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INFLUENCE OF VARIOUS MEDIA AND NUTRIENT SOURCES ON *ALTERNARIA SOLANI* CAUSE EARLY BLIGHT DISEASE IN TOMATO

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ABSTRACT

Early blight of tomato (*Solanum lycopersicum* L.) incited by *Alternaria solani* is highly destructive causing yield loss up to 78 per cent. The fungus was tested with different media along with the host extract and different nutrient sources for their growth and development in *in vitro.* Twelve different media were tested on the growth of *A. solani*, among them potato dextrose agar + host leaf extract recorded maximum radial mycelial growth of *A. solani* (89.57mm) and potato dextrose broth + leaf extract has maximum mycelial dry weight (613mg). Six carbon and nitrogen sources amended media were tested. Among carbon sources, glucose recorded maximum radial mycelial growth (74.65mm) and mycelia dry weight (709.17mg). Among the nitrogen sources, ammonium nitrate has the enhanced the radial mycelail growth (84.56 mm) and high mycelial dry weigh (654.27mg). This study will be helpful for further investigations on the physiology of the fungus and management of the disease.

Keywords: culture media, early blight**,** *Alternaria solani, Solanum lycopersicum*

INTRODUCTION

Tomato (*Solanum lycopersicum* L.), native from South America, is one of the most common horticultural crops cultivated throughout the world. The vegetable plays an important role in human nutrition, providing essential amino acids, vitamins and minerals (Sainju *et al*., 2003). Tomato contains lycopene, a very potent antioxidant that prevents cancers (Agarwal and Rao, 2000).

There are several diseases on tomato caused by fungi, bacteria, viruses, nematodes and abiotic factors (Balanchard, 1992). Among the fungal diseases, early blight also known as target spot disease incited by *Alternaria solani* (Ellis and Martin) Jones and Grout is one of the world's most catastrophic disease of tomato (Abada *et al*., 2008). The causal organism is responsible for early blight, collar rot and fruit rot of tomato (Datar and Mayee, 1981). The loss due to this disease was estimated up to 80 per cent (Mathur and Shekawat, 1986). The Early blight is one of the most common and destructive diseases of tomato in areas of heavy dew, rainfall and relative humidity. A severe epidemic in tomatoes in India resulted in decrease of 78 per cent in fruit yield (Datar and Mayee, 1981). The disease becomes wide spread and serious, causing large economic loss to the growers when the season begins with abundant moisture or frequent rains followed by warm and dry weather which are unfavourable for the host and help in rapid disease development (Agrios, 1988; Rex *et al*., 2019a).

Species of the genus *Alternaria* are cosmopolitan, surviving both as saprophytes as well as weak parasites. The genus is characterized by the formation of polymorphous conidia either singly or in short or longer chains and provided with cross, longitudinal as well as oblique septa and having longer or short beaks. The spores of these polyphagus fungi occur commonly in the atmosphere and also in soil. The telomorphs (sexual stage) were known in a very few species and placed in the genus *Pleospora,* in which sleeper-shaped, muriform ascospores were produced in bitunicate asci (Verma and Verma, 2010).

The fungus, *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 favour the isolation of the *A. solani* fungus which supports the radial growth, dry weight growth and sporulation of the fungus (Diba *et al*., 2007; Zain *et al*., 2009). 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*.

MATERIALS AND METHODS

Isolation of the fungus

Leaves of tomato showing typical symptoms of dark

coloured lesion with concentric rings were collected separately from tomato growing areas of Tamil Nadu. The infected leaf was first washed with tape water to remove dust and other contaminants. The periphery of the lesions were cut into small bits and surface sterilized with 10 per cent sodium hypochlorite for 5-10 min. In order to remove the residue of the chemical, the tissue bits were washed with three changes of sterile distilled water. The surface sterilized bits were placed on potato dextrose agar (PDA) medium in sterilized Petri dishes. These plates were incubated at room temperature (28 ± 2^0C) for seven days. After incubation the cultures were purified by hyphal tip method (Sinclair and Dhingra, 1985) and the fungal cultures were maintained separately in agar slants/ Perti plates (Rex *et al.*, 2019b).

Selection of suitable medium

Growth on solid media

Various nutrient media used for the growth of the pathogen were: potato dextrose agar (PDA), PDA + host (leaf) extract, PDA+ host (stem) extract, PDA + host (fruit) extract, Czapek's Dox agar, Czapek's Dox agar+ host (leaf) extract, Czapek's Dox agar + host (stem) extract, Czapek's Dox agar + host (fruit) extract and Richard's agar, Richard's agar + host (leaf) extract, Richard's agar + host (stem) extract, Richard's agar + host (fruit) extract. Fifteen ml of sterilized medium was poured in sterile Petri dish (90 mm) and allowed to solidify. The pathogen was inoculated at the centre of the plate by placing a seven days old nine mm culture disc of the pathogen. Three replications were maintained. The plates were incubated at room temperature (28±2°C). The radial mycelial growth of the fungus was measured daily.

Growth on liquid media

Twelve liquid media *viz.*, potato dextrose broth, potato dextrose broth with host extracts viz., leaf, stem and fruit, Richard's broth, Richard's broth with host extracts viz., leaf, stem and fruit, Czapek's Dox broth and Czapek's Dox broth with host extracts viz., leaf, stem and fruit were prepared. From the prepared broth 100 ml was taken in 250 ml Erlenmeyer flasks and autoclaved at 1.05 kg cm-2 for 30 min. and cooled. The flasks were inoculated with a seven days old nine mm culture disc of the pathogen. Nine days after incubation the mycelial mat was filtered through a pre weighed Whatman No.1 filter paper, dried in hot air oven at 100°C until constant weight was obtained. The mycelial dry weight was obtained by subtracting the weight of the filter paper.

Utilization of different carbon and nitrogen sources on solid medium

The Richard's agar medium was substituted with different carbon sources *viz*. glucose, sucrose, maltose, fructose, lactose, starch and different nitrogen sources *viz*., ammonium nitrate, ammonium sulphate, potassium nitrate, peptone, sodium nitrate and ammonium dihyrogen phosphate. The different carbon and nitrogen sources were added into Richard's medium at 21.053 g of carbon and 1.385 g of nitrogen per liter of the medium. The medium containing without carbon and nitrogen sources served as control. The sterilized 15 ml warm medium was poured in the sterilized Petri dish and allowed to solidify and was inoculated with seven days old nine mm culture disc of the pathogen. Three replications were maintained. The plates were incubated at the room temperature (28+2°C) for nine days. The radial mycelial growth of fungus was recorded.

Utilization of different carbon and nitrogen sources on liquid medium.

The Richard's broth was substituted with different carbon sources *viz*., glucose, sucrose, maltose, fructose, lactose, starch and different nitrogen sources *viz*., ammonium nitrate, ammonium sulphate, potassium nitrate, peptone, sodium nitrate and ammonium dihyrogen phosphate. The different carbon and nitrogen sources were added into Richard's broth at 21.053 g of carbon and 1.385 g of nitrogen per litre of the medium. The broth containing without carbon and nitrogen sources served as control. Thirty millilitres of the medium dispensed in 150 ml conical flasks were sterilized and used for inoculation with the fungus. Then the warm broth was inoculated with seven days old nine mm culture disc of the pathogen. Three replications were maintained. The flasks were incubated at the room temperature (28+2°C) for nine days and then the mycelial dry weight was recorded.

RESULT

Effect of different media on the growth of *A. solani*

Various media *viz.,* Potato dextrose agar, Richards agar and Czapeck's Dox agar with leaf, stem and fruit extracts were used to study the growth of *A. solani*. The result of the experiment was presented in table 1. The result of the experiment indicated that potato dextrose agar + host leaf extract recorded maximum radial mycelial growth of *A. solani* (89.57mm). This was followed by potato dextrose agar + host fruit extract (89.98mm), Richard's agar + host leaf extract (86.08mm), potato dextrose agar (68.07), Richard's agar (67.17mm), potato dextrose agar + host stem extract (56.18mm), Czapeck's Dox agar (53.48mm), Richard's agar + host fruit extract (42.19mm), Czapeck's Dox agar + host leaf extract (41.19mm), Richard's agar + host stem extract (40.09mm), Czapeck's Dox agar + host fruit extract (39.69mm) and Czapeck's Dox agar + host stem extract (38.00mm).

Table 1. Effect of different solid media on radial mycelial growth of *A. solani*

S.No.	Different media	Radial mycelia growth (mm)*
1.	PDA	68.07^{ab}
2.	PDA + host leaf extract	89.57^{a}
3.	PDA + host stem extract	56.18^{cd}
4.	PDA + host fruit extract	86.98^{b}
5.	Richard's agar	67.17^{bc}
6.	RA + host leaf extract	86.08^{cde}
7.	RA + host stem extract	40.09^{hi}
8.	RA + host fruit extract	42.19^{fg}
9.	Czapeck's dox agar	53.48^{f}
10.	CDA + host leaf extract	41.19^{gh}
11.	CDA + host stem extract	38.00^{kl}
12.	CDA + host fruit extract	39.69^{hij}

*Mean of three replications
Means in a column followed by same superscript are not significantly different by DMRT at P 0.05

Effect of different liquid media on the mycelial dry weight of *A. solani*

The effect of various liquid media *viz.,* potato dextrose broth, Richards's broth and Czapeck's Dox broth with leaf, stem and fruit extracts were studied and the result was presented in table 2. The result of the experiment indicated that potato dextrose broth + leaf extract recorded maximum mycelial dry weight of 613mg and this was followed by potato dextrose broth (558.56mg), Czapeck's Dox (508.69mg), potato dextrose + fruit extract (478.76mg), Czapeck's Dox + host leaf extract (474.77mg), Richard's + host leaf extract (455.82mg), Richard's broth (436.87mg), potato dextrose + stem extract (424.90mg), Czapeck's Dox + fruit extract (418.92mg), Richard;s + fruit extract (396.98mg) and Richard's + stem extract (386.00mg).

Effect of different carbon sources on radial mycelial growth of *A. solani* on Richard's agar medium

The influence of various carbon sources *viz.,* glucose, sucrose, maltose, fructose, lactose and starch on radial mycelial growth of *A. solani* on Richard's agar solid medium was studied and the result was presented in table 3. Among the carbon sources glucose recorded maximum radial mycelial growth of 74.65. This was followed by maltose (69.66mm), sucrose (65.14mm), lactose (64.31mm), starch (63.54) and control (61.10mm).

Table 2. Effect of different liquid media on mycelial dry weight of *A. solani*

S. No.	Media	Mycelial dry weight * (mg)
1.	Potato dextrose broth	558.56^{b}
2.	Potato dextrose + host leaf extract	613.42^{a}
3.	Potato dextrose + host stem extract	424.90^{gh}
4.	Potato dextrose + host fruit extract	478.76^{d}
5.	Richard's broth	436.87^{g}
6.	Richard's + host leaf extract	455.82^{f}
7.	Richard's + host stem extract	386.00^{kl}
8.	Richard's + host fruit extract	396.98^{jk}
9.	Czapeck's Dox broth	508.69^{c}
10.	Czapeck's Dox + host leaf extract	474.77^{de}
11.	Czapeck's Dox+ host stem extract	399.47^{j}
12.	Czapeck's Dox + host fruit extract	418.92^{hi}

*Mean of three replications
Means in a column followed by same superscript are not significantly different by DMRT at P 0.05

Table 3. Effect of different carbon sources on radial mycelial growth of *A. solani* on Richard's agar medium

S.No.	Media	Mycelial mycelial Growth* (mg)
1.	Glucose	74.65^{a}
2.	Sucrose	65.14^{c}
3.	Maltose	69.66^{b}
4.	Fructose	61.95^{f}
5.	Lactose	64.31^{cd}
6.	Starch	63.54^{de}
7.	Control	61.10^{fg}

*Mean of three replications
Means in a column followed by same superscript are not significantly different by DMRT at P 0.05

Effect of different carbon sources on mycelial dry weight of *A. solani* on Richard's liquid medium

Mycelial dry weight of *A. solani* due to various carbon sources *viz.,* glucose, sucrose, maltose, fructose, lactose, and starch was studied in liquid media and result was presented in table 4. Maximum mycelial dry weight of 709.17mg was recorded in glucose amended liquid medium. This was followed by maltose (682.24mg),

lactose (623.39mg), fructose (588.48mg), starch (580.50mg), sucrose (573.52 mg) and control (416.92 mg).

Table 4. Effect of different carbon sources on mycelial dry weight of A. solani on Richard's liquid medium

S.No.	Media	Mycelial dry weight * (mg)
1.	Glucose	709.17[a]
2.	Sucrose	573.52[def]
3.	Maltose	682.24[b]
4.	Fructose	588.48[d]
5.	Lactose	623.39[c]
6.	Starch	580.50[e]
7.	Control	416.92[g]

*Mean of three replications
Means in a column followed by same superscript are not significantly different by DMRT at P 0.05

Effect of different nitrogen sources on radial mycelial growth of *A. solani* on Richard's agar medium

The radial mycelial growth of *A. solani* on Richard's solid medium due to incorporation of various nitrogen sources *viz.,* ammonium nitrate, ammonium sulphate, potassium nitrate, peptone, sodium nitrate, ammonium dihydrogen phosphate was studied and result was presented in table 5. Among the nitrogen sources, ammonium nitrate enhanced the radial mycelail growth (84.56 mm) and this was followed by peptone (82.29mm), ammonium dihydrogen phosphate (80.15mm), sodium nitrate (79.37mm), potassium nitrate (69.09mm), ammonium sulphate (64.51) and control (61.22mm).

Table 5. Effect of different nitrogen sources on radial mycelial growth of *A. solani* on Richard's agar medium**.**

S.No.	Nitrogen source	Radial mycelial growth (mm)*
1.	Ammonium nitrate	84.56[a]
2.	Ammonium sulphate	64.51[f]
3.	Potassium nitrate	69.09[e]
4.	Peptone	82.29[b]
5.	Sodium nitrate	79.31[cd]
6.	Ammonium dihydrogen phosphate	80.15[c]
7.	Control	61.22[g]

*Mean of three replications
Means in a column followed by same superscript are not significantly different by DMRT at P 0.05

Effect of different nitrogen sources on mycelial dry weight of *A. solani* on Richard's liquid medium

The effect of various nitrogen sources *viz.,* ammonium nitrate, ammonium sulphate, potassium nitrate, peptone, sodium nitrate, ammonium dihydrogen phosphate was studied and the result was presented in table 6. The result of the experiment clearly showed that ammonium nitrate recorded high mycelial dry weight of 654.27mg. This was followed by peptone (620.28mg), sodium nitrate (598.00mg), ammonium dihydrogen phosphate (560.00mg), potassium nitrate (540.27 mg), ammonium sulphate (511.00 mg) and control (428.00 mg).

Table 6. Effect of different nitrogen sources on mycelial dry weight of *A. solani* on Richard's liquid medium

S.No.	Nitrogen source	Mycelial dry weight* (mg)
1.	Ammonium nitrate	654.27[a]
2.	Ammonium sulphate	511.00[f]
3.	Potassium nitrate	540.27[e]
4.	Peptone	620.28[b]
5.	Sodium nitrate	598.00[c]
6.	Ammonium dihydrogen phosphate	560.00[d]
7.	Control	428.00[g]

*Mean of three replications
Means in a column followed by same superscript are not significantly different by DMRT at P 0.05

DISCUSSION

A. solani isolated from tomato was grown on twelve solid media to study the variation in growth characteristic. Among the twelve solid media used for the growth of *A. solani* maximum growth was observed in potato dextrose agar + host leaf extract and potato dextrose agar, followed by potato dextrose agar + host fruit extract and Richard's agar after 9 days of inoculation which may be attributed to complex nature of natural media supporting good fungal growth (Table 1). The results are on par with the results obtained in an experiment conducted by Gemawat and Ghosh (1920), where PDA showed good growth with excellent sporulation after 10 days of inoculation. Kumar *et al.,* (2008) observed PDA as the best medium for *Alternaria* sp. Studies of liquid media revealed that (Table 2) *A. solani* growth was best on potato dextrose broth + leaf extract (613mg) and this was followed by potato dextrose broth (558.56mg) Czapeck's Dox broth (508.60mg) and potato dextrose broth+ fruit extract (478.76mg). Kumar *et al.,* (2008) reported that *A. solani* growth was best on potato dextrose broth.

Carbon is the most important and essential structural

component of frame work of the fungal cells. Fungi exhibit carbon heterotrophy and obtain the carbon requirement for various organic sources and the nature of organism largely determines the range of substrates (Steinberg 1950; Bilgrami and verma 1978). Carbon comprises about 50 per cent of total mycelial dry weight in fungus (Bilgrami and verma, 1978). Among the various carbon sources tested glucose revealed highest radial mycelial growth (69.99mm) and high mycelial weight of 709.17mg (Tables 3 and 4). These results are in agreement with results obtained by Gupta *et al.,* (1970) where in out of eight carbon sources tested glucose supports maximum growth of *Alternaria brassicae*.

Saleh and Ei-Garni (2001) reported that monosaccharide glucose appeared to be most favourite carbon sources. This could be attributed to the fact that glucose is the simplest form of carbon which is readily soluble and easily available to fungus. Nitrogen is a very important element for protein synthesis but all the sources of nitrogen are not equally good for the growth of fungi. In the present study among the various nitrogen sources tested (Tables 3 and 4), Ammonium nitrate recorded maximum radial mycelial growth (84.56mm) and mycelial dry weight (654.27mg) and this was followed by peptone (82.27mg; 620.28mg). Ammonium nitrate and peptone were found to be good sources nitrogen for both vegetative and reproductive growth. Similar results were obtained of Mishra and Mahamood (1960). The growth in nitrogen sources indicates the direct utilization of these compounds in protein synthesis (Lilly and Barnett, 1951).

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