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Effect of pH & Different Media on Mycelial Growth of *Alternaria solani* Causing Early Blight of Tomato in Manipur and *In-vivo* Evaluation of Native *Trichoderma* spp., One Chemical Fungicide (Mancozeb) on *Alternaria solani* Causing Early Blight of Tomato

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Abstract

The tomato is the edible, often red berry of the plant Solanum lycopersicum, commonly known as a tomato plant. The species originated in western South America and Central America. The area under tomato cultivation in Manipur accounts for about 0.15 million hectares with an average production of 2.10 million tonnes and productivity of 12.02 tonnes ha^{-1} during 2016-17. Present research was carried out to study the effect of different culture media and pH on mycelia growth of *Alternaria solani*. Among seven culture media that were tested, the fungus grew best on Richards agar, OatMeal Agar and Coon's media. The most suitable pH for growth of fungus was 6.0 and 7.0. Native *Trichoderma* spp. has good effect on controlling early blight of tomato in filed conditions.

Keywords: *Alternaria solani, Trichoderma* spp, Different pH, Different Media and Early blight of Tomato

1. Introduction

Tomato is an important source of nutrients as it has high nutritive value of vitamin A, B, C, E and other important nutrients *viz.*, protein, carbohydrates, fiber, fat, biotin, malic acid, citric acid, oxalic acid etc. It contains fibers and is known as free of cholesterol. Lycopene is a very potent antioxidant that prevents cancers and it avert humans from free radicals that degrade many parts of the body. Tomatoes are utilized more in the developed countries than in the developing countries and hence it may be referred to as a luxury crop (Anon., 2013).

Tomato cultivation has become more popular since mid-nineteenth century because of its varied climatic adaptability and high nutritive value. It is cultivated in an area of 4.78 million hectares all over the world with production of 177.04 million tonnes and an average yield of 19.57 tonnes ha^{-1} (FAO Stat 2016).

In Manipur state it is cultivated between November to May. It occupies an area of 0.15 million hectares with the production of 2.10 million tonnes and productivity of 12.02 tonnes ha^{-1} during 2016-17 ((NHB database 2013).

Koley and Mahapatra (2015) tested the growth of the fungi under culture in twelve different liquid and solid media and compared with each other. Potato dextrose agar and oat meal agar media solid media and Richard's broth and Sabouraud's broth liquid media appeared be better than other media for growth of tomato early blight causing fungi.

Alhussaen (2012) observed that the optimum pH level for the growth of *Alternaria solani* grow in vitro was 6 to 7. Maximum growth of *Alternaria solan* was recorded at 6.5 pH level on PDA medium under continuous light condition.

Kay and Stewart (1994) found that *T. viride* and *T. harzianum* were capable of reducing white rot of onion caused by *S. rolfsil* when they were applied as soil additive at the rate of 0.1 percent wheat bran/g dry soil. Application of *T. harzianum* to the root zone of tomato controlled *Alternaria solani* infection in naturally infested soil and further, on transplanting the treated plant showed reduced disease incidence to the tune of 93 percent.

2. Materials and Methodologies

2.1 Isolation of Fungus

Early blight infected tomato plant samples were collected from farmer's field of different locations and isolation, identification of the causal pathogen was carried out in the Department of Plant Pathology, College of Agriculture, CAU, Imphal. Diseased samples were lacerated to small pieces with the help of sterilized scalpel. The lacerated pieces were surface sterilized using 1% sodium hypochlorite solution for 1 minute followed by rinsing the pieces in three phases of sterile distilled water in order to remove the traces of sodium hyphochlorite. Later the pices were blot dried using blotting paper. The sterile pieces were aseptically transferred to sterilized petri dishes containing Potato dextrose agar (PDA). The petri dishes were incubated at 27 ± 1^{0}C in BOD incubator and were observed periodically for the fungal growth. Purified cultures of the fungus ware obtained by hyphal tip culture methods. Identification was done according to the key of (Leslie and Summerell, 2006).

2.2 Effect of different culture media on mycelial growth of *Alternaria solani* causing Early blight of Tomato

Ascerting suitable medium for mycelial growth formation of *Alternaria solani*, different media for culturing were taken under *in-vitro* study. Three replications for each medium were maintained. Different media like coon's medium, corn meal agar medium, Elliots Agar, OatMeal Agar, Potato Dextrose Agar, Richards Agar, Water Agar were used. The mycelial growth of the fungus was studied and the data was statistically analyzed.

2.3 Effect of different pH on mycelial growth of *Alternaria solani* causing Early blight of Tomato

To estimate the mycelial growth of the *Alternaria solani* at different pH levels, 20ml of potato dextrose broth was taken in 150ml conical flasks. Then the pH of the medium was altered by using 1N HCl and 1N NaOH to acidic and basic respectively and the pH was checked by pH meter and sterilized at 1.1kg/cm2 of pressure for 20 min. The sterilized broth flasks were then inoculated with 5mm disc of 4 days old culture and were incubated at

room temperature for 10 days. Three replications of each pH were maintained and weighed was measured as per procedure data was analysed statistically.

2.4 *In-vivo* evaluation of Native *Trichoderma* spp., one chemical fungicide (Mancozeb) on *Alternaria solani* causing Early blight of Tomato

Three *Trichoderma* spp., one chemical fungicide (mancozeb) will also be tried in the field for controlling diseases of tomato. Tomato variety Indam 3001 will be used as test plant. Each treatment will be replicated 4 times, plot size of 2 x 3.3 m with spacing of 60 cm x 45 cm plot to plot. Root dip method and spraying will be done. Plants sprayed with water will be kept as control. Observation on disease incidence will be recorded.

3. Results and Discussions

3.1 Effect of different culture media on mycelial growth of *Alternaria solani* causing Early blight of Tomato

The investigation results revealed that *Alternaria solani* showed highest maxima radial growth in Richards agar medium 80.93mm, coons and oat meal agar medium showed same amount of radial growth 55.33 and 55.93mm, corn meal showed 37.81mm, Elliots agar showed 37.84mm, water agar showed 23.18mm. lowest radial growth was showed by PDA 22.93mm. Coons medium showed fluffy growth with irregular shape light colour, corn meal showed thick colour with regular shape, Elliots agar showed regular shape with fluffy, oat meal agar showed soft transparent growth, PDA showed irregular shape, Richard agar showed thick colour with white margin and water agar showed transparent light colour. these results are found to be similar with Rahmatzai et al. (2016) reveal that maximum mycelial growth of *Alternaria solani* was noted with Sabouraud's Agar medium (9 cm) followed by host agar medium (8.7cm) and PDA (7.9cm). While, the maximum linear growth of AS2 was recorded with Richard's Agar (9cm) followed by Czapeck's Agar (8.6cm) and Sabouraud's Agar (8.5cm). Isolates of *A. solani* showed highly variation in pigmentation, sporulation and feature of mycelial growth such as colony surface, growth margin and zonation.

3.2 Effect of different pH on mycelial growth of *Alternaria solani* causing Early blight of Tomato

Alternaria solani showed Highest growth at pH 7 (98.43%), pH 6, pH 8 and pH 5 showed 94.68, 91.87 and 90.62%, pH 9 showed 67.18%. lowest growth was showed at pH 10 (51.25%). these results are found to be similar with Samuel and Govindaswamy (1972) demonstrated that good mycelial growth and sporulation of *A. solani* was between pH 4.0 to 8.0 and pH 5.0 was the best for mycelia growth and pH 7.0 for sporulation. Rangaswami and Sambandam (1960) observed that various species of Alternaria isolated from solanaceous hosts are capable of growing over a wide range of hydrogen-ion concentrations. The optimum pH for *Alternaria solani* from potato found to be at 6.0.

3.3 *In-vivo* evaluation of Native *Trichoderma* spp., one chemical fungicide (Mancozeb) on *Alternaria solani* causing Early blight of Tomato

Three *Trichoderma* spp one chemical fungicide (mancozeb) will also be tried in the field for controlling diseases of tomato. Among the results revealed that for Early blight disease incidence highest was showed by *Trichoderma harzianum* (47.5%), followed by *Trichoderma viride* (40%), Mix (*Trichoderma harzianum* + *Trichoderma asperellum*) (37.5%) *Trichoderma asperellum* (35%) and lowest early blight disease incidence was mancozeb 15% these results are found to be similar with Kay and Stewart (1994) found that *T. viride* and *T. harzianum* were capable of reducing white rot of onion caused by *Alternaria solani* when they were applied as soil additive at the rate of 0.1 percent wheat bran/g dry soil. Application of *T. harzianum* to the root zone of tomato controlled *Alternaria solani* infection in naturally infested soil and further, on transplanting the treated plant showed reduced disease incidence to the tune of 93 percent.

Table 1. Effect of different culture media on radial growth of *Alternaria solani*

Culture media	***Alternaria solani***
Coons media	55.33
Corn meal	37.81
Elliot's Agar	37.84
Oat Meal Agar	55.93
PDA	22.93
Richards agar	80.93
Water Agar	23.18
SE(d)±	0.03
CD (0.05)	0.11

*Mean of three replications

Table 2. Effect of different pH on mycelial growth of *Alternaria solani*

Different pH	***Alternaria solani***
5	90.62
6	94.68
7	98.43
8	91.87
9	67.18

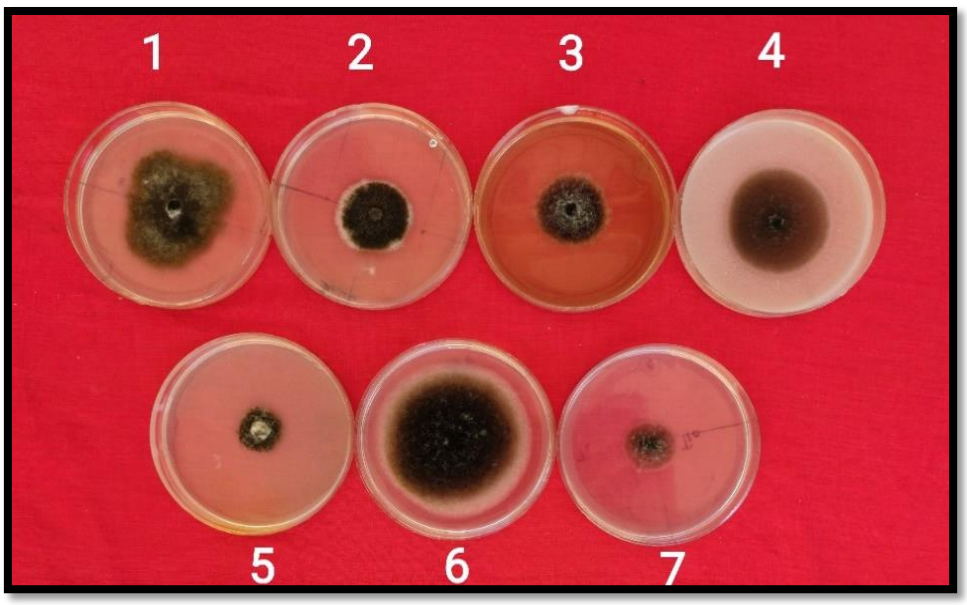
10	51.25
SE(d)±	0.05
CD (0.05)	0.15

*Mean of three replications

Table 3. *In vivo* management of Early blight disease of tomato

Treatments	**Disease incidence**
	Early blight
Trichoderma asperellum	35
Trichoderma harzianum	47.5
Trichoderma viride	40
Mix (T1+T2)	37.5
Mancozeb	15
SE(d)±	0.31
CD (0.05)	0.93

*Mean of Four replications



1.Coons media, 2. Corn meal, 3. Elliot's Agar, 4. Oat Meal Agar, 5. Potato Dextrose Agar, 6. Richards agar and 7. Water Agar

Plate 1. Effect of different culture media on radial growth of *Alternaria solani*

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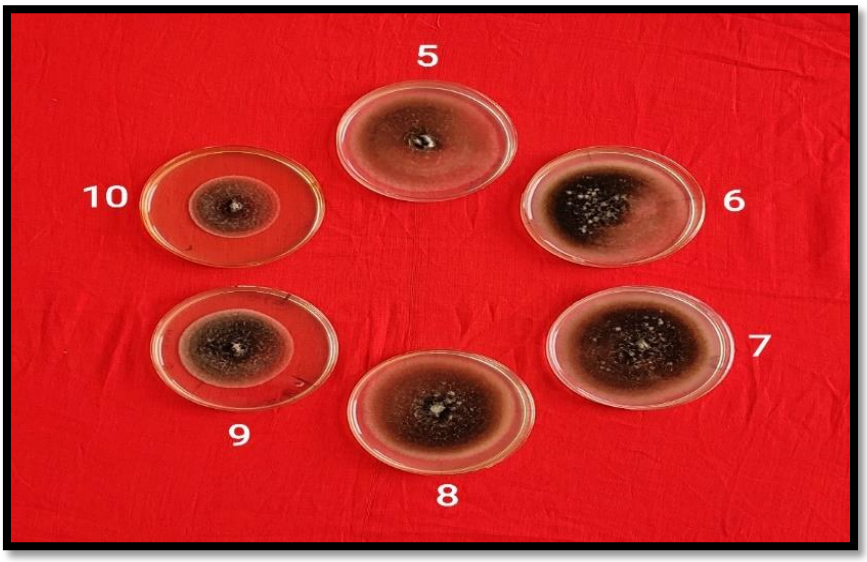
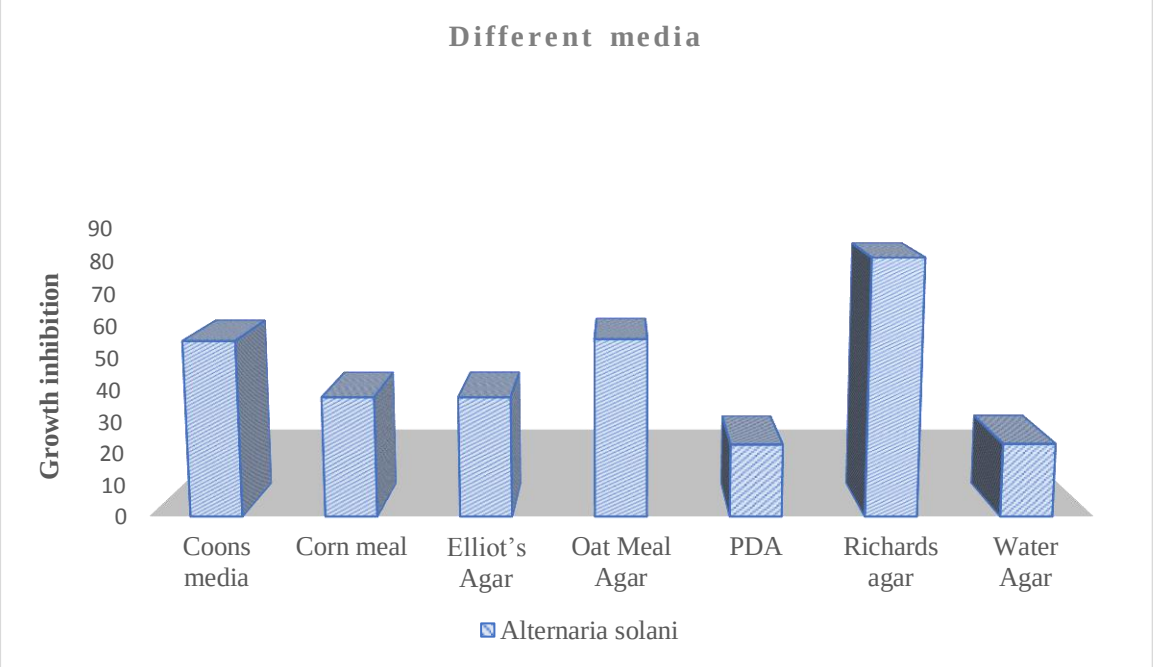


Plate 2. Effect of different pH on mycelial growth of *Alternaria solani*

Graph 1. Effect of different culture media on radial growth of *Alternaria solani*



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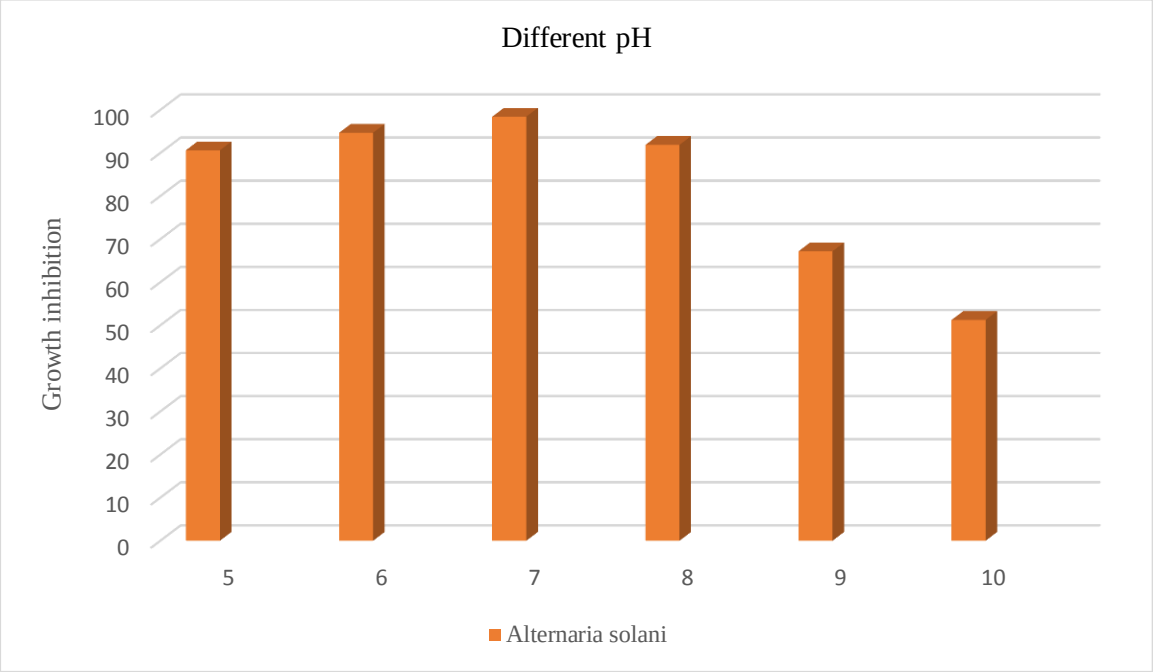
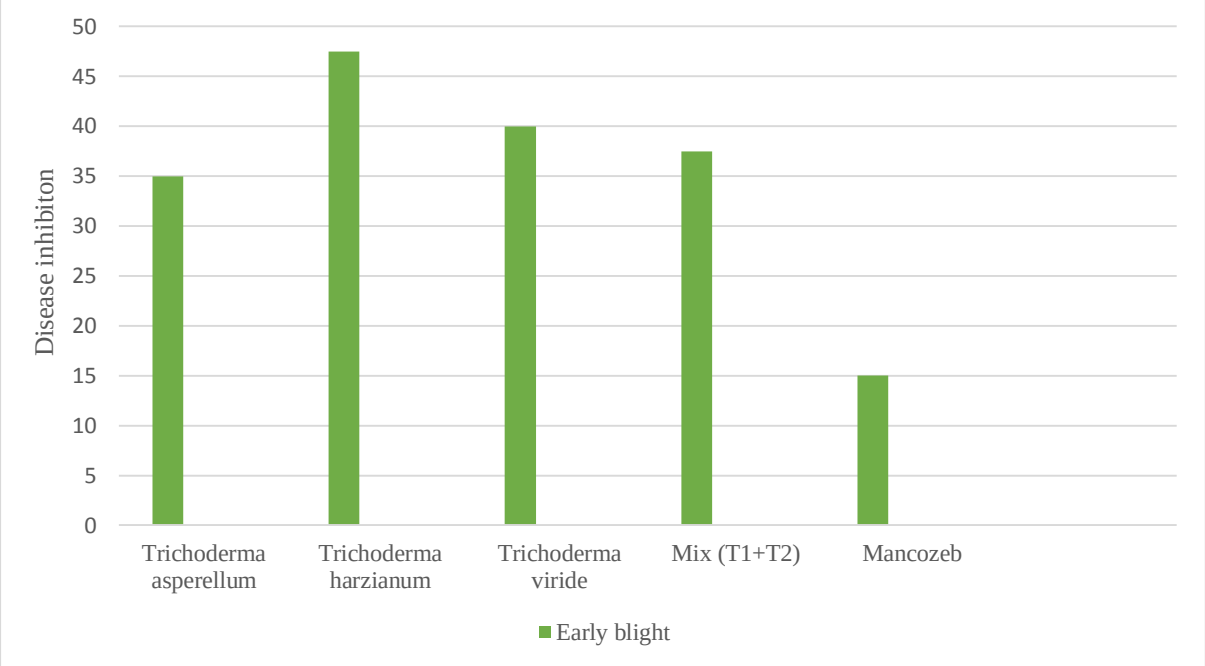
Graph 2. Effect of different pH on mycelial growth of *Alternaria solani*



Graph 3. *In vivo* management of Early blight disease of tomato



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4. Conclusion

The results showed that different media and pH has clear cut effect on the mycelial growth of *Alternaria solani*. These findings showed that for management of *Alternaria solani* was also done by adjustment of pH. Native *Trichoderma* spp. has clear cut effect on disease management.

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