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Survey of fruit rot of tomato caused by *Alternaria solani* in Manipur and evaluation of different cultural media for its growth characteristics

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Abstract
Fruit rot of tomato caused by *Alternaria solani* is an important disease causing constraint and yield loss in tomato production in Manipur. Surveys on the incidence of fruit rot of tomato were conducted during November to April, 2015-2016 at different locations in the valley districts of Manipur where tomato are grown viz., Imphal East, Imphal West, Thoubal and Bishnupur. The disease incidence ranged from 44.5 to 76.67%. The district wise maximum intensity of disease was found in Imphal West district (60.78%) and minimum in Thoubal (55.60%). The effect of different cultural media on mycelial growth and sporulation of *A. solani* showed that maximum linear growth of the fungus was found in Oat meal agar (83.67mm) and least in Elliote's agar (28.00mm) in solid media. In case of liquid media maximum growth was found in Potato dextrose broth (433.13 mg) and minimum in Host extract broth (13mg). However, the maximum sporulation was found in Potato dextrose agar ($40X10^3$spores/ml). This study will help to report the situation of fruit rot disease of tomato in Manipur and provide useful ideas for further investigations on the physiology and taxonomic study of the fungus.

Keywords: Fruit rot, *Alternaria solani*, survey, cultural media

Introduction
Tomato is regarded as the world's largest vegetable crop next to potato. It is also known as protective food because of its nutritive value and widespread production around the world. Tomato is a rich source of vitamins A, C and E, minerals, organic acids and dietary fibres that is regarded as an anticancer food and protect the body against diseases (Taylor, 1987 and Kamble *et al.*, 2009) [1, 2]. Since it is a short duration crop giving comparatively higher yield, it is considered as an economically attractive crop and the area under cultivation is increasing day-to-day. Among the fungal diseases, fruit rot of tomato caused by *Alternaria solani* causes around 50 to 86 per cent loss in fruit yield (Mahantesh *et al*., 2017) [3]. *A. solani* is most destructive disease of tomato in tropical and subtropical countries causing fruit rot in storage, transportation and marketing. Shrivastava and Tondon (1966) [4] reported *Alternaria solani* as a pathogen for fruit rot disease of tomato in India. The maximum incidence of fruit rot disease has been observed in ill drained and low lying fields, where water lodging is common and soil moisture is high (Chaurasia *et al*., 2013) [5]. The pathogen *A. solani* produces disease symptoms starting with small irregular to circular dark brown spots on lower leaves appear, measuring 0.5 mm in size. Later concentric rings form are as a result of irregular growth patterns by the organism in the leaf tissue and fruits giving the lesion a characteristic 'target spot' or 'bulls eye' appearance (Ganie *et al*., 2013) [6].

The disease appears on leaves, stems, petiole, twig and fruits under favourable conditions resulting in defoliation, drying off of twigs and premature fruit drop (Mathur and Shekhawat, 1986) [7]. *A. solani* is also most damaging on tomato plants growing in regions with heavy dew, rainfall, high humidity, and fairly high temperatures (Rotem, 1964) [8]. Different pathogen thrive at different environments and have variety of growth requirements; like nutrients, pH, osmotic conditions and temperature (Basu *et al.*, 2015) [9]. All fungi require several specific elements for growth and reproduction. There are many media which can support the isolation, radial growth, dry weight and sporulation of the fungus. Though the nutrient requirements for good growth of the fungus do not necessarily confirm the nutrient requirements for good sporulation, so it is often necessary to try several types of media when attempting to identify a fungus in culture.

The composition of any media has been known to influence the colony morphology, growth of *A. solani*. Hence the present study is focused on the status and severity of *A. solani* causing fruit rot of tomato in different valley districts of Manipur and influence of various culture media on the growth, sporulation rate and colony characteristics of *A. solani* and may help in taxonomical and physiological study of the fungus.

Materials and methods

Survey for fruit rot disease of tomato

Surveys were conducted during November to April, 2015-2016 at different locations in the valley districts of Manipur where tomato was grown viz., Imphal East, Imphal West, Thoubal and Bishnupur. Observation on the disease incidence was taken by random sampling technique from 100 plants from the farmer's field by infection index method as below:

Scale	Disease intensity
¼	: below 25% of fruit area infected
½	: from 25% to 50% fruit area infected
¾	: from 50% to 75% fruit area infected
1	: from 75% to more than 90% fruit area infected

$$\text{Percent disease incidence (PDI)} = \frac{\text{Total fruit area infected}}{\text{Total number of infected fruits examined}} \text{ X } 100$$

Diseased fruit samples were collected from the field and brought to the laboratory. The diseased portion from fruits were cut with sterilized blade into small pieces of 2-3mm size containing 50% of diseased and 50% healthy portion. The sterilized pieces were then inoculated on Potato Dextrose Agar slants. The inoculated slants were incubated at 26 ± 1 ºC for seven days.

Media preparation

To find out the suitable medium for the growth and sporulation of *A. solani*, seven solid and liquid cultural media namely Potato dextrose agar/ Potato dextrose broth, Richard's agar/ Richard's broth, Elliott's agar/Elliott's broth, Malt extract aga/Malt extract broth, Oat meal agar/oat meal broth, Czapek's dox agar/Czapek's dox broth and Host extract agar/Host extract broth were selected for *in vitro* studies. The Culture Media were prepared by autoclaving at 121 °C, 15 psi pressure for twenty minutes.

The compositions of various cultural media are given below:

1. Potato dextrose agar (PDA) /Potato dextrose broth

- Peeled potato 200g
- Dextrose 20g
- Agar 20g*
- Distilled water 1000ml

2. Richard's agar / Richard's broth

- Ferric Chloride 0.02g
- Magnesium sulphate 2.50g
- Potassium dihydrogen phosphate 5.00g
- Potassium nitrate 10.00g
- Sucrose 50.00g
- Agar 15g*
- Distilled water 1000ml

3. Elliott's agar / Elliott's broth

- Potassium nitrate 3.5g
- Potassium hydrogen phosphate 1.75g
- Magnesium sulphate 0.76g
- Glucose 5.00g
- Agar 15g*
- Distilled water 1000ml

4. Malt extract agar/ Malt extract broth

- Malt extract 30.00g
- Peptone 5.00g
- Agar-agar 15g*
- Distilled water 1000ml

5. Oat meal agar/ oat meal broth

- Oat meal 20.00g
- Glucose 20.00g
- Agar -agar 20.00g*
- Distilled water 1000ml

6. Czapeks dox agar /Czapeks dox broth

- Dipotassium phosphate 1.00g
- Ferrous sulphate 0.01g
- Magnesium sulphate 0.50g
- Potassium chloride 0.05g
- Sodium nitrate 2.00g
- Sucrose 30.00g
- Agar -agar 15.00g*
- Distilled water 1000ml

7. Host extract agar / Host extract broth

- Ripen fruit tissue 200g
- Agar-agar 15g*
- Distilled water 1000ml

*Agar is not added incase of liquid or broth media

Measurement of growth and sporulation in solid and liquid media

Incase of solid media 20ml of each medium were poured in 90 mm Petri plates and inoculated with 5mm mycelia disc of the fungus cut with the help of cork borer from young (5 days) actively growing culture by placing it on the middle of the each pre poured medium and incubated at 25±1 ºC. Each treatment was replicated three times. The diameter of the growth or radial growth of the fungus was measured 7 days after inoculation. The colony characteristics namely colony colour, type of margin and zonation were also studied for the different media. For sporulation 1sq.cm block of the fully grown mycelium was cut from the periphery. The mycelium was scraped off with the help of a sterilized blade and was then put into a test tube containing 5ml of distilled water and shake properly to make a homogenous spore suspension. The spore counts were done with the help of haemocytometer. The best culture medium for the fungus was sorted out and used for further studies.

The seven different culture broth media were prepared and 50 ml of each medium were poured into a 100ml Erlenmeyer conical flask and sterilized at 15 lb/ inch^2 for 20 minutes. A five mm mycelial disk was cut from an actively growing colony of 7 days old culture and transferred aseptically to each flask. Each treatments were replicated three times. The inoculated flasks were then incubated at 25 ± 1 ºC for 10 days. The mycelial mats were then harvested by filtering through a pre-weighed Whatmann No.40 filter paper. The fungal mats along with the filter paper were collected and oven dried at 60 ^{0}C for 3 days (72 hrs) and then cooled in a

desiccator. The dried and cooled filter papers with mycelial mats were then reweighed to estimate the fungal growth on dry weight basis by using the following formula:

$W = W_2 - W_1$

Where, W= the weight of the mycelial mat

W_1= the weight of the filter paper

Results and discussions

Survey for fruit rot disease of tomato at different locations in the valley districts of Manipur

The fruit rot disease of tomato was found to be well distributed in the surveyed valley districts of Manipur. Initially small, pale brown, water soaked spots were developed on the skin of fruit. With the age, the size of spot has been increased and it became dark brown in colour and almost rounded in shape. Concentric rings in the form of ridges were seen on brown developing spot and different degrees of its severity were found on tomato fruit (Figure 1 and 2). The disease was found occurring in all the districts throughout the period of November to April 2015-2016. The disease incidence ranged from 44.5 to 76.67%. The maximum disease incidence was recorded during the month of April in Imphal West (76.67%), followed by Thoubal (75.00%), Bishnupur (72.70%) and Imphal East (63.80%).The district wise distribution and intensity of disease were found maximum in Imphal West district (60.78%) followed by Bishnupur (58.59%) and least disease intensity was recorded in Thoubal (55.60%) (Table 1). Similarly fruit rot of tomato as most common and destructive in yield and quality of fruits was stated by Gwary and Nahunnaro (1998) [10], Khurana *et al*. (1998) and Rashmi and Vishunavat (2012) [12] in Nigeria and India. The present findings are also in accordance with the findings of Gudmestad *et al*. (2013) [13] who reported that period of profuse moisture from rain, dew and temperature ranging from 5-30 ºC were favourable for *A. solani* sporulation and disease development.

Fig 1: Symptom of fruit rot starting from the stalk

Fig 2: Different degrees of severity of fruit rot of tomato

Table 1: Distribution and per cent disease intensity of fruit rot of tomato in the valley districts of Manipur (2015-2016)

Disease intensity (%)					
Month	**Thoubal**	**Imphal west**	**Imphal east**	**Bishnupur**	**Mean**
November	44.51	55.00	47.72	56.25	50.87
December	47.72	53.36	58.33	47.23	51.66
January	56.25	51.71	58.23	55.35	55.385
February	62.50	63.34	62.50	60.80	62.285
March	58.20	70.20	56.20	70.12	63.68
April	75.00	76.67	63.80	72.70	72.04
Mean	55.60	60.78	57.04	58.59	

Effect of cultural media on growth and sporulation of *A. solani*

Solid media

The data presented in Table 2 showed that when *A. solani* was grown in Richard's agar medium it produced Milky white to cream colony later becoming light greyish with smooth irregular margin and concentric zonation. In case of potato dextrose agar the colony color was found to be dark brown or olivacious grayish black with irregular margin and concentric zonation. In case of Oatmeal agar no zonation was found. The data in Table 3 showed that the maximum (83.67mm) linear growth of the fungus was found in Oat meal agar which was followed by Potato Dextrose Agar (58.33mm) and Czapeck's Dox Agar (57.33mm). The least (28.00mm) growth was found in Elliott's Agar. It was found that all the media showed a significant different effect on the linear mycelial growth of the fungus. It further indicated that different media induced marked variability in sporulation. However, the maximum ($40X10^3$spores/ml) sporulation was found in Potato Dextrose Agar followed by Czapeck's dox agar ($28\ X10^3$ spores/ml) and Host Extract Agar ($24X10^3$spore/ml). There was no sporulation in Elliott's Agar medium. The present findings are in agreement with those of Koley and Mahapatra (2015) [14] who found that maximum mycelial growth was found in Oat meal agar and Potato dextrose agar. The present findings are also in agreement with the findings of Somappa *et al*. (2013) [15] who reported that maximum sporulation of *A. solani* was found in Potato Dextrose Agar ($13.2X10^6$ spores/ml). Variation in the colour of colony, margin of colony and zonation of mycelium on 7 different solid media add the important information which may help in taxonomic identification of *A. solani*.

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

Solid media	Colony colour	Type of margin	zonation
Richard's Agar	Milky white to cream later becoming light greyish	Smooth irregular	Concentric zonation
Malt extract Agar	Greenish brown at the centre and white at the margin	Smooth regular	Concentric zonation
Oat meal Agar	Whitish grey or light grey	Thin flat,irregular	No zonation
Host extract Agar	Dark brown or olivacious black	Smooth,irregular	Concentric zonation
Czapek's Dox Agar	Greyish olivacious	Smooth,irregular	Concentric zonation
Potato Dextrose Agar	Dark brown or olivacious grayish black	Irregular	Concentric zonation
Elliote's Agar	Light Grey to olivacious brown	Smooth,irregular	Concentric zonation

Table 3: Effect of different solid media on mycelial growth and sporulation of *A. solani*

Treatments	Growth(mm)*	Sporulation(cfu/ml)
Richard's Agar	38.67 (6.26)	$16X10^3$
Malt extract Agar	44.33 (6.70)	$20X10^3$
Oat meal Agar	83.67 (9.17)	$20X10^3$
Host extract Agar	30.67 (6.34)	$24X10^3$
Czapek's Dox Agar	57.33 (7.60)	$28X10^3$
Potato Dextrose Agar	58.33 (7.67)	$40X10^3$
Elliott's Agar	28 (5.3)	_ (no sporulation)
SE (d)±	0.082	
$CD_{(0.05)}$	0.18	

*All insertion is an average of three replications

Figures in parenthesis are ($\sqrt{x+0.5}$) transformed values.

Liquid media

The data presented in Table 4 showed the effect of different liquid culture media on growth and sporulation of *Alternaria solani*. The data revealed that maximum growth of the fungus was found in Potato dextrose broth (433.13 mg) followed by Richard's broth medium (311.7mg) and Malt extract broth (297.00mg). But the least growth of the fungus was found in Host extract broth (13mg). However all the treatments were found to be statistically significant to each other. Similary, Somappa *et al*. (2013) [15] found best growth of *A. solani* on potato dextrose broth (34.1mg). Mohapatra *et al*. (1977) [16] also reported that maximum growth of *A. sesame* (infecting sesame) on PDB followed by Richard's, Czapek's dox and oat meal broth media. Potato which contains more nutrients might have supported maximum growth of *A. solani* than synthetic media. The diameter growth quality of the test fungus on the solid media does not always correlate to the dry weight growth quality in the liquid media.

Table 4: The relative abundance of mycelial dry weight of *A. solani* in different liquid media

Treatments	Growth (mg)*
Richard' Broth	311.77 (17.60)
Malt extract Broth	297 (17.01)
Oat meal Broth	276 (16.51)
Host extract Broth	13 (3.13)
Czapek's Dox broth	202.21 (14.23)
Potato Dextrose broth	433.13 (20.64)
Elliott's Broth	91.93 (9.58)
SE (d)±	1.38
$CD_{(0.05)}$	3.01

*All insertion is an average of three replications

Figures in parenthesis are ($\sqrt{x+0.5}$) transformed values.

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