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Characterization of *Fusarium oxysporum* causing wilt disease in groundnut

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Abstract
Richard's agar and carrot dextrose agar supported the maximum mycelial growth of 9.00 cm at 7 DAI. Richard's broth yielded the maximum mycelial dry weight of 914.00 mg. when carbon sources glucose ranked first in solid as well as liquid broth to promote the fungal growth with a mean mycelial diameter of 9.00 cm and mycelial dry weight of 453.40 mg respectively. In nitrogen sources, ammonium nitrate was found to be effective for the growth of *F. oxysporum*in solid and liquid media. The fungus grew well in pH 6.5 to 7.0.

Keywords: *Fusarium oxysporum*, groundnut, richard's

Introduction
Groundnut or peanut (*Arachis hyphogaea* L.) is an important leguminous oil seed crop, belonging to the family Fabaceae. Groundnut is grown on a large scale in almost all the tropical and subtropical countries of the world. Including China, India, Nigeria, Sudan and USA. Groundnut occupied on area of 26.46 million ha with an annual production of 26.4 million tonnes in the world (FAO, 2007) [2]. The crop was introduced into India by Portuguese. In India it is one of the most important food and cash crop with valuable source of all nutrients. India ranks first in the world with regard to area of groundnut (26.11 million ha) and second in its production (7.54 million tonnes). (www.india stat.com, 2011).
In India, Gujarat is the largest producer of groundnut contributing to 25 per cent of its productionfollowedby Tamil Nadu with 22.48 per cent, while in productivity Tamil Nadu ranks first. In the State groundnut is cultivated in 11.29 lakh ha accounting for a production of 18.04 lakh tonnes with an average yield of 2681 kg/ha (www.india stat.com, 2011).
Groundnut is susceptible to many foliar and soil-borne fungal diseases including early leaf spot caused by *Cercosporaarachidicola* Hori., late leaf spot caused by *Phaeoisariopsispersonata* (Bark and Curt.) v.Arx., rust incited by *Puccinia orachidis* Speg., dry root rot caused by *Macrophominaphaseolina* (Tassi) Goid., stem rot incited by *Sclerotium rolfsii* Sacc. and wilt caused by *Fusarium oxysporum*. Among these the soil- borne diseases viz., dry root rot, stem rot and wilt cause serious losses to the crop which is extensively grown under rainfed conditions (Kannaiyan *et al*., 1989) [11].
The pathogen *Fusarium oxysporum* that causes wilt of groundnut was first reported in Tansania by Armstrong *et al.* (1975) [3]. *Fusarium oxysporum* is a root pathogen colonizing the xylem and blocking them completely to effect wilting (Bateman *et al.*, 1996) [4]. Fusarium induces vascular diseases in crops such as watermelon, cucumber, tomato, pepper, muskmelon, bean, cotton and groundnut (Rasheed *et al.*, 2004) [16].
In a survey carried out in groundnut growing areas of Theni district, the crop was found to exhibit a disease syndrome which caused sudden wilting in plants. During sudden wilting, leaves of an entire plant turn greyish green and the taproots show vascular browning. The fungus isolated from the stem on potato dextrose agar (PDA) produced cottony white mycelium with microconidia, macroconidia and chlamydospores. The present investigation was undertaken with the following objectives. 1. Survey on the incidence of wilt in groundnut growing areas. 2. Isolation, identification of the pathogen, symptomatology and confirmation of pathogenicity. 3. Studies on the morphology and growth characters of the pathogen.

Materials and Methods
Collection of samples
A survey was conducted during 2011 to assess the occurrence of Fusarium wilt of groundnut at Madurai, Sivagangai and Theni districts of Tamil Nadu. The disease incidence was assessed by counting the number of affected plants out of total number of plants in each field.

In each area three fields were assessed and the disease incidence was calculated. Diseased samples of plants were collected from these areas.

Isolation of pathogen

The pathogen causing wilt in groundnut was isolated from the samples by tissue segment method on potato dextrose agar (PDA) and the fungus was purified by single spore isolation and maintained on PDA. The causal organism was identified based on colony morphology, colour and conidial features.

Pathogenicity

Detached seedling technique

A five- mm culture disc of *F.oxysporum* was placed closer to the root region of either a pinpricked or non pinpricked healthy and cleaned groundnut seedling, kept in 150-mm-dia Petri dish over a layer of moistened cotton. An empty five-mm disc of PDA served as control. Three replications were maintained and the plates were incubated at room temperature (28 ± 2º C). The formation of lesion on stem was closely monitored and the lesion length was recorded at regular intervals up to16 days.

Pathogenicity in glasshouse

Multiplication of inoculum

The fungus was multiplied on sand-maize medium (Riker and Riker, 1936). The medium containing 1900 g of sand and 100 g of maize powder (19:1) was mixed, moistened with 400 ml of water kg^{-1} and filled in empty salaine bottles. The bottles were sterilized at 1.4 kg cm^{-2} pressure for two h for two alternate days. Each bottle was inoculated with two nine- mm culture disc of actively growing *F.oxysporum* and incubated at room temperature (28±2°C) for 30 days to be used as inoculum.

Earthen pots of 25 cm- dia were filled with five kg of potting medium (red soil:sand: FYM @ 1:1:1). The pot mixture was sterilized in an autoclave at 1.4 kg cm^{-2} pressure for two h on two successive days and inoculated with 5 g inoculum of *F.oxysporum* multiplied on sand maize medium. Healthy groundnut seeds were sown in pots with proper control. The pots were maintained in glasshouse by uniform and judicious watering and plants were constantly observed for the development of symptoms. The pathogen was reisolated from plants showing symptoms of wilt.

Morphological features and growth of *F.oxysporum* isolates on PDA

Five isolates of *F.oxysporum* collected during the survey were grown on PDA to study their growth and variability in colony characters. From the seven-day-old culture plates, five- mm disc of the fungus was cut by a sterilized cork borer and placed at the center of each sterile Petri dish (90-mm-dia) containing 15 ml of sterilized and solidified PDA. The plates were incubated at room temperature (28±2ºC) for seven days. The mycelial growth colony morphology and colour were recorded seven days after inoculation (DAI). One of the five isolates that was fast in growth was carried for further studies.

Standardization of culture media for the rapid growth of *F.oxysporum*

*F.oxysporum*was grown on PDA, Richard's agar, carrot dextrose agar, starch agar, Czapek's Dox agar, oatmeal agar and Martin's rose bengal agar to standardize the one which supported rapid growth of the pathogen. The sterilized warm medium was poured @15 ml in sterile Petri dishes and medium was allowed to solidify. A seven-day-old, five-mm culture disc of the fungus was inoculated at the centre of the plate. Theplates were incubated at room temperature (28±2ºC) and three replications were maintained for each treatment. The radial growth of the fungus was measured 7 DAI.

Growth of *F.oxysporum*on different liquid media

Liquid broths *viz.*, potato dextrose, Richard's, carrot dextrose, beetroot dextrose starch, Czapek's Dox, oatmeal and Martin's rose bengal were prepared and 100 ml of medium was distributed in 250 ml Erlenmeyer flasks. The flasks were autoclaved at 1.4 kg $cm^{-2}$for 20 min, cooled and inoculated with seven-day-old, five-mm culture disc of the pathogen. After seven days, the mycelial mat was filtered through pre weighed Whatman No.1 filter paper, dried in hot air oven at 100º C until constant weight was obtained.

Effect of carbon, nitrogen sources and the growth of *F.oxysporum*

Richard's agar mediumas well as broth was substituted with different carbon sources *viz.*, mannitol, glucose, starch, sucrose, fructose, maltose and carboxy methyl cellulose (CMC) and nitrogen sources such as peptone, urea, potassium nitrate, ammonium oxalate, sodium nitrate, ammonium sulphate and ammonium nitrate and sterilized. The medium without carbon and nitrogen source served as control. To study the growth, the fungus was inoculated in solid media as mentioned in 3.6. and in liquid media as cited in 3.7. The colony diameter of the fungus was measured 5 DAI and 7 DAI and the mycelial weight was recorded 7 DAI.

p^H levels and thegrowth of *F.oxysporum*

Sterilized PDA was distributed in 250 ml Erlenmeyer flasks @100 ml per flask and the $p^H$of the medium was adjusted to levels of5.0, 5.5, 6.0, 6.5, 7.0, 7.5 and 8.0 with 0.1N HCl or 0.1N NaOH and autoclaved at 1.4 kg $cm^{-2}$for 20 min. Fifteen ml of the medium from each p^H level was poured onto sterilized Petri dishes and allowed to solidify. The pathogen was inoculated as mentioned in 3.6. and the mycelial growth was measured 5 DAI and 7 DAI.

Results and Discussion

Wilt of groundnut is caused by *F. oxysporum*. A survey was conducted in three southern districts of Tamil Nadu for the occurrence of Fusarium wilt of groundnut. The disease incidence was recorded up to 66.00 per cent in Theni district.

Culture media suited for the growth of *F. oxysporum*

The results from the present study showed that Richard's agar as well as broth promoted the maximum mycelial growth of *F.oxysporum* followed by carrot dextrose agar. The results are in agreement with that of Major (1923) who observed that Richard's agar promoted the maximum production of aerial mycelium of *F. solani*. PDA followed by Richard's agar was suitable for culturing *F.o*.f.sp.*nevium* (Jamaria, 1972) [10]. Khare *et al*. (1975) [13] reported that the maximum growth of *F. o.* f. sp. *lentis* was on PDA followed by lentil extract and Richard's agar. Both host leaf extract agar and Richard's agar were best for the growth and sporulation of *F. oxysporum* f. sp. *ciceri.* Godage (1979) [7] and Kewate (1986) [12]. Anjaneya (2002) [1] observed the maximum growth of *F. udum* on Richard's agar and PDA. Naik *et al.* (2010) reported that *F. o.* f. sp. *vanillae* grew well in Richard's agar and PDA.

Carbon, nitrogen sources and the growth of *F.oxysporum*

Among the carbon sources tested, glucose was found to promote fast growth of *F.oxysporum* in solid media and yielded more mycelial weight in liquid culture. Sowmya (1993) [18] reported that glucose was the best carbon source for the growth of *F. o.* f. sp. *cubense*. Imrankhan *et al*. (2011) observed that glucose followed by maltose was found to be the best carbon source for culturing *F. o.* f. sp. *ciceri*.

In the present study when the nitrogen sources were compared, ammonium nitrate stood first followed by potassium nitrate for culturing *F.oxysporum*. Both were equally efficient in promoting the fast growth of the fungus on solid medium and yielding more mycelium in liquid broth. These results are similar to the findings of Mathur (1960) who reported that *L.asparagines* and potassium nitrate were effective sources for the growth of *Fusarium* spp. Peptone followed by potassium nitrate was the best source of nitrogen for the growth of *F. oxysporum* (Farooq *et al*., 2005) [6]. Naik *et al.* (2010) observed that L.asparagines followed by potassium nitrate was found to be very good nitrogen source for the growth and development of *F. o.* f. sp. *Vanilla*. In contrast, Ramteke *et al*. (2001) reported calcium nitrate as best for culturing *F. solani* followed by sodium nitrate, ammonium nitrate and potassium nitrate.

pH levels and the growth of *F. oxysporum*

In the present study, the maximum mycelial growth of *F.oxysporum* was observed at pH 6.5 followed by pH 7.0 and the least growth was at pH 8.0. These results are in conformity with that of Chaudhary (1971) and Prasad *et al.* (1992) [14] who reported pH 6.0 as the best for the growth and sporulation of *F.moniliforme v. subgluitinanse.* Sowmy (1973) reported pH 7.0 as suitablefor culturing *F. o.* f. sp. *cubense*. Shaikh (1974) and Hayes (1978) [8] observed that *F. o.* f. sp. *ciceri* could tolerate a wide range of pH ranging from 5.0 to 6.5. El-Sayad *et al.* (2008) observed that *Fusarium*spp. grew well at a pH range of 6.5 to 7.0.

Table 1: Growth of *F. oxysporum*on different solid and liquid media

S. No.	Media	Mycelial growth (7 DAI) *	
		Colony diameter (cm)	Dry mycelial weight (mg)
1	Potato dextrose agar	8.30	542.00
2	Carrot dextrose agar	9.00	851.00
3	Beetroot dextrose agar	8.90	274.00
4	Oatmeal agar	7.00	181.00
5	Richard's agar	9.00	914.00
6	Czapek'sDox agar	7.50	772.00
7	Martins rose Bengal agar	7.50	42.60
CD		0.14	33.50

*DAI - days after inoculation

Table 2: Effect of different carbon sources on the growth of *F. oxysporum*

S. No.	Carbon sources	Mycelial growth (cm)		Dry mycelial weight (mg)
		5 DAI *	7 DAI *	
1	Sucrose	6.30	8.30	432.20
2	Fructose	5.70	7.80	359.10
3	Glucose	7.80	9.00	453.40
4	Mannitol	3.70	5.80	80.00
5	Carboxy methyl cellulose	4.10	6.60	306.10
6	Starch	5.10	7.40	326.60
7	Maltose	3.20	6.20	142.60
8	Control	2.10	4.70	49.00
CD		0.15	0.17	17.79

*DAI - days after inoculation

Table 3: Effect of different nitrogen sources on the growth of *F. oxysporum*

S. No.	Nitrogen sources	Mycelial growth (cm)		Dry mycelial weight (mg)
		5 DAI *	7 DAI *	
1	Peptone	6.10	8.70	347.00
2	Urea	4.10	6.10	211.00
3	Ammonium nitrate	6.90	9.00	386.00
4	Potassium nitrate	6.80	8.90	378.00
5	Ammonium sulphate	4.30	6.90	218.00
6	Sodium nitrate	4.70	7.00	322.00
7	Ammonium oxalate	4.90	7.10	336.00
8	Control	6.80	8.20	195.00
CD		0.15	0.14	18.17

*DAI - days after inoculation

Table 4: Effect of p^H levels on the growth of *F. oxysporum*

S. No.	p^H	Mycelial growth (cm)	
		5 DAI *	7 DAI *
1	5.0	6.30	6.50
2	5.5	6.60	6.90
3	6.0	7.30	7.70
4	6.5	8.40	9.00
5	7.0	7.70	8.10
6	7.5	6.90	7.30
7	8.0	5.60	5.80
CD		0.20	0.18

*DAI - days after inoculation

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