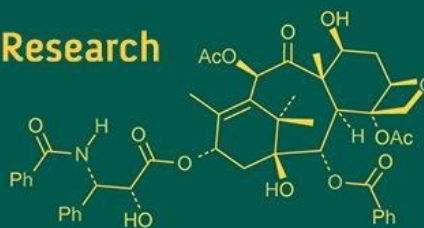


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Kartikey Pandey
Department of Plant
Protection, Faculty
of Agricultural Sciences,
Aligarh Muslim University,
Aligarh, Uttar Pradesh, India

Ziaul Haque
Department of Plant
Protection, Faculty
of Agricultural Sciences,
Aligarh Muslim University,
Aligarh, Uttar Pradesh, India

Anita Kumari Pandey
Department of Plant
Pathology, Rajmata
Vijayaraje Scindia Krishi
Vishwavidyalaya, Gwalior,
Madhya Pradesh, India

Sachin Kumar Rathor
Department of Plant
Pathology, Rajmata
Vijayaraje Scindia Krishi
Vishwavidyalaya, Gwalior,
Madhya Pradesh, India

Jaya Jeevitha SRM
Department of Plant
Protection, Faculty
of Agricultural Sciences,
Aligarh Muslim University,
Aligarh, Uttar Pradesh, India

Uma Shanker
Department of Plant
Protection, Faculty
of Agricultural Sciences,
Aligarh Muslim University,
Aligarh, Uttar Pradesh, India

Corresponding Author:
Kartikey Pandey
Department of Plant
Protection, Faculty
of Agricultural Sciences,
Aligarh Muslim University,
Aligarh, Uttar Pradesh, India

Effects of different culture media on mycelial growth and sporulation of *Fusarium oxysporum* f. sp. *Lycopersici*

Kartikey Pandey, Ziaul Haque, Anita Kumari Pandey, Sachin Kumar Rathor, Jaya Jeevitha SRM and Uma Shanker

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Abstract

Tomato (*Lycopersicon esculentum* Mill.) is a popular, easily grown plant with highly tasty Vegetable. Tomato wilt is a warm weather disease caused by fungus *Fusarium oxysporum* f. sp. *lycopersici*. The first indication of disease in small Plant is the drooping of lower leaves with lose of green color followed by wilting & death of the plant. The present study was undertaken to check the best suitable growth of *Fusarium oxysporum* f. sp. *lycopersici*. Six different synthetic and semi-synthetic media were screened to know out the suitable medium for the radial growth and sporulation of *Fusarium oxysporum* f. sp. *lycopersici* was revealed that among the Six different synthetic and semi-synthetic solid media viz., King B Medium, Czapek dox Agar, Rose Bengal Agar, Richard Synthetic Agar, Czapek Yeast Extract Agar and Potato Dextrose Agar. the Potato Dextrose Agar medium was observed significantly superior in supporting maximum mycelial growth (86.00 mm) and abundant sporulation of the fungus at the 7th day of incubation at 25±1 °C followed by Richard's synthetic agar medium (82.00 mm) followed by czapek dox agar (76.00 mm). The minimum growth of the fungus was recorded on czapek yeast extract agar (63.00 mm) followed by on rose bengal agar (65.00 mm). PDA is one of the most commonly used culture media because of its simple formulation and its ability to support mycelial growth of a wide range of fungi.

Keywords: Tomato, *Fusarium oxysporum* f. sp. *lycopersici*, media, mycelial growth and sporulation

Introduction

Tomato (*Lycopersicon esculentum* Mill.) is one of the most popular, highly nutritious, commercial vegetable crop grown all over the world. It is a Solanaceous family vegetable and origin in Tropical America (Thompson and Kelly, 1957) ^[1]. It is grown extensively and marketed throughout the world including tropical and temperate regions. Fusarium wilt is one of the most serious diseases affecting tomato plants throughout the world, especially in upland areas. *Fusarium oxysporum* F. sp. *lycopersici* is a devastating pathogen of tomato ^[11]. Management of Fusarium wilt can be done by many ways. Crop rotation with non-solanaceous crops (non-host) for four to six years is usually recommended to reduce the inoculum level of FOL in the field ^[12]. The crop should be rotated with cereals crops wherever possible ^[3]. Physical methods are quite effective but an important hindrance that limits their use is low efficiency and high labour intensive. Synthetic chemicals are commonly used for the management of FOL in tomatoes. Treatment with chemical fungicides especially prochloraz, carbendazim, propiconazole, thiabendazole, benomyl, thiophanate, fuberidazole and benzimidazoles considerably reduce the wilt incidence in tomatoes ^[4, 5]. Khan and Khan ^[6] reported that the root-dip treatment of carbendazim on tomato seedlings infected with FOL led to a 24% increase in yield. However, the use of fungicides is a bit expensive as well as environmentally undesirable due to damaging effects on the agroecosystem, human beings and animals ^[7]. Under this situation, all hopes are set on the exploration of biological management of plant diseases. Biological control is one of the best and safest methods of disease management and is devoid of residual toxic effects ^[12]. This method is based on the idea that pathogen's populations can be decreased by influencing naturally occurring living microbial species, altering the environment, or introducing antagonists. Several antagonists of FOL are known and their utilization resulted in a substantial decrease in the disease

severity and correspondingly increase in the crop yield. *Trichoderma* spp. are well known, Frequently used biocontrol agents [8-10].

Origin, Taxonomy and Morphology of *Fusarium*

Fusarium oxysporum f. sp. *lycopersici* comprise that are widely distributed worldwide in soils. Taxonomically *Fusarium oxysporum* f. sp. *lycopersici* belongs to the Kingdom-Fungi, Phylum- Ascomycota, Subphylum-Pezizomycota, Class -Sordariomycetes, Subclass -Hypocromycetidae, Order-Hypocreales, Family-Nectriaceae and Genus - *Fusarium*. It produces three types of asexual spores: Microconidia, Macroconidia and Chlamydospores.

Macroconidia

Macroconidia were also different shape and size like fusiform, sickle shaped hyaline with mostly 2-5 septate and measured 20.7-28.5 x 2.8-6.09 µm in size.

Microconidia

The microconidia were vary from round to cylindrical shaped with hyaline, single celled (0-2 septate), ovoid to ellipsoidal in shape, straight to slightly curved and measured 5.7-12.7 x 2.8-4.3 µm in size.

Chlamydospores

The chlamydospores produced in 2-3 week old culture were typically intercalary and produced in individual or in a chain. They were globose to sub-globose, aseptate thick walled and smooth surfaced. The pathogen enters through the epidermis of root, later spreads through the vascular tissue and inhabits the plant xylem vessels, resulting in vessel clogging, and severe water stress as a result wilt like symptoms appear (Singh *et al.*, 2017) [13]. The dormant chlamydospore of *Fusarium oxysporum* f. sp. *lycopersici* in infested soil can survive indefinitely in the absence of host (Khan *et al.*, 2017 and Cha *et al.*, 2016) [14].

Materials and Methods

Isolation, Purification and characterization *Fusarium oxysporum* F. spp *Lycopersici*

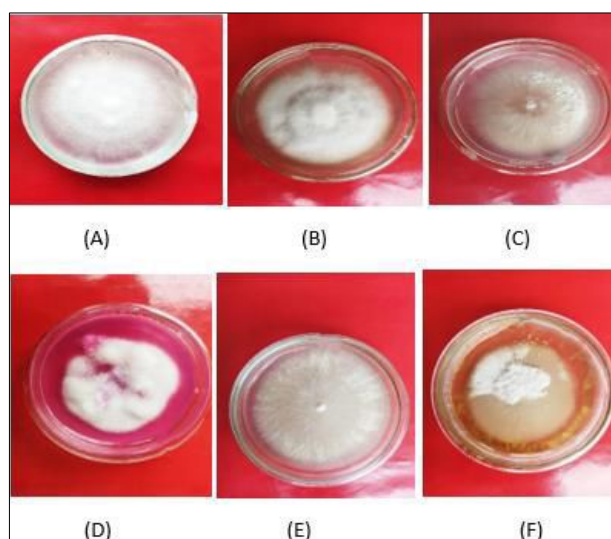
To isolate of the indigenous isolate of test fungus *Fusarium oxysporum* f. sp. *lycopersici* (FOL), soil samples were collected from agricultural field in and around, Aligarh. The fields from where the samples collected was under tomato cultivation. Soil was collected from the site having continuously good moisture contents. The samples were collected from a depth of 2-4 cm and 10-15cm after removing skin of soil from the rhizosphere of the tomato field. The fungus was isolated using serial dilution technique. Association of *FOL* with the soil samples was confirmed on the basis of its colony and morphological characteristics. First 10 g soil (properly dried) were taken in sterilized flask with 90 ml water and then stirred with a magnetic stirrer for 20-30 minutes. A commercial blender was used for blending the samples. While the suspension is in motion, withdraw 10 ml and add to 90 ml sterile water in a screw cap flask. Shake for 1 minute and transfer 10 ml of the suspension to another 90 ml sterile water blank. Repeat the process until the desired dilution of 10^5 was obtained. The final 1 ml of diluted samples were spread on a solidified PDA (Potato dextrose agar, HiMediaTM, India) in a Petriplate under a laminar flow using sterile pipette. To avoid the growth of bacteria Streptomycin were added to PDA media. Petri plates were incubated at 25 ± 1 °C for 7 days. After incubation the fungal colonies were observed

under a microscope and matched with FOL characteristics. Isolates were further purified on another petri dish containing PDA and identified by morphological characters. Relative growth of *Fusarium oxysporum* f. sp. *lycopersici* on different media The cultural characters of *F. oxysporum* f.sp. *lycopersici* were conducted on the following six synthetic and semi-synthetic media viz., King B medium, Czapek dox agar, rose bengal agar, Richard synthetic agar, Czapek yeast extract agar and potato dextrose agar. To prepare the solid media, Agar-agar was melted in 500 ml distilled water. The other ingredients were dissolved in the remaining 500 ml of distilled water. The two solutions were mixed thoroughly and the volume was made up to one litre. The medium was sterilized at 15 PSI /lbs for 20 minutes at 121°C in an autoclave. Twenty ml of each solid media was poured into 90 mm diameter Petri plates and three replicates were maintained. Inoculation was made by transferring ½ cm disc of mycelium taken from the periphery seven days old pure. Culture of *FOL*. The plates were incubated at 25°C for seven days. The cultural characteristics and the colony diameter (mm) on each of the mediums were recorded and results were analyzed statically.

S. No.	Medium	Constituents	Quantity
1.	Potato Dextrose Agar	Agar Dextrose Peeled Potato Distilled Water	20.00 g 20.00 g 250.00 g 1000 ml
2.	Czapek's dox Agar	Agar Sucrose Distilled water Dipotassium phosphate Magnesium Potassium chloride Sodium nitrate Ferrus Sulphate	20.00 g 30.00 g 1000 ml 1.00 g 0.50 g 0.50 g 2.00 g 0.01 g
3.	Rose Bengal Agar	Rose Bengal Distilled water Agar Soya peptone Dextrose Monopotassium Phosphate Magnesium sulphate	0.05 g 1000 ml 15 g 5.00 g 10.00 g 1.00 g 0.05 g
4.	Richard's synthetic Agar	Sucrose Potassium nitrate Potassium dihydrogen phosphate Magnesium Sulphate Ferric chloride Agar Distilled water	20.00 g 2.00 g 1.00 g 0.50 g 0.01 g 20.00 g 1000 ml
5.	Czapek's Yeast Extract agar	Distilled water Agar Copper sulphate Ferrous sulphate Zinc sulphate Sucrose Yeast extract Dipotassium hydrogen phosphate Sodium nitrate Potassium chloride Magnesium sulphate	1000 ml 15.00 g 0.0005 g 0.001 g 0.001 g 30.00 g 5.00 g 1.00 g 0.30 g 0.05 g 0.05 g
6.	King B Medium	Distilled water Agar Dipotassium hydrogen phosphate Protease peptone Magnesium Sulphahte	1000 ml 20.00 g 1.50 g 20.00 g 1.50 g

Results and Discussion

Six different synthetic and semi-synthetic media were screened to know out the suitable medium for the radial growth and sporulation of *Fusarium oxysporum* f. sp. *lycopersici* (FOL). The data revealed that among the six different synthetic and semi-synthetic solid media, the Potato Dextrose Agar medium exhibited maximum mycelial growth (86.00 mm) and abundant sporulation of the fungus at the 7th day of incubation at $25\pm 1^\circ\text{C}$ and was significantly superior over other media. Next in supporting the mycelial growth of FOL was Richard's synthetic agar medium (82.00 mm) followed by Czapek dox agar (76.00 mm). The minimum growth of the fungus was recorded on rose Bengal agar (65 mm) followed by on Czapek yeast extract agar (63.00 mm). the relative mycelial growth and sporulation of *F. oxysporum* f. sp. *lycopersici* were evaluated on six synthetic and semi-synthetic media under *in vitro* conditions. Lazarotto also observed that the mass production of macroconidia of *F. chlamydosporum* was very high on potato sucrose agar (PSA) and PDA media, corroborating the results obtained in the present work. Silva and Teixeira (2012) [15] stated that carbohydrate-rich media induces an increase in the production of conidia of *F. solani*. The Potato Dextrose Agar (PDA) was the best media for maximum mycelial growth and abundant sporulation of the *fusarium oxysporum* f. sp. *lycopersici*.



(A) Potato Dextrose Agar (B) King B Medium Base (C) Czapek Dox Agar (D) Rose Bengal Agar Base (E) Richard's Synthetic Agar (F) Czapek Yeast Extract Agar

Table 2: Effect of different culture media on Mycelial Growth and Sporulation of *Fusarium oxysporum* f.sp. *lycopersici* at 7 days incubation at $25\pm 1^\circ\text{C}$

S. No.	Solid Medium	Myceliu growth (mm)	Sporulation
1.	Potato Dextrose Agar	86	++++
2.	King B Medium	75	+++
3.	Czapek Dox Agar	76	+++
4.	Rose Bengal Agar	65	++
5.	Richard Synthetic Agar	82	+++
6.	Czapek Yeast Extract Agar	63	+
	LSD(P $\geq$ 0.05)	6.23	

Summary and Conclusion

From the present research investigation out of Six different tested solid synthetic or semisynthetic media, the Potato

Dextrose Agar (PDA) was recorded to the best media for maximum mycelial growth and abundant sporulation of the *Fusarium oxysporum* f. sp. *lycopersici*, followed by Richard's synthetic agar medium, Followed by Czapek Dox agar.

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