



Investigation of optimum conditions for the growth of *Fusarium solani* EGY1 causing root rot of guar (*Cyamopsis tetragonoloba* L.)

Simranjit Singh¹ and Upasana Rani², U.S. Tiwana², Devinder Pal Singh² and Asmita Sirari²

¹Department of Plant Pathology, Punjab Agricultural University, Ludhiana-141004 (Punjab), INDIA

²Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana-141004 (Punjab), INDIA

*Corresponding author. E-mail: simran.badhan27@gmail.com

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Abstract: Guar gum (Galactomannan) is extracted from *Guar* (Cluster bean), which is extensively used in petroleum, food and pharmaceutical industry. Root rot of guaris caused by *Fusarium solani* EGY1 under Punjab, having sub-tropical climatic conditions. This study was undertaken to evaluate different culture media, grain substrates (sorghum, maize, cowpea, guar and pearl millet), temperatures (20, 25, 30, 35°C), pH levels (5.0, 6.0, 7.0, 8.0), light and darkness for the identification of optimum conditions for the growth and sporulation of the fungus. Czapek's dox media was found to be best for growth (84.65 mm) and sporulation (1.8×10^4 microconidia and 3.0×10^4 macroconidia) of fungus. For mass multiplication of the fungus, sorghum grains proved to be the best substrate. The fungus showed maximum radial growth at temperature of 25°C (84.36 mm) and pH of 6.0 (84.43 mm) whereas sporulation was highest at 30°C (2.0×10^4 microconidia and 3.2×10^4 macroconidia) and pH of 8.0 (1.8×10^4 microconidia and 3.1×10^4 macroconidia) respectively. Continuous light favoured radial growth (84.62 mm) whereas sporulation (1.8×10^4 microconidia and 3.1×10^4 macroconidia) was favoured by darkness.

Keywords: *Fusarium solani* EGY1, Light, Media, pH, Temperature

INTRODUCTION

Cluster bean [*Cyamopsis tetragonoloba* (L.) Taub.], a legume commonly known as *guar*, is rich in nutrients with crude protein content and total digestible nutrients on dry matter basis of 18.1 and 60.0 per cent respectively. It is widely used for fodder and vegetable purposes (Ayub *et al.*, 2011). It has an economic importance in international market as its important component glactomannan known as guar gum is extensively used in petroleum, food and pharmaceutical industry (Sortino and Greata, 2007). Area under cluster bean in India was 5.6 million ha with production and productivity of 2.7 million tonnes and 485 kg per ha, respectively during 2013-14. Rajasthan is the major cluster bean growing states of India. Punjab and Haryana also contributes significantly towards the production of cluster bean (Anonymous, 2014). Although, cluster bean being a hardy crop is very sensitive to biotic and abiotic stresses. The crop succumbs to number of diseases caused by fungi, bacteria and viruses. Of all the diseases, pathogen associated with root rot of guar is identified and confirmed as *Fusarium solani* strain EGY 1 by Singh *et al.*, (2016). It causes heavy losses in summer sown crop and damage is visible even on young plants. Attacks of the *Fusarium* wilt pathogen can destroy the crop completely and can cause significant annual yield losses (Pareek and Varma, 2015).

Under Punjab conditions, this disease is associated with *Fusarium solani* EGY1. Present work depicts the role of different pH, temperature, substrate and alternative light and darkness to understand ecological survival of pathogen which will be helpful in the management strategy and for further laboratory evaluation studies.

MATERIALS AND METHODS

Isolation, purification and identification of pathogen: Plants of guar (*Cyamopsis tetragonoloba* L.) showing symptoms of root rot were collected from guar fields of PAU, Ludhiana. The samples for isolation were made free from dirt, cut into small bits and surface sterilized with mercuric chloride (HgCl_2) (Singh *et al.*, 2016). The culture isolated on PDA was purified by single spore isolation (Choi *et al.*, 1999) and identified on the basis of cultural, morphological and molecular characteristics. The isolated pathogen was used for further laboratory studies.

Effect of different cultural media on growth and sporulation of pathogen: Five nutrient media *viz.* (I) potato dextrose agar [PDA] (peeled potatoes 200.0g, dextrose 20.0g, agar 20.0g, distilled water 1000 ml and pH 5.6 ± 0.2); (II) oat meal agar [OMA] (Oat meal 30.0g, agar 20.0g, distilled water 1000.0 ml, pH 6.5 ± 0.03); (III) guar leaf extract agar [GLEA] (Guar

leaves 1000.0g, agar 20.0g, distilled water 1000.0 ml, pH 7.1±0.02); (IV) Czapek's dox agar [CDA] (Sodium nitrate (NaNO₃) 2.0g, Dipotassium hydrogen phosphate [DHP](K₂HPO₄) 1.0g, Magnesium sulphate (MgSO₄.7H₂O) 0.50g, Ferrous sulphate (FeSO₄.7 H₂O) 0.01g, Sucrose (C₁₂ H₂₂O₁₁) 30.0 g, agar 20.0g, distilled water 1000 ml, pH7.3±0.02); (V) Richard's agar medium [RA](Potassium nitrate (KNO₃) 10.0g, Potassium monobasic phosphate (KH₂PO₄) 5.0g, Magnesium sulphate (MgSO₄.7H₂O) 2.5 g, Ferric chloride (FeCl₃ 6H₂O) 0.02g, Sucrose (C₁₂H₂₂O₁₁)50.0g, agar 15.0g, distilled water 1000 ml, pH 6.8±0.03) were tested for growth of pathogen under laboratory conditions at 25±1°C. The contents of PDA, OMA and GLEA were boiled in water and filtered through muslin cloth. For the preparation of CDA and RA, sucrose was steam sterilized in an autoclave separately. The agars along with other constituents of the media were dissolved in water and autoclaved separately. The sterilized sugar solution was added to the media aseptically. The agar was added to the extract after filtering through double layered muslin cloth. Final volume of all nutrient medium was made up to 1.0 liter and sterilized. The sterilized media was poured in Petri plates, inoculated with actively growing bit of the fungus and incubated at 25±1°C.

Effect of different substrates on growth and sporulation of pathogen: Five different substrates i.e. grains of sorghum, maize, pearl millet, cowpea and guar were tested for mass culturing of the fungi for further studies. One hundred gram of each substrate was soaked in water overnight in 250 ml Erlenmeyer flasks. The excess water which is left after maximum absorption by different grain substrate was drained off and the flasks containing soaked grains were plugged and autoclaved at 15 psi pressure for 30 minutes for three consecutive days. Four flasks of each substrate were used as replicates. The substrate in flasks was inoculated with actively growing 5 mm mycelial disc of the pathogen under aseptic conditions and inoculated flasks were incubated at 25±1°C in BOD incubator.

Effect of different temperatures on growth and sporulation of pathogen: The growth of the pathogen was determined at minimum, optimum and maximum range of temperature. For this purpose 20 ml of optimized medium was poured into the Petri plates. The Petri plates were inoculated with 5 mm mycelial discs of inoculum cut with the help of sterilized cork borer from 8 days old culture of pathogen raised on PDA. The inoculated Petri dishes were incubated for 8 days at different temperatures *viz.*, 20, 25, 30 and 35°C maintained in separate BOD incubators.

Effect of different pH levels on growth and sporulation of pathogen: To determine the optimum level of pH for growth of the associated pathogen, 100 ml of the optimized medium was dispensed in Erlenmeyer flasks of 250 ml capacity, and the different pH levels of 5, 6,

7 and 8 were adjusted by adding 0.1 NHCl or NaOH solutions and measured by digital pH meter (ELTOP 3030). The flasks were autoclaved and sterilized Petri plates with nutrient medium were inoculated with 5 mm mycelial discs of inoculum cuts with the help of sterilized cork borer from 8 days old culture raised on the medium. The inoculated Petri dishes were maintained under similar laboratory conditions.

Effect of light and darkness on growth and sporulation of pathogen: To determine effect of light, darkness and alternate light and darkness cycle of 12 hours each on growth of the pathogen associated with wilt, 100 ml of the best medium was dispensed in Erlenmeyer flasks of 250 ml capacity. The flasks were plugged and sterilized in an autoclave at 15 psi pressure for 15 minutes. After autoclaving, the lukewarm media was poured in Petri plates under aseptic conditions and the Petri plates were inoculated with 5 mm mycelial discs of inoculum cut with the help of sterilized cork borer from 8 days old culture of the pathogen raised on the medium. The inoculated Petri dishes were incubated at 25±1°C maintained in BOD incubator.

Observations recorded and statistical analysis: The observations on radial growth (mm), sporulation (number of spores ml⁻¹) and colony colour of the pathogen were recorded continuously after every 24 hours of incubation for 8 days. The colour and quantity of actively growing mycelium was categorized on the basis of visual observations (Kornerup and Wancher, 1978). Each treatment was replicated four times. The sporulation was measured with haemocytometer after 8 days of incubation. Spore suspension was obtained by eluting 5 mycelial discs of 5 mm size in 5 ml of sterilized distilled water in test tubes. Three tubes were used for each treatment. The tubes were thoroughly shaken for two to three minutes and the eluted spores were counted with the help of haemocytometer. The number of spores per ml was calculated with a haemocytometer, using the formula given by Pathak (1984).

$$\text{No. of spores per ml} = \frac{N \times 1000}{X}$$

where:

N = Total No. of spores counted/No. of squares,

X = Volume of mounting solution between the cover glass and above the squares counted.

For each of the above experiments LSD was calculated at 5 per cent level of significance.

RESULTS AND DISCUSSION

Identification of the pathogen: On the basis of cultural, morphological and molecular characterization, the pathogen was identified as *Fusarium solani* strain EGY 1 (Singh *et al.*, 2016).

Effect of different media on growth and sporulation of the pathogen: The results revealed that EGY 1 strain of *Fusarium solani* showed good growth on all

Table 1. Effect of different culture media on mycelial growth, colour and sporulation of *Fusarium solani* EGY 1.

Culture media	Radial growth of mycelium in mm (days)									Colony Colour	Sporulation (conidia ml ⁻¹)	
	1 st	2 nd	3 rd	4 th	5 th	6 th	7 th	8 th	Mean		Micro	Macro
Potato dextrose agar	8.30	21.00	30.12	43.87	53.75	60.62	69.75	73.52	45.12	Creamy white	1.5 x 10 ⁴	3.1 x 10 ⁴
Richard's agar	7.40	13.50	27.12	37.37	50.31	61.31	72.00	75.80	43.10	Pale yellow	1.1 x 10 ⁴	2.5 x 10 ⁴
Czapek's dox agar	7.80	20.40	32.50	48.12	62.00	71.25	80.87	84.65	50.94	Cottony white	1.8 x 10 ⁴	3.0 x 10 ⁴
Guar leaf extract	8.20	18.70	30.62	42.18	56.62	65.12	66.93	70.70	44.89	Creamy white	6.0 x 10 ³	1.9 x 10 ⁴
Oat meal agar	8.00	18.50	31.18	42.43	56.62	65.62	73.12	79.00	46.56	Creamy white	1.6 x 10 ⁴	2.7 x 10 ⁴
Mean	7.93	18.41	30.36	42.80	55.86	64.78	72.53	76.26				

LSD (p= 0.05), Culture medias = 0.26, Intervals (Days) = 0.33, Culture medias x Intervals (Days) = 0.73

Table 2. Effect of different substrates on mycelia growth, colour and sporulation of *Fusarium solani* EGY 1.

Substrates (grains)	Mycelial growth	Mycelial colour	Sporulation after 15 days of incubation (conidia ml ⁻¹)	
			Micro	Macro
Maize	Moderate	Creamy white	1.7 x 10 ⁵	2.9 x 10 ⁵
Pearl millet	Abundant	Creamy white	1.9 x 10 ⁵	3.2 x 10 ⁵
Cowpea	Abundant	Cottony white	2.0 x 10 ⁵	3.0 x 10 ⁵
Guar	Scanty	Light brown	8.2 x 10 ⁴	1.9 x 10 ⁴
Sorghum	Abundant	Cottony white	2.1 x 10 ⁵	3.4 x 10 ⁵

Table 3. Effect of different temperatures on mycelial growth, colony colour and sporulation of *Fusarium solani* EGY 1.

Temperatures (°C)	Radial growth of mycelium in mm (days)									Colony Colour	Sporulation (conidia ml ⁻¹)	
	1 st	2 nd	3 rd	4 th	5 th	6 th	7 th	8 th	Mean		Micro	Macro
20	5.00	8.09	15.77	26.34	35.26	42.96	48.72	53.42	29.44	Cottony white	1.5 x 10 ³	3.6 x 10 ³
25	7.90	20.62	32.93	48.31	62.06	71.62	81.00	84.36	51.10	Cottony white	1.8 x 10 ⁴	3.1 x 10 ⁴
30	7.75	20.12	32.00	47.12	61.43	70.62	80.00	82.81	50.23	Cottony white	2.0 x 10 ⁴	3.2 x 10 ⁴
35	6.98	17.06	27.68	41.31	56.58	65.46	73.84	76.59	45.69	Creamy white	8.2 x 10 ³	2.2 x 10 ⁴
Mean	6.91	16.48	27.10	40.77	53.84	62.67	70.89	74.30				

CD (p= 0.05), Temperatures = 0.17, Intervals (Days)= 0.25, Temperatures x Intervals (Days)= 0.51

Table 4. Effect of different pH levels on mycelial growth, colour and sporulation of *Fusarium solani* EGY 1.

pH levels	Radial growth of mycelium in mm (days)									Colony colour	Sporulation (conidia ml ⁻¹)	
	1 st	2 nd	3 rd	4 th	5 th	6 th	7 th	8 th	Mean		Micro	Macro
5.0	7.09	19.62	31.68	46.12	60.62	69.56	79.18	82.37	49.27	Creamy white	1.3 x 10 ⁴	2.7 x 10 ⁴
6.0	7.90	20.62	32.93	48.31	62.06	71.62	81.00	84.43	51.11	Cottony white	1.7 x 10 ⁴	3.0 x 10 ⁴
7.0	7.09	19.31	31.31	46.12	60.43	69.06	78.75	82.06	50.80	Creamy white	1.6 x 10 ⁴	2.9 x 10 ⁴
8.0	7.90	20.37	32.62	47.87	61.75	71.37	80.56	83.93	49.54	Cottony white	1.8 x 10 ⁴	3.1 x 10 ⁴
Mean	7.50	19.98	32.14	47.11	61.22	70.41	79.88	83.20				

CD (p= 0.05), pH levels = 0.18, Intervals (Days) = 0.25, pH levels x Intervals (Days) = 0.51

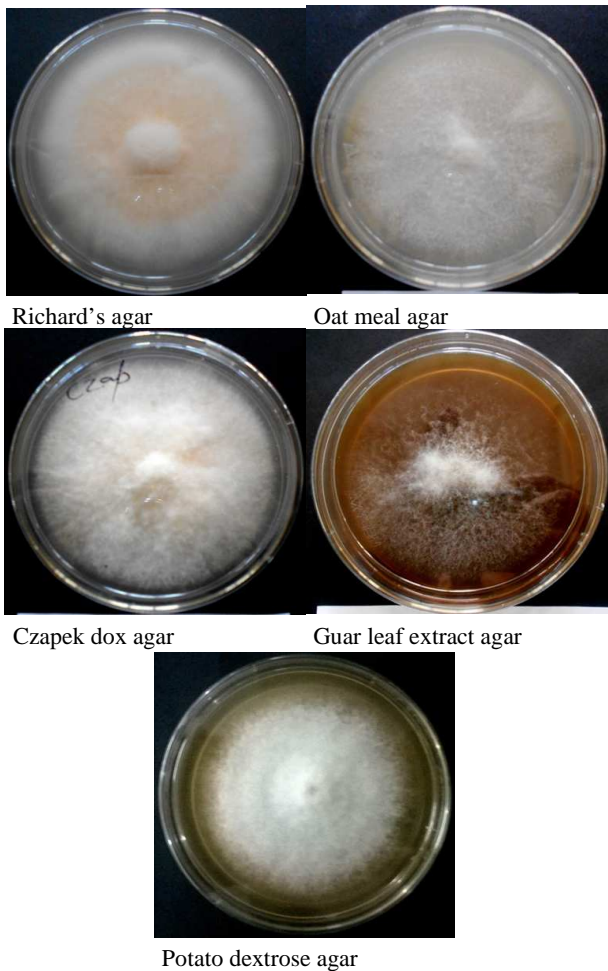
Table 5. Effect of light and darkness on mycelial growth, colony colour and sporulation of *Fusarium solani* EGY 1.

Conditions	Radial growth of mycelium in mm (days)									Colony Colour	Sporulation (conidia ml ⁻¹)	
	1 st	2 nd	3 rd	4 th	5 th	6 th	7 th	8 th	Mean		Micro	Macro
Darkness	7.75	20.37	32.75	48.12	62.00	71.25	80.87	84.31	50.93	Creamy white	1.8 x 10 ⁴	3.1 x 10 ⁴
Light	7.75	21.50	33.56	49.50	62.62	72.31	81.81	84.62	51.71	Creamy white	1.7 x 10 ⁴	2.9 x 10 ⁴
Alternating (12 hrs each)	7.09	19.62	31.21	45.12	60.52	69.56	79.77	82.36	49.53	Creamy white	1.5 x 10 ⁴	2.7 x 10 ⁴
Mean	7.53	20.50	32.66	47.91	61.75	71.04	80.62	83.77				

CD (p= 0.05), Conditions = 0.21, Intervals (Days) = 0.33, Conditions x Intervals (Days) = 0.58

the tested media (Plate 1). The mean radial growth varied from 43.10 ± 24.30 to 50.94 ± 26.71 mm on all the tested media (Table 1). The maximum vegetative growth of 84.65 mm was supported by Czapek's dox agar after 8th day of inoculation. This was followed by oat meal agar with radial growth of 79.00 mm and Richards agar with radial growth of 75.80 mm. Least

vegetative growth was observed on PDA and guar leaf extract with radial diameter of 73.52 and 70.70 mm respectively. All the tested media differed statistically and significantly among themselves, when tested on 5 per cent significant level. Based on visual observations, the colour of the fungal mycelium varied from cottony white on Czapek's dox agar, pale yellow on



Richard's agar

Oat meal agar

Czapek dox agar

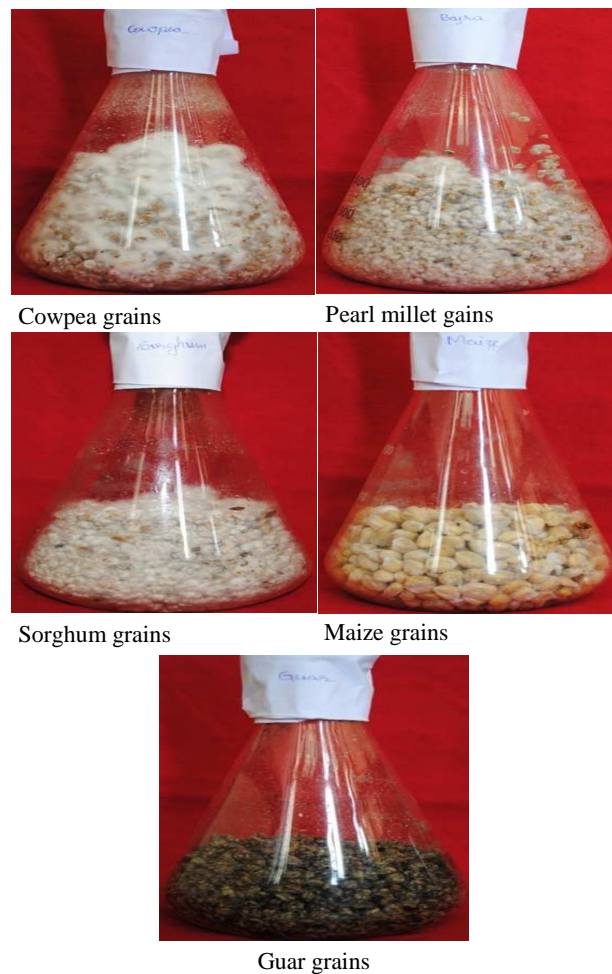
Guar leaf extract agar

Potato dextrose agar

Plate 1. Growth pattern of *Fusarium solani* EGY 1 on given five semi-solid media.

Richard's agar and creamy white on potato dextrose agar, guar leaf extract and oat meal agar respectively. Sporulation varied from 1.9×10^4 to 3.1×10^4 macroconidia ml^{-1} and 6.0×10^3 to 1.8×10^4 microconidia ml^{-1} on different tested media. All the media supported the production of both microconidia and macroconidia. Maximum sporulation was achieved on Czapek's dox agar medium with 1.8×10^4 microconidia ml^{-1} and 3.0×10^4 macroconidia ml^{-1} respectively and minimum sporulation was observed on guar leaf extract medium with 6.0×10^3 microconidia ml^{-1} and 1.9×10^4 macroconidia ml^{-1} respectively.

The nutrient contents in Czapek's Dox agar, oat meal agar and Richard's agar might be favourable for the growth and development of *Fusarium solani* EGY 1. Chittem and Kulkarni (2008) also observed that maximum radial growth of *F. oxysporum* f. sp. *gerberae* was on oat meal agar (90.00 mm) followed by Richard's agar (86.00 mm), Czapek's Dox agar (84.67 mm) and potato dextrose agar (83.67 mm). Sporulation was also abundant on Czapek's Dox agar and gerbera leaf extract media. Czapek's Dox agar and potato dextrose agar were also found the best medium by Khilare and



Cowpea grains

Pearl millet grains

Sorghum grains

Maize grains

Guar grains

Plate 2. Effect of different substrates on mycelial colour and growth of *Fusarium solani* EGY1.

Ahmed (2012) for growth of *F. oxysporum* f. sp. *ciceri*. **Effect of different substrates on growth and sporulation of pathogen:** The substrates viz., soaked grains of sorghum, pearl millet, maize, cowpea and guar were used to find out the best substrate for mass culturing of the fungus. The visual observations recorded after 15 days of incubation showed that colour of mycelium was fluffy white on sorghum and cowpea grains, suppressed creamy white on pearl millet and maize grains and light brown on guar grains (Plate 2). Abundant mycelial growth was found on sorghum, cowpea and pearl millet grains, whereas growth was moderate on maize grains and scanty on guar grains. The sporulation varied from 8.2×10^4 to 2.1×10^5 spores ml^{-1} and 1.9×10^4 to 3.4×10^5 spores ml^{-1} respectively in terms of both microconidia and macroconidia production. The maximum production of microconidia and macroconidia was found on sorghum grains with 2.1×10^5 and 3.4×10^5 spores ml^{-1} respectively. Sporulation on sorghum grains was followed by pearl millet grains (1.9×10^5 microconidia ml^{-1} and 3.2×10^5 macroconidia ml^{-1}), cowpea grains (2.0×10^5 microconidia ml^{-1} and 3.0×10^5 macroconidia ml^{-1}) and maize grains (1.7

$\times 10^5$ microconidia ml^{-1} and 2.9×10^5 macroconidia ml^{-1}). Least sporulation was observed on guar grains with microconidia production of 8.2×10^4 spores ml^{-1} and macroconidia production of 1.9×10^4 spores ml^{-1} respectively showed in Table 2.

Both the mycelial growth as well as sporulation was maximum on sorghum grains. The nutritional composition as well as size of the grains might be suitable for growth and sporulation of the fungus. Similarly Diarra *et al.* (1996) also observed that highest production of macroconidia occurred on sorghum glumes, whereas the highest microconidia production occurred on sorghum fibre. Sorghum grains were also used as nutritional substrate by Luckew *et al.* (2012) for mass culturing of *Fusarium virguliforme* causing sudden death syndrome in soybean.

Effect of different temperatures on growth and sporulation of the pathogen: It is evident from Table 3 that the mean radial growth of the fungus was found to be significantly different at all the tested temperatures i.e. 20, 25, 30 and 35°C on Czapek's dox agar medium as temperature has marked effect on growth of the fungus. The mean mycelial radial growth varied from 29.44 ± 17.36 mm at 20°C to 51.10 ± 26.63 mm at 25°C . The maximum radial growth of 84.36 mm was observed at 25°C followed by 82.81 mm at 30°C on 8th day of incubation as it is the optimum temperature range for growth of most of the mesophilic fungi. Least radial growth (53.42 mm) was recorded at 20°C whereas radial growth of 76.59 mm was found at 35°C . At low temperatures the growth is retarded due to low metabolic rate of the fungus whereas at high temperatures the metabolism increases which results in exhaustion of nutrients. The colour of mycelium was cottony white at 20°C and 30°C , whereas it was creamy white at 35°C .

It is evident from the data that the pathogen produced various extents of sporulation on Czapek dox agar at different temperatures of incubation. The highest sporulation was recorded at 30°C (2.0×10^4 microconidia ml^{-1} and 3.2×10^4 macroconidia ml^{-1}) followed by 25°C (1.8×10^4 microconidia ml^{-1} and 3.1×10^4 macroconidia ml^{-1}) and 35°C (8.2×10^3 microconidia ml^{-1} and 2.2×10^4 macroconidia ml^{-1}). Minimum sporulation (1.5×10^3 microconidia ml^{-1} and 3.6×10^3 macroconidia ml^{-1}) was observed at 20°C .

According to present studies maximum mycelial growth and sporulation was achieved at 25°C and 30°C respectively. Chaturvedi *et al.* (2003) also found that optimum temperature for growth and sporulation of *F. oxysporum* and *F. solani* was 25°C . Gangadhara *et al.* (2010) also observed that maximum growth of *Fusarium oxysporum* f.sp. *vanilla* after seven days of incubation was at 25°C which was drastically reduced below 15°C and no growth observed at 40°C , because low temperature inhibits the normal metabolic processes of the fungus. Similarly Khilare and Ahmed

(2012) observed that the growth and sporulation of *Fusarium oxysporum* f. sp. *ciceri* was maximum at 30°C (24.7 conidia μl^{-1}) after seven days of inoculation, which was reduced drastically below 15°C and above 35°C . Adeniyi *et al.* (2011) also reported that the optimum temperature for growth of *Fusarium* was 25°C . Similarly Merlin *et al.* (2013) and Kausar *et al.* (2009) also found that temperature of $25 \pm 2^\circ\text{C}$ supported the maximum growth of *F. solani*.

Effect of different pH levels on growth and sporulation of the pathogen: The investigations showed that in Table 4, there were significant differences among pH levels viz., 5.0, 6.0, 7.0 and 8.0 respectively and the mean radial growth of the pathogen raised on Czapek's dox agar. The mean radial growth of *Fusarium solani* EGY 1 varied from 49.27 ± 26.23 to 51.11 ± 26.64 mm. Increasing trend in radial growth was noticed from pH 5.0 to 6.0 and thereafter growth showed a decreasing trend from pH 7.0 to 8.0. Maximum radial growth of 84.43 mm with mean 51.11 ± 26.64 was recorded at pH 6.0 after 8th day of incubation. Minimum mean radial growth of 49.27 ± 26.23 mm was found at pH 5.0. At pH 7.0 to 8.0, mean radial growth of 50.80 ± 26.53 and 49.54 ± 26.14 mm was observed respectively. Colony colour also varied from cottony white to creamy white. Maximum sporulation was recorded at pH 8.0 in terms of production of 1.8×10^4 microconidia ml^{-1} and 3.1×10^4 macroconidia ml^{-1} followed by pH 6.0 (1.7×10^4 microconidia ml^{-1} and 3.0×10^4 macroconidia ml^{-1}) and pH 7.0 (1.6×10^4 microconidia ml^{-1} and 2.9×10^4 macroconidia ml^{-1}) respectively. Least sporulation (1.3×10^4 microconidia ml^{-1})

El-Sayed *et al.* (2008) observed that best growth of *F. oxysporum* f. sp. *Lycopersici* and *F. solani* was promoted at pH 8.0. Most suitable pH level according to Gangadhara *et al.* (2010) for growth of *F. oxysporum* f.sp. *vanillae* isolates was also 5.0 to 6.0. Gupta *et al.* (2010) also found that maximum mycelial mass production of *F. oxysporum* f.sp. *psidii* and *F. solani* isolates was 5.5 followed by 5.0 while maximum sporulation was obtained at pH 6.0 to 6.5 which indicated that *Fusarium* spp. favours acidic pH for its growth and sporulation.

Effect of light and darkness on growth and sporulation of the pathogen: Data presented in Table 5 revealed that statistical differences in mean radial growth of fungus *Fusarium solani* EGY were observed when grown under light, darkness and alternating conditions (Plate 9) on Czapek's dox agar. Maximum mean creamy white growth of 51.71 ± 26.72 mm was achieved under light conditions, whereas 50.93 ± 26.65 mm radial growth of fungus was observed when kept under dark condition. Alternating conditions of 12-12 hours light-dark cycle showed radial growth of 49.53 ± 22.41 mm. Data on sporulation also indicated that maximum sporulation in terms of microconidia (1.8×10^4 spores ml^{-1}) and macroconidia (3.1×10^4

spores ml⁻¹) production was achieved under dark conditions. Light conditions recorded sporulation of 1.7 x 10⁴ microconidia ml⁻¹ and 2.9 x 10⁴ macroconidia ml⁻¹ followed by alternating conditions with 1.5 x 10⁴ microconidia ml⁻¹ and 2.7 x 10⁴ macroconidia ml⁻¹ respectively.

The results revealed that maximum fungal growth and sporulation were observed under continuous light conditions. This might be due to more responsiveness of fungus under light conditions. Kausar *et al.* (2009) found that continuous light was more suitable for maximum growth of *Fusarium solani* with colony diameter of 16.67 mm when exposed for seven days. The colony diameter at continuous darkness was decreased to 11.5 mm, whereas it was 8.9 mm when exposed to alternate 12 hrs of light and 12 hrs of darkness. El-Sayed *et al.* (2008) also observed that continuous light induced the maximum growth in case of *F. oxysporum* f.sp. *Lycopersici* and *F. solani*. Continuous light and white light were also found ideal by Bhale (2012) for maximum radial growth and spore germination of *F. oxysporum* f. sp. *spinaciae*. The dark conditions were found to be favourable by Velmurugan *et al.* (2009) as incubation in total darkness increased the biomass, extracellular and intracellular pigment production in all the tested fungi including *F. verticilloides*. Adeniyi *et al.* (2011) found that alternating 12 hours light plus 12 hours darkness with colony diameter of 61.0 mm was more suitable for growth of *Fusarium* than continuous darkness with colony diameter of 33.5 mm.

Conclusion

Czapek's dox media was found to be best for growth and sporulation of fungus *F. solani* EGY 1. For mass multiplication of the fungus, sorghum grains were proved to be the best substrate. Slightly acidic pH of 6.0 at 25°C favoured the radial growth of the fungus under continuous light conditions, whereas sporulation was highest at slightly alkaline pH of 8.0 at 30°C in dark.

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