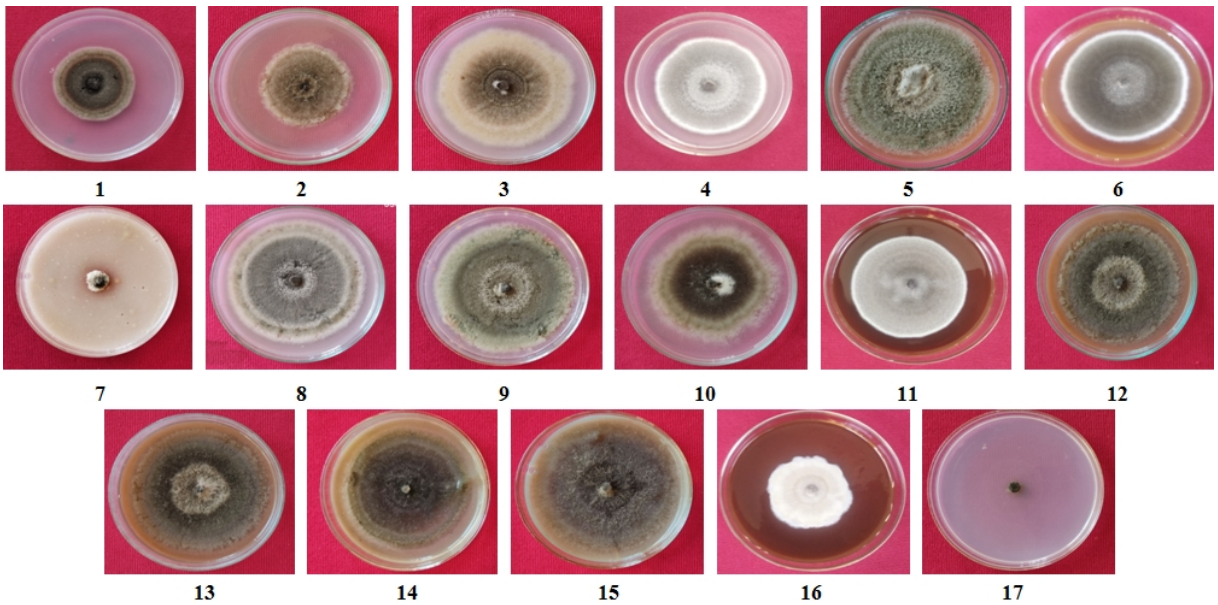
By 12 DAI, Richard's agar medium, PDA and host based media recorded full growth of 9 cm. The least growth of 1.72 cm was noticed in PDA+ $CaCO_3$ medium, followed by water agar (4.95 cm) and V8 juice agar medium (5.00 cm), which were on par with each other. A medium mycelial growth range of 6.45 to 8.80 cm was noticed in the rest of tested culture media.

On examination of data on sporulation of *A. solani* on different culture media, water agar showed excellent sporulation with 56.67 spores per microscopic field. Whereas poor sporulation was observed in czapak's dox agar medium with an average of 5.22 spores per microscopic field, followed by a carrot dextrose agar medium with moderate sporulation of 14.00 spores per microscopic field. The remaining tested culture media were discovered to be the best media for good to excellent sporulation, with 22.33 to 42.89 spores per microscopic field, and they were significantly comparable to each other.

Observations pertaining to colony colour and characteristic growth pattern of *A. solani* on different solid culture media at the end of the 12th day of incubation are presented in Table 3 and Plate 3.

Table 3: Cultural characteristics of *A. solani* on different culture media.

Sr. No.	Medium	Colony colour	Type of growth	Zonations
1.	Asthana and Hawkers's medium	Grayish brown with light brown margin	Uniform fluffy with regular smooth and flat margin	Present
2.	Carrot dextrose agar medium	Light grayish brown with pale white margin	Uniform central fluffy with regular smooth and raised margin	Absent
3.	Conns agar medium	Pale white with central brown	Light light flyffy growth with irregular smooth and flat margin	Absent
4.	Czapak's Dox agar medium	Light gray with white margin	Uniform fluffy with regular smooth and raised margin	Present
5.	Malt extract agar medium	Pale whitish gray	Uniform granular fluffy with regular smooth and flat margin	Absent
6.	Oat meal agar medium	Light gray with central pale white	Uniform light fluffy with regular smooth and flat margin	Present
7.	PDA+ $CaCO_3$ medium	Pale white	Submerged sparse growth	Absent
8.	Potato carrot agar medium	Dark grayish brown with light gray margin	Uniform light granular fluffy with regular smooth and flat margin	Present
9.	Potato Dextrose agar	Grayish brown	Uniform fluffy with regular smooth and flat margin	Absent
10.	Richard's agar medium	Dark brown with central and white margin	Uniform fluffy with irregular smooth and flat margin	Present
11.	Sabourd's agar medium	Grayish brown with white margin	Central granular fluffy with regular rough and smooth margin	Present
12.	Tomato leaf extract agar medium	Grayish brown with central light gray	Light flyffy growth with regular rough and raised margin	Absent
13.	Tomato leaf dextrose agar medium	Grayish brown with central light gray	Light flyffy growth with regular rough and raised margin	Absent
14.	Tomato stem extract agar medium	Dark grayish brown	Sparse mycelium growth with regular smooth and flat margin	Present
15.	Tomato stem dextrose agar medium	Dark grayish brown	Uniform fluffy growth with regular smooth and flat margin	Absent
16.	V8 juice agar medium	White	Uniform fluffy with irregular smooth and flat margin	Present
17.	Water agar medium	Pale white	Uniform very sparse growth	Absent



1. Asthana and Hawkers's medium; 2. Carrot dextrose agar medium; 3. Conns agar medium; 4. Czapak'sDox agar medium; 5. Malt extract agar medium; 6. Oat meal agar medium; 7. Potato dextrose agar+ $CaCO_3$ medium; 8. Potato carrot agar medium; 9. Potato dextrose agar; 10. Richard's agar medium; 11. Sabourd's agar medium; 12. Tomato leaf extract agar medium; 13. Tomato leaf dextrose agar medium; 14. Tomato stem extract agar medium; 15. Tomato stem dextrose agar medium; 16. V_8 juice agar medium; 17. Water agar.

Plate 3: Effect of different culture media on growth and sporulation of *A. solani.*

The results noticed in the present study are confirmed with finding of the several workers. In the current investigation, pathogen growth and sporulation were excellent on PDA and host based medias (non-synthetic medias). This may be attributed to the inherent complex nature of natural media supporting good fungal growth owing to provision of some additional nutrints as reported by several workers (Bonde, 1929; Neergaard, 1945; Rotem, 1965; Barksdale, 1968; Cheema *et al*., 1976; Arunakumara, 2006; Hubballi *et al*., 2010; Somappa *et al*., 2013; Chohan *et al.,* 2015; Koley and Mahapatra, 2015; Yadav *et al*., 2015; Kumar *et al*., 2016; Rahmatzai *et al*., 2016; Parvin *et al*., 2021). Among different synthetic media tested, Richard's agar medium recorded good growth (9.00 cm at 12 DAI), which would be due to good quality of sugar (sucrose - a disaccharide) present in the media for fungal growth and sporulation as reported by Shahin and Shephard (1978); Rahmatzai *et al*. (2016); Balakrishnan *et al*., (2020).

Sporulation was higher when PDA was supplemented with $CaCO_3$ in this study, as it was in Prasad, (2002), while there were no significant differences between PDA with and without $CaCO_3$. Despite evidence that adding $CaCO_3$ to a medium promotes sporulation, it is unclear whether the effect is due to the pH adjustment or the calcium supplementation.

Other culture media also supporting good growth and sporulation of fungus have been reported namely, carrot medium and carrot + potato agar medium (Balakrishnan *et al*., 2020), corn meal agar and glucose peptone agar (Arunakumara, 2006), malt extract agar (Arunakumara, 2006), Martins media and water agar (Somappa *et al*., 2013), oat meal agar (Arunakumara, 2006; Koley and Mahapatra, 2015), Sabouraud's agar medium (Rahmatzai *et al*., 2016), V-8 juice agar medium (Parvin *et al*., 2021), Walksman agar (Balakrishnan *et al*., 2020). In this study, it was also noticed that Czapek's agar fungus showed good growth (Arunakumara, 2006; Somappa *et al*., 2013; Rahmatzai *et al*., 2016) and poor sporulation. Balakrishnan *et al*. (2020) stated that there was no sporulation noticed on Czapeck's dox agar medium.

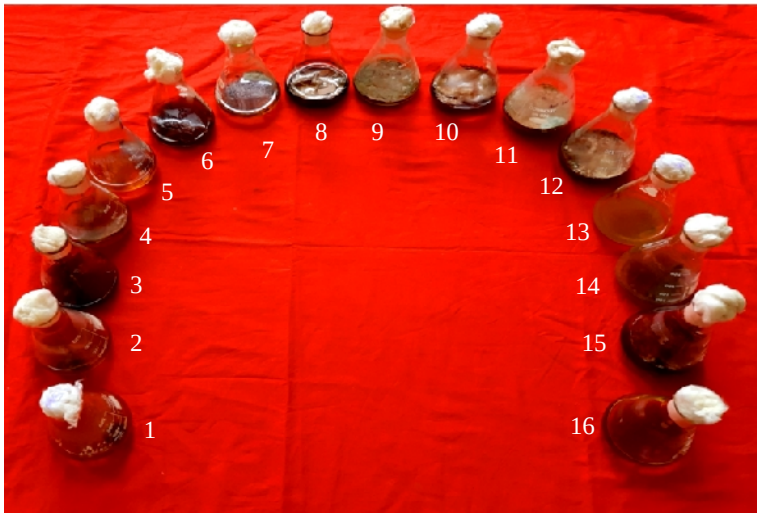
Several workers noticed the diversity in cultural characteristics of *A. solani* in different media, such as colony colour, type of growth, growth rate, margins, topography and sporulation (Babu *et al*., 2000; Arunakumara, 2006; Anand and Bhaskaran, 2009; Hubballi *et al*., 2010; Chohan *et al*., 2015; Koley and Mahapatra, 2015; Yadav *et al*., 2015; Rahmatzai *et al*., 2016; Balakrishnan *et al*., 2020; Parvin *et al*., 2021).

Effect of different liquid culture media on mycelium dry weight of *A. solani*: *A. solani* biomass was recorded on 16 different liquid culture media after the 12th day of incubation (Plate 4).

Table 4: Effect of different liquid culture media on mycelial dry weight of *A. solani*.

Sr. No.	Medium	Mycelial dry weight (mg)*	Category
1.	Asthana and Hawkers's broth	108.59[a]	Good
2.	Carrot dextrose broth	279.04[d]	Excellent
3.	Conns broth	316.20[fg]	Excellent
4.	Czapak's Dox broth	149.62[b]	Good
5.	Malt extract broth	299.99[e]	Excellent
6.	Oat meal broth	328.81[ij]	Excellent
7.	PDA+ $CaCO_3$ broth	270.26[c]	Excellent
8.	Potato carrot broth	295.62[e]	Excellent
9.	Potato dextrose broth	331.82[ij]	Excellent
10.	Richard's broth	320.39[gh]	Excellent
11.	Sabourd's broth	333.68[j]	Excellent
12.	Tomato leaf extract broth	311.13[f]	Excellent
13.	Tomato leaf dextrose broth	325.25[hi]	Excellent
14.	Tomato stem extract broth	309.45f	Excellent
15.	Tomato stem dextrose broth	328.41[ij]	Excellent
16.	V8 juice broth	315.64[fg]	Excellent
SEm(±)		**1.85**	
CD (P≤0.01)		**5.36**	

***** Means of three replications at 12th day of incubation and Values in the column followed by common letters are non-significant at p = 0.01 as per DMRT: Mycelial dry weight (mg): >150 (Excellent), 100-150 (Good), 50-100 (Fair) and <50 (Poor)



1. Asthana and Hawkers's broth; 2. Carrot dextrose broth; 3. Conns broth; 4. Czapak's dox broth; 5. Malt extract broth; 6. Oat meal broth; 7. Potato dextrose broth; 8. PDA+ $CaCO_3$ broth; 9. Potato carrot broth; 10. Richard's broth; 11. Sabourd's broth; 12. Tomato leaf extract broth; 13. Tomato leaf dextrose broth; 14. Tomato stem extract broth; 15. Tomato stem dextrose broth; 16. V8 juice broth.

Plate 4: Effect of different liquid culture media on mycelial dry weight of *A. solani*.

The data presented in Table 4 revealed that there was a significant variation in the mycelial dry weight of *A. solani* in different liquid media. The highest mycelium dry weight of 333.68 mg was observed in Sabourd's broth, followed by potato dextrose broth (331.82 mg), oat meal broth (328.81 mg) and tomato stem dextrose broth (328.41 mg) and they were statistically on par with each other. The least mycelium dry weight (108.59 mg) was observed in Asthana and Hawkers's broth, followed by czapak's dox broth (149.62 mg), PDA + $CaCO_3$ (270.26 mg) broth and carrot dextrose broth (279.04). The remaining liquid culture media also showed an excellent mycelium dry weight of 295.62 to 325.25 mg (Table 4). The present findings are in agreement with the reports made by several workers that Czapeck's medium and potato dextrose broth (Somappa *et al*., 2013), Sabouraud's broth and Richard's broth (Koley and Mahapatra, 2015), were found to be the best media for growth of tomato early blight causing fungus.

CONCLUSION

In the current investigation, a total of 17 different culture media were tested to assess their influence on *A. solani* cultural characters and sporulation. Colony diameter (9 cm) was significantly highest on Richard's agar medium, PDA and host based media at 12 DAI. Similarly, water agar showed excellent sporulation with 56.67 spores per microscopic field and Sabourd's broth recorded maximum mycelium dry weight of 333.68 mg at 12 DAI.

APPENDIX-I

Composition of different culture media

1. Asthana and Hawker's Medium:
Composition:
Potassium Nitrate (KNO_3) : 3.50 g
Potassium Monobasic Phosphate (KH_2PO_4) :1.75 g
Magnesium Sulphate ($MgSO_4.7H_2O$) : 0.75 g
Glucose : 5g
Agar : 20g
Distilled Water : 1000ml

2. Carrot Dextrose Agar Medium:
Composition:
Carrot : 200 g
Dextrose : 20 g
Agar : 20g
Distilled water : 1000 ml

3. **Conns agar medium**
Composition:
Potassium nitrate : 2 g
Magnesium sulphate : 1.2 g
Potassium dihydrogen phosphate : 2.7 g
Maltose : 7.2 g
Potato starch : 10 g
Agar : 15 g
Distilled water : 1000 ml

4. Czapek's Dox Agar:
Composition:
Sodium nitrate ($NaNo_3$) : 2 g
Potassium dihydrogen phosphate (K_2HPO_4) : 1 g
Magnesium sulphate ($MgSO_4.7H_2O$) : 0.5 g
Ferrous sulphate ($FeSO_4.7H_2O$) : 0.01 g
Sucrose ($C_{12}H_{22}O_{11}$) : 20 g
Agar : 20g
Distilled water : 1000 ml

5. Malt extract Agar:
Composition:
Malt extract : 20 g
Glucose : 5 g
Peptone : 1 g
Agar : 20 g
Distilled Water : 1000 ml

6. Oat meal Agar Medium:
Composition:
Rolled oats : 40 g
Agar : 20 g
Distilled water : 1000 ml

7. Potato Dextrose Agar Medium:
Composition:
Potato : 200 g
Dextrose : 20 g
Agar : 20g
Distilled water : 1000 ml

8. Potato + $CaCO_3$ Agar Medium:
Composition:
Potatoes : 20 g
$CaCO_3$: 3 g
Agar : 20g
Distilled Water : 1000 ml.

9. Potato Carrot Agar Medium:
Composition:
Potato : 200 g
Carrot : 20 g
Agar : 20 g
Distilled water : 1000 ml

10. Richard's agar medium
Composition:
Potassium nitrate : 10 g
Monopotassium dihydrogen phosphate : 5g
Magnesium sulphate: 2.5 g
Iron (III) Chloride : 0.02 g
Sucrose : 50 g
Agar : 15 g
Distilled water : 1000ml

11. Sabourd's dextrose agar medium
Composition:
Dextrose : 40 g
Peptone : 10 g
Agar agar : 20 g
Distilled water : 1000 ml

12. Tomato leaf extract agar medium:
Composition:
Healthy tomato leaves (Green) : 200 g
Agar : 20 g
Distilled water : 1000 ml

13. Tomato leaf dextrose agar medium:
Composition:
Healthy tomato leaves (Green) : 200 g
Dextrose : 20 g
Agar : 20 g
Distilled water : 1000 ml

14. Tomato stem extract agar medium:
Composition:
Healthy tomato stem pieces : 200 g
Agar : 20 g

Distilled water : 1000 ml

15. Tomato Stem Dextrose Agar Medium:

Composition:

Healthy tomato stem pieces : 200 g
Dextrose : 20 g
Agar : 20 g
Distilled water : 1000 ml

16. V8 juice agar medium

Composition:

V-8 juice (100 ml) : 8.3 g
L-Asparagine : 10 g
Yeast extract : 2 g
Calcium carbonate : 2 g
Glucose : 2 g
Agar : 20 g
Distilled water : 1000 ml

17. Water Agar:

Composition:

Agar : 20 g
Distilled Water : 1000 ml

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