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Yaragorla Hanumantha Rao

Department of Plant Pathology,
College of Agriculture, Central
Agricultural University, Imphal,
Manipur, India

PH Sobita Devi

Department of Plant Pathology,
College of Agriculture, Central
Agricultural University, Imphal,
Manipur, India

Vishnu Vardhani Vemavarapu

Department of Plant Pathology,
College of Agriculture, Central
Agricultural University, Imphal,
Manipur, India

K Rishitha Chowdary

Department of Plant Pathology,
College of Agriculture, Central
Agricultural University, Imphal,
Manipur, India

Corresponding Author:**Yaragorla Hanumantha Rao**

Department of Plant Pathology,
College of Agriculture, Central
Agricultural University, Imphal,
Manipur, India

Effect of media & Ph on mycelial growth of *Fusarium oxysporum* f.sp. *lycopersici* causing fusarium wilt of tomato in Manipur under *in-vitro* condition and effect of native *Trichoderma* spp. one chemical fungicide (Mancozeb) on growth parameters of tomato in field conditions

Yaragorla Hanumantha Rao, PH Sobita Devi, Vishnu Vardhani Vemavarapu and K Rishitha Chowdary

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Abstract

Tomato, (*Solanum lycopersicum*), flowering plant of the nightshade family (Solanaceae), cultivated extensively for its edible fruits. Labelled as a vegetable for nutritional purposes, tomatoes are a good source of vitamin C and the phytochemical lycopene. 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 *Fusarium oxysporum* f.sp. *lycopersici*. Among seven culture media that were tested, the fungus grew best on Oat meal 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 growth parameters like plant height, plant canopy, no of branches and yield in kgs.

Keywords: Mycelial, *Fusarium oxysporum*, *Trichoderma*, Mancozeb, parameters

Introduction

Tomato, (*Solanum lycopersicum*), flowering plant of the nightshade family (Solanaceae), cultivated extensively for its edible fruits. Labelled as a vegetable for nutritional purposes, tomatoes are a good source of vitamin C and the phytochemical lycopene. Tomato holds second rank next to potato in world acreage although it is first among processing crops.

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) [2]. In India, 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) [3].

Bhagwat (1969) [4] found Oat meal agar as the best medium for the mycelial growth and sporulation of *Fusarium oxysporum*. Mycelial growth of *Fusarium oxysporum* f.sp. *nivenum* were also good in eight different solid media viz., potato dextrose agar, Oat meal agar and Coon's media (Jamaria, 1972) [5].

Nair (1957) [7] found that incidence of wilt appeared most at soil pH of 5.5 to 7.5 than at higher or lower levels. Cochran (1958) [6] reported that several species of *Fusarium* grew and sporulated in a pH range of 5.0 to 6.5.

Barnwal *et al.*, (2011) [8] reported from Jharkhand that minimum fusarium wilt incidence was recorded in tomato plants by seed priming with *T. viride* at 4g / kg seed plus soil application of neem oil cake at 250 kg/ha with highest number of branches per plant (15.0), number of flowers of plant (119.8), plant height (82.3cm), number of fruits per plant (32.6), highest fruit yield (162.7q /ha) and increases in fruit yield over control of 38.1 percent with rupee return of 7.9 percent.

Materials and Methodologies

Isolation of fungus

Fusarium wilt 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 hypochlorite. Later the pieces 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 $27 \pm 1^\circ\text{C}$ in BOD incubator and were observed periodically for the fungal growth. Purified cultures of the fungus were obtained by hyphal tip culture methods. Identification was done according to the key of (Leslie and Summerell, 2006) [9].

Effect of different culture media on mycelial growth of *Fusarium oxysporum*

Ascerting suitable medium for mycelial growth formation of *Fusarium oxysporum*, 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, Oat Meal Agar, Potato Dextrose Agar, Richards Agar, Water Agar were used. The mycelial growth of the fungus was studied and the data was statistically analyzed.

Effect of different pH on mycelial growth of *Fusarium oxysporum*

To estimate the mycelial growth of the *Fusarium oxysporum* 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.

Effect of Native *Trichoderma* spp., one chemical fungicide (mancozeb) on growth parameters of Tomato in field conditions.

Three *Trichoderma* spp., one chemical fungicide (mancozeb) was tried in the field for controlling diseases and checking the effect on growth parameters 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 taller plant height, larger canopy area, number of branches per plant and more yield in kgs will be recorded.

Results and Discussions

Effect of different culture media on mycelial growth of *Fusarium oxysporum*

For studying the mycelial growth of *Fusarium oxysporum*, seven media were used. The results are given in Table 1. *Fusarium oxysporum* showed highest maximum radial growth

in Oat agar medium 83.15mm, coons medium showed 81.56mm, Elliots agar showed 75.93mm, PDA showed 58.78mm, corn meal showed 55.93 mm and Richard agar showed 44.06mm.

Lowest radial growth was observed in water agar 33.96mm. Coons medium showed regular shape white colour, corn meal showed white colour with regular shape, Elliots agar showed regular shape with fluffy, oat meal agar showed loose fluffy with regular growth, PDA found as regular shape, Richard agar showed light yellow colour with irregular margin and water agar showed low transparent growth these results are found to be similar with Thamaria (1972) [10] also observed Richard's agar and Potato dextrose agar medium to be the best for the mycelial growth of the fungus

Effect of different pH on mycelial growth of *Fusarium oxysporum*

Fusarium oxysporum showed Highest mycelial growth at pH 6 (90.93%), pH 7 showed 85.3%, pH 8 and pH 9 showed 57.5 and 48.12%, pH 5 showed 42.5%. lower most growth was showed at pH 10 (38.75%) these results are found to be similar with Korobomokova (1961) [11] also reported that pH 6 to 8 were sustable for the pathogen.

Further, increase in the pH level showed retarding effect on growth and sporulation. Recently, Singh *et al.*, (2016) [12] reported that maximum growth of the *Fusarium oxysporum* f. sp. *lentis* is at pH 6.0

Effect of Native *Trichoderma* spp one chemical fungicide (mancozeb) on growth parameters of Tomato in field conditions

1. Plant height

Highest plant height was observed in *Trichoderma viride* treatment with 26.93cm, followed by *Trichoderma harzianum* treatment showed 23.38cm, Mix (*Trichoderma harzianum* + *Trichoderma asperellum*) with 22.83cm and Mancozeb showed 20.56. lowest plant height recorded in *Trichoderma asperellum* treatment with 19.20 cm.

2. Plant canopy

Field results also showed that canopy area was highest is observed in *Trichoderma viride* treatment with 4.96 cm², followed by *Trichoderma harzianum* treatment showed 4.51 cm², Mix (*Trichoderma harzianum* + *Trichoderma asperellum*) with 4.39 cm² and Mancozeb showed 4.22 cm². lowest plant height recorded in *Trichoderma asperellum* treatment with 4.03 cm²

3. Number of Branches per plant

Data showed that Highest number of branches is observed in *Trichoderma viride* treatment with 6.4 branches/plant, followed by *Trichoderma harzianum* treatment showed 5.31 branches/plant, Mix (*Trichoderma harzianum* + *Trichoderma asperellum*) with 5.5 branches/plant and Mancozeb showed 5.24 branches/plant. lowest plant height recorded in *Trichoderma asperellum* treatment with 5.25 branches/plant

4. Fruit yield

Results also showed that fruit yield was highest is observed in *Trichoderma viride* treatment with 3.5kgs, followed by *Trichoderma harzianum* treatment showed 2.7kgs, Mix (*Trichoderma harzianum* + *Trichoderma asperellum*) with 1.47 kgs and Mancozeb showed 1.42kgs. Lowest plant height recorded in *Trichoderma asperellum* treatment with 0.8kgs

Table 1: Effect of different media on mycelial growth of *Fusarium oxysporum*

Culture media	<i>Fusarium oxysporum</i>
Coon's media	81.56
Corn meal	55.93
Elliot's Agar	75.93
Oat Meal Agar	83.15
PDA	58.78
Richards agar	44.06
Water Agar	33.90
SE(d)±	0.02
CD (0.05)	0.08

*Mean of three replications

Table 2: Effect of different pH on mycelial growth of *Fusarium oxysporum*

Different Ph	<i>Fusarium oxysporum</i>
5	42.5
6	90.93
7	85.31
8	57.5
9	48.12
10	38.75
SE(d)±	0.03
CD (0.05)	0.11

*Mean of three replications

Table 3: Effect of different *Trichoderma* spp and a chemical fungicide on growth parameters of tomato in filed conditions

Treatment	Plant height (in cm2)	Plant canopy (in cm2)	No of branches	Yield in Kgs
<i>Trichoderma asperellum</i> (25)	13.20	4.03	5.23	0.85
<i>Trichoderma harzianum</i> (69)	23.38	4.51	5.31	2.7
<i>Trichoderma viride</i>	26.94	4.96	6.43	3.5
Mix (T1+T2)	22.83	4.39	5.50	1.47
Mancozeb	20.56	4.22	5.24	1.42
SE(d)	0.54	2.67	0.308	0.26
CD (0.05)	1.64	8.02	0.92	0.79

*Mean of Four replications

Plate 1: Effect of different media on mycelial growth of *Fusarium oxysporum***Plate 2:** Effect of different pH on mycelial growth of *Fusarium oxysporum* **Graph 1:** Effect of different media on mycelial growth of *Fusarium oxysporum* **Graph 2:** Effect of different pH on mycelial growth of *Fusarium oxysporum* **Conclusion**

The results showed that different media and pH has clear cut effect on the mycelial growth of *F. oxysporum*. These findings showed that for management of *F. oxysporum* was also done by adjustment of pH. Native *Trichoderma* spp has clear cut effect on growth parameters of tomato like taller plant height, larger canopy area, number of branches per plant and more yield in kgs

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