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Effects of different culture media on the growth and sporulation of *Fusarium oxysporum* f. sp. *lycopersici* causing tomato Wilt

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Abstract
Fusarium wilt is considered as one of the most important diseases of tomato (*Lycopersicon esculentum* Mill) in green house as well as open field cultivation. Fusarium wilt were found associated with commercial grown cultivars with disease incidence ranging from 10.21 to 22.63 per cent in different location of Rajasthan. The mycelial growth and sporulatiuon of *Fusarium oxysporum* f. sp. *lycopersici* was studied at different culture growth medium. Potato Dextrose Agar (PDA) was found most significant to support the maximum mycelial growth with 88.04mm and abundant sporulation at 7th day of incubation at 25±1 ^{0}C followed by Oat meal agar medium with 81.14mm mycelial growth which was found statistically at par with Czapeck's Dox medium (78.49 mm). The radial growth of fungus was also recorded on Corn meal medium and on Martins medium as 58.49 mm and 67.95 mm respectively.

Keywords: Tomato, *Fusarium oxysporum* f. sp. *lycopersici*, Isolation, Identification, Mycelial growth and Sporulation

Introduction
Tomato (*Lycopersicon esculentum* Mill.) is one of the most popular and important commercial vegetable crop grown all over the world. It is a Solanaceous family vegetable believed to have its origin in Tropical America (Thompson and Kelly, 1957) [16]. It is grown extensively and marketed through-out the world including tropical and temperate regions. Fusarium is one of the more troublesome genera of fungal plant pathogens, causing devastating diseases like Fusarium wilt and Fusarium root/stem rot in numerous economically important crops. It is one of the most serious diseases affecting tomato plants throughout the world, especially in upland areas. The incidence of wilt was recorded as 10-22 per cent at different places of tomato growing in Rajasthan during survey in 2019-20. The causal agent of Fusarium wilt is soil borne pathogen which can persist many years in the soil without a host. Most infections originate from the population associated with infected tomato debris. Healthy plants can become infected by *Fusarium oxysporum* if the soil in which they are growing is infested with the pathogen (Farr *et al*., 1989) [5] However, pathogenic fungi of the genus *Fusarium* that is the causal agents of tomato vascular wilt cause root and basal stem deterioration and result in the wilting of vegetable plants. Browning of the vascular tissue is strong evidence of Fusarium wilt (Snyder and Hansen, 2003). Some isolates of this fungus are pathogenic only too specific plant species (forma specialis) and there are also a large number of physiological races within each of these specialized forms, all of which make the selection for resistance to this pathogen more difficult (Armstrong and Armstrong, 1981) [1]. Evaluating tomato material for the resistance, Djordjevic *et al*. (2011) [3] showed that race 1 of fusarium wilt is not a limiting factor for successful tomato production, but race 3 of *Fusarium oxysporum* f. sp. *lycopersici* occurs in Serbia and can seriously endanger tomato production (Djordjevic *et al*., 2011) [3]. Presence of *Fusarium* sp. on tomato fruits can cause great damages and economic losses. Also, it can cause human health problems because of production of mycotoxins.
Saccardo first described *Fusarium* spp. on tomato. Fungus that was isolated from tomato fruits originated from northern Italy and was named *Fusarium oxysporum* (Schl.) f. sp. *lycopersici* Sacc. (Walker, 1971) [17].

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* (Fry, 2004). It produces three types of asexual spores: Microconidia, Macroconidia and Chlamydospores (Agrios, 1988).

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) [15]. The disease in morphologically identified by wilted plants bearing yellow colored leaves with minimal or absent crop yield. 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) [9].

Materials and Methods

Isolation of the pathogen

The diseased samples of tomato plants showing the symptoms of tomato wilt were collected from different tomato growing district of Rajasthan.

The pathogen was isolated by root tissue segment isolation method from infected tomato roots collected from farmer (Khemram Jat) field, Takarda village of Chomu tehsil (Jaipur). Sections of roots (1cm) exhibiting symptoms of Fusarium wilt were washed gently under tap water to remove adhering soil and then rinsed in sterile water. The bits of roots were surface sterilized with one per cent sodium hypochlorite solution for one minute and transferred to sterilized distilled water for three successive washings to remove the traces of sodium hypochlorite solution from tissue. The surface sterilized root sections were blotted dry with sterile paper towel and then placed aseptically on Potato Dextrose Agar (PDA) plates. The inoculated plates were incubated at 25±1°C in BOD incubator. Pure cultures obtained by single spore technique isolation with the help of dummy objective, was transferred on PDA slants and allowed to grow at 25±1^{0}C temperature for 7 days. The culture so obtained was stored in refrigerator at 4^{0}C and sub cultured periodically once in a month from uncontaminated peripheral growth was made on PDA slants.

Purification and identification of the pathogen

For the purification of the fungus, single spore technique was used. After sporulation, conidial suspension was made in sterile water and the dilution was adjusted such that in one loop full, 20-25 conidia could be counted under low power objective of microscope. One such loop full was mixed with 20 ml melted and sterilized agar (2%) and poured in sterile petri plates. After 12 hours of incubation at 25±1 °C, the single germinating conidium was cut with the help of dummy objective and transferred to PDA slants. They were subsequently allowed to grow and sporulation. Monoconidial culture established in this way was maintained by periodical transfer on PDA slants. After purification, fungus was allowed to sporulation. The isolated fungus was identified based on the morphological characters and visual observations. The mycelium was stained with lactophenol blue and observed under binocular research microscope (40x) for septation, hyphal branching pattern, macro and micro conidia and chlamydospores and compared with standard description given by booth (1971) in key "The genus *Fusarium*", the associated pathogen was identified as *Fusarium oxysporum* f. sp. *lycopersici.*

Pathogenicity test of the pathogen:

To make a sure certain, the ability of the pathogen to cause disease in tomato plant. The isolated and purified fungus from diseased roots of tomato was tested for its pathogenicity. The pathogenicity of *Fusarium oxysporum* f. sp. *lycopersici* was tested following by Koch's postulates method under pot conditions by sick soil inoculation technique. Apparently healthy surface sterilized seeds of tomato were grown in nursery. One month old seedlings of tomato were used for transplanting in sick pot. The pathogen (*Fusarium oxysporum* f. sp. *lycopersici*) multiplied on sorghum grain at 25±1°C was used as the soil inoculums. Prior to transplanting, pots were sterilized with copper sulphate solution and filled with sterilized soil (Soil: FYM = 3:1) sterilized at 1.045 kg cm^2 for one hour for three consecutive days.These pots were inoculated with fungus inoculum multiplied on sorghum grain medium. Seven apparently healthy and surface sterilized tomato seedlings were transplant in each pot and replicated five times. Root surface sterilized seedlings were also transplanted in un-inoculated sterilized soil served as check. These pots were kept in pot house and watered regularly as and when required and maintained under identical condition. Observation of symptoms appearance and disease progression were record and per cent disease incidence was calculated as follows.

$$\text{Per cent disease incidence} = \frac{\text{Number of diseased plants}}{\text{Total number of plants}} \text{ X } 100$$

The pathogen was re-isolated and resulting culture were compared with the original one with respect to colony characters, colony colour, conidial morphology and septation and identified as *Fusarium oxysporum* f. sp. *lycopersici* further confirmed by ICTV New Delhi.

Every living microorganism needs a specific food for growth and reproduction. No one microbe is an exception to this phenomenon (Kiryu, 1939) [10]. Fungi sheltered food and energy from the medium upon which they grow in the nature and laboratory. In directive to culture the fungi in the controlled condition, it is necessary to provide those essential rudiments and compound in the medium which are essential for their growth and other existence course. Neither all media

are equally good for all fungi nor there can a common substrates or artificial synthetic medium on which the fungi grown well. In present studied five different media were taken to find out the best growth medium for the well growth of *Fusarium oxysporum* f.sp. *lycopersici*.

Growth on solid media was determined by measuring the colony diameter along with the two diagonals passing through the center of colony by excluding initial diameter (5 mm) of bit. Five solid media whose composition is given below were taken for *in vitro* studies. Petri plates having sterilized medium were inoculated with 5 mm disc of mycelial growth with the help of sterilized cork borer and incubated at 25±1 ºC in incubator for 7 days with four replications. Observations on mycelial growth (radial growth) were taken after 7 days of incubation. Medium giving best radial growth of mycelium used for further studies.

To find out the suitable media for growth of the test fungus, following five solid media were assessed at 25±1 ^{0}C.

Table 1: Constituents of different media

S. No.	Medium	Constituents	Quantity
1.	Potato Dextrose Agar	Agar agar	20.00 g
		Dextrose	20.00 g
		Peeled potato	250.00 g
		Distilled water	1000 ml
2.	Czapeck`s Dox Agar medium	Agar agar	15.00 g
		Sucrose	30.00 g
		Distilled water	1000 ml
		Dipotassium phosphate	1.00 g
		Magnesium	0.50 g
		Potassium chloride	0.50 g
		Sodium nitrate	2.00 g
		Ferrus sulphate	0.01g
3.	Oat meal medium	Agar agar	15.00 g
		Dextrose	20.00 g
		Oat meal	20.00 g
		Distilled water	1000 ml
4.	Corn meal medium	Agar agar	20.00 g
		Corn meal	20.00 g
		Glucose	20.00 g
		Distilled water	1000 ml
5.	Martin'sAgar medium	Distilled water	1000 ml
		Dipotassium phosphate	4.00 g
		Monopotassium phosphate	1.00 g
		Sodium chloride	5.00 g
		Agar agar	12.0 g

Result and Discussion

Five 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*. On perusal of data (Table-2 and Plate-1) was revealed that among the five different synthetic and semi-synthetic solid media, the Potato Dextrose Agar medium was observed significantly superior in supporting maximum mycelial growth (88.04 mm) and abundant sporulation of the fungus at 7th day of incubation at 25±1 ^{0}C followed by Oat meal agar medium (81.14 mm) which was found statistically at par with Czapeck's Dox medium (78.49 mm). The minimum growth and poor sporulation of the fungus was recorded on Corn meal medium (58.49 mm) followed by on Martins medium (67.95 mm).

Table 2: Effect of different culture media on mycelial growth and sporulation of *Fusarium oxysporum* f. sp. *lycopersici* at 7 days of incubation at 25±1 °C

S. No.	Solid medium	Mycelial growth (mm)*	Sporulation
1.	Potao Dextrose Agar	88.04	++++
2.	Czapeck's dox Agar	78.49	+++
3.	Oat meal Agar	81.14	+++
4.	Corn meal Agar	58.49	+
5.	Martins Agar	67.95	++
	S.Em+	1.05	
	CD (5%)	3.19	
	CV	2.81	

*Average of Four replications

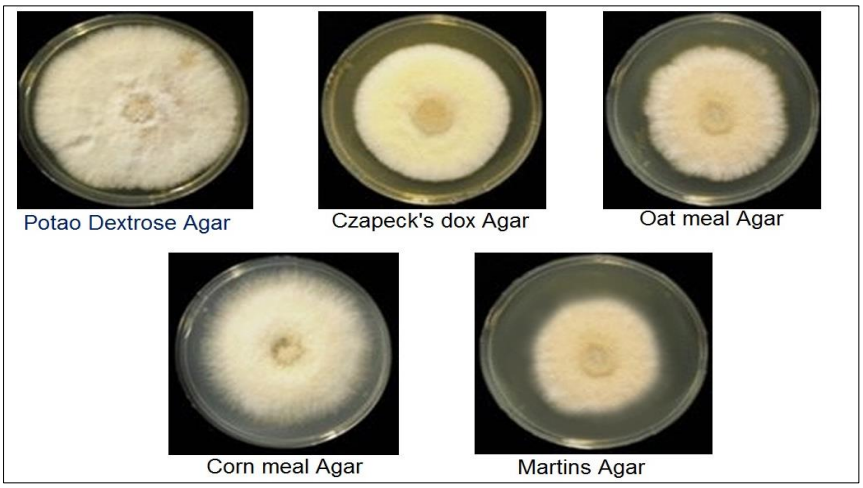

Plate 1: effect of different solid media on mycellial growth of *Fusarium oxysporum* f. sp. tycopersici

The present existed experiment results agreed with the finding of Imran Khan *et al*., (2011) [8] the effect of media on growth and sporulation of *Fusarium oxysporum* f. sp. *ciceri* and they observed that PDA was the best for the growth sporulation of different isolates of FOC followed by Czapek's Dox agar. Similary Nath *et al*., (2017) [11] were found the Oat meal agar medium was the best growth medium followed by Czapek's Dox agar which gave 90.00 mm and 84.50 mm mycelial growth respectively. In a study conducted by El-Sayed *et al*., (2008) [4] PDA induced the best linear growth for *Fusarium oxysporum* f. sp. *lycopersici*. Osman *et al*., (1992) [12] studies the effect of various culture media on *Fusarium oxysporum* and PDA was the best culture media. These results were in confirmation with Ingole (1995) [7] who reported that PDA and Richard's agar supported best mycelial growth of *Fusarium udum*. In the present study our findings agree with that. 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.

Summary and Conclusion

From the present research investigation out of five 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 oat meal agar medium (OMA), which was significantly at par with Czapeck Dox medium.

Declaration

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Conflict of interest: There is no conflict of interest.
Further Research: *Fusarium oxysporum* f. sp. *lycopersici* is a widely distributed as whereas tomato grown, it's basic need of the future to understand the pathogen from its genetically form and develop the systemic resistance in Tomato plants.

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