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Effect of physiological parameters on the growth of *fusarium sp.*

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Abstract
An *in vitro* experiment was conducted in UAS, GKVK Bengaluru to study the effect of various physiological parameters on the growth and development of *fusarium* isolate F-2 which is causing stalk rot of maize crop, especially in the southern state of karnataka. The results indicated that definite impact of physiological parameters like media, temperature and ph on the growth and development of *Fusarium sp.*

Keywords: physiological, parameters, growth, *fusarium*

Introduction
The genus *Fusarium* is one of the most economically important genera of fungi and includes many pathogenic species that cause a wide range of plant diseases (Nelson *et al.,* 1981) [1]. It also includes endophytic and saprophytic species found in association with plants in agricultural and natural ecosystems (Leslie, *et al.,* 2004a; Summerell *et al.,* 2003a) [2, 3].
The genus has widespread distribution and occur in all major regions of the world. Some species have a cosmopolitan geographic distribution whereas others tend to occur predominantly in tropical and subtropical regions or cool to warm temperate regions. Many *Fusarium* species are particularly common in soil and persist as chlamydospores or as hyphae in plant residues and organic matter. Several species produce airborne conidia and are common colonisers of stems, leaves and floral parts (Burgess, 1981) [4].
The maximum radial growth of *F. solani* was observed in Potato Dextrose Agar (90.00 mm) which was followed by oat meal agar (69.66 mm), least growth was observed in corn meal agar (6.33 mm) (Raghu, 2014) [5].
Potato Dextrose Agar Medium was the best medium for linear and amount of growth for all tested isolates of *Fusarium oxysporum* F. sp. *cucumerinum*, whereas the rate of growth varied between the different tested isolates with the different tested media (Tuwaijr, 2015a) [6].
Nirmaladevi and Srinivas (2012a) studied the cultural variability among 114 isolates of *F. oxysporum* F. sp. *lycopersici* causing wilt of tomato. The colour and pigmentation of the isolates on PDA medium varied between white, creamish white to cream, light pink to pink and light purple to violet. On the basis of the mycelial growth pattern, the isolates could be categorized into two groups *i.e.,* fluffy growth and adherent smooth growth. Most of the isolates showed fluffy growth while other isolates revealed adherent growth on the medium.

Temperature
Among the temperature levels 30°C was the common optimum temperature for the growth of *Fusarium oxysporum, Fusarium ciceri* which was followed by 25°C. Temperature below 25 °C and above 30 °C reduced the growth of fungus drastically (Khan *et al.,* 2011a) [8, 18].
Khilare and Ahmed (2012) [9, 17] studied the effect of different culture media, pH and temperature levels on mycelial growth of *F. oxysporum, Fusarium ciceri*. The maximum growth was recorded at 30 °C was found effective and least in 15°C and above 35 °C.
Optimum temperature for the fungal linear growth was 27 °C, followed by 28 °C, while the best amount of growth was obtained at 25 °C for all tested isolates of *Fusarium oxysporum, Fusarium cucumerinum* (Tuwaijr, 2015c) [6].

PH
Bai *et al.* (1988c) [10] after studying several *Fusarium* species isolated from maize plants in northern china said that the optimum pH for growth was 6.7 to 7.5 with a range of 4.3 to 9.8

along with fructose, insulin, xylose and glucose as carbon sources. Desai (1992a) working with four isolates of *F. oxysporum, Fusarium* sp. *ciceri* recorded maximum growth of all its four races at pH 6.

Jadhav *et al.* (2000) [11] reported that the fungus *F. chlamydosporum* recorded maximum growth at pH 6.5 which was followed by pH 6.0 and 5.5. Rawal *et al.* (2003) noticed that the pH 6.5 favoured maximum growth and sporulation of *Fusarium* sp. The most suitable pH level for growth of *F. oxysporum* was 7.0 and 6.0 (Farooq *et al.*, 2005b) [12].

Jaruhar and Prasad (2011) [13] reported that pH level 6.0 was found optimum for the growth as well as sporulation of the *F. oxysporum, Fusarium lentis.* Sporulation of chlamydospore was found best in the pH 4.0 further increase in pH level showed retarding effect on growth, sporulation and size of the spores increased with increase in pH range. The optimum pH for growth of the pathogen was ranged from 6.5 to 7.0 (Khan *et al.*, 2011b). The maximum growth of *Fusarium* species was observed in pH 8 after 8 days of incubation (Selvi and Sivakumar, 2013b) [14].

With all these findings a study was conducted in order to determine the ideal physiological conditions like media, temperature and ph for the growth and development of stalk rot causing pathogen *Fusarium sp.* under *in-vitro* condition.

Materials and Methods

In the present study after the detailed survey, isolated ten *Fusarium sp.* from different places were designated as given below and studied for variability.

Designation of different isolates of *Fusarium* sp. causing post flowering stalk rot of maize

Location	Code of the Isolate
Gauribidanur	F-1
Thondebhavi	F-2
Periyapatna	F-3
Channapatna	F-4
Channarayapatna	F-5
Tarikere	F-6
Kallalli	F-7
Gama	F-8
Hinduvalli	F-9
Hirekerur	F-10

Identification of the fungus

Among the ten pathogens isolated, F-2 isolate has shown highest disease incidence hence, selected for further work under *in-vitro*.

The pathogen F-2 was identified as *Fusarium solani* based on mycelial and conidial characteristics by sending the isolate to National Centre of Fungal Taxonomy.

Growth characters of *Fusarium solani* (F-2) on different solid media

The growth characters of F-2 was studied on eight different solid media *viz*., Corn meal, Host extract, Potato dextrose, Potato carrot, Oat meal, Richards' agar, Sabouraud maltose and V-8 juice agar media. All the media were sterilized at 1.1 kg/cm^2 pressure and 121.5 °C for 15 min. To carry out the study, 20 ml of each of the medium was poured in 90 mm Petri plates. Such plates were inoculated with five mm discs cut from periphery of actively growing culture and incubated at 28±2 °C. Each treatment was replicated thrice. Observations were taken when the fungus covered entire petridish in any one of the medium. The colony diameter was recorded by averaging mycelial growth in two directions for each plate. The fungus colony colour, type of growth and sporulation were also recorded. The data on radial growth was analyzed statistically.

Physiological studies

Effect of temperature on the growth of *Fusarium solani* (F-2)

Both PDA and PDB medium were used in this experiment. Petriplates and 150 ml flasks containing 30 ml sterilized media were inoculated with 5 mm mycelial discs taken from periphery of 7 days old pure culture grown on PDA in petriplate and incubated at different temperature levels *viz.*, 15, 20, 25, 30, 35, 40 and 45 °C. In each case, three replications were maintained. The colony diameter at each temperature level was recorded in solid media whereas in liquid media the dry mycelial weight was recorded as described earlier. The results were analyzed statistically.

Effect of hydrogen ion concentration (pH) on the growth of *Fusarium solani* (F-2)

The potato dextrose broth was used in this experiment; pH of the liquid media was adjusted by using 0.1 N alkali (NaOH) or 0.1 N acid (HCl). The reaction of the medium was adjusted to the desired pH by using dihydrogen phosphate citric acid buffer according to schedule of Vogel (1951). The pH of the medium used was 3 to 11. Each treatment was replicated three times. The 100 ml flasks each containing 30 ml medium of respective hydrogen ion concentration was sterilized at 1.1 kg/cm^2 pressure for 15 minutes. They were inoculated with *F. solani* and incubated. Dry mycelial weight was recorded and analyzed statistically.

Results and Discussion

Cultural variability

Growth characters of *Fusarium solani* (F-2) on different solid media

Every living being requires food for its growth and reproduction and the fungi are not an exception. Fungi derive the food from substrate upon which they grow. In order to culture the fungi artificially, it is necessary to supplement the medium with essential nutrients required for their growth. The cultural character's of *F. solani* was studied on different solid media to know the best media. The effect of different solid media on the growth of *Fusarium solani* was found significant.

Cultural characters of *F. solani* were studied in eight different solid media to identify the best medium. The radial growth of the pathogen was measured when the maximum growth was observed in any of the media. Observations on various cultural characters were recorded. The results are presented in Table 1.

Table 1: Growth characters of *Fusarium solani* (F-2) on different agar media at 7th day of incubation.

Media	Colony characters				Sporulation
	Radial growth (mm)	Type of growth	Margin of colony	Colour	
Corn meal agar	72.17	Submerged	Rough	White with pink margin	+

Host extract agar	75.50	Merged	Rough	Light grayish	++++
Oatmeal agar	83.00	Aerial	Smooth	Cottony white	++++
Potato carrot agar	74.15	Aerial	Smooth	Whitish	+
Potato dextrose agar	90.00	Aerial	Smooth	Light pink	++
Richards' agar	63.00	Raised	Smooth	Cottony white	+
Sabouraud maltose agar	65.33	Aerial	Smooth	Dark pink	+++
V-8 juice agar	76.33	Aerial	Smooth	Whitish	+
S.Em. ±	1.05				
CD at 1%	4.22				

Sporulation Conidia / microscopic field
++++ >40
+++ 30-40
++ 20-30
+ <20

Media had significant effect on the growth of the pathogen. Among the eight solid media tested, the maximum radial growth of *F. solani* was observed in Potato Dextrose Agar (90.00 mm) which was significantly superior to all other treatments. This was followed by Oat meal agar (83.00 mm), V-8 juice agar (76.33 mm) which was statistically on par with host extract dextrose agar (75.50 mm) and potato carrot agar (74.15 mm). The least radial growth was observed in Richards' agar (63.00 mm). Oat meal agar and host extract agar recorded the maximum sporulation (>40 conidia /microscopic field) this was followed by Sabouraud maltose agar (30 to 40 conidia /microscopic field) and minimum sporulation was recorded in corn meal agar, potato carrot agar and V-8 juice agar (<20 conidia/microscopic field).

PDA contains a simple sugar (dextrose) and Potato which contains huge amount of starch. PDA also contains some vitamins which are essential for the growth of fusarium. Both these factors are responsible for the luxurious growth of the fungus. The least growth was observed in Corn Meal Agar (6.33 mm). The poor growth may be attributed to the quality of sugar present in them. These investigations are in confirmatory with the earlier reports of Raghu (2014) [5] and Tuwaijr (2015) [6] both the scientists who recorded the maximum radial growth of *Fusarium oxyspourm* in Potato Dextrose Agar in their study.

Oat meal agar recorded the maximum sporulation (>40 Conidia /microscopic field), moderately low sporulation (20 to 30 Conidia/microscopic field) was recorded in PDA and minimum sporulation was observed in Corn Meal Agar, Potato Carrot Agar and V-8 juice agar (<20 Conidia/microscopic field). These investigations are in contrary to the earlier findings of Saheb *et al.* (1987) in *F. oxysporum*, *Fusarium* sp. *lupine* who observed maximum sporulation in PDA.

Physiological studies

Effect of temperature on growth of *F. solani* on PDA

Among the environmental factors, temperature plays an important role on growth and reproduction of fungi. Each fungus has its own optimum temperature requirement. In the present study, an attempt was made to study the effect of different incubation temperature on the growth of maize stalk rot pathogen both on Potato Dextrose Agar and Potato Dextrose Broth.

The observations on the effect of different temperature on the radial growth of

F. solani were found to be statistically significant. The maximum radial growth (90.00 mm) of *F. solani* was recorded at 20 °C which was on par with 25 °C and 30 °C and significantly superior to all other treatments. No radial growth was recorded at 40 and 45 °C (Table 1). Similarly, Khilare and Ahmed (2012) [9, 17] observed that the maximum growth of *F. oxysporum* and *Fusarium* sp. *ciceri* at 30 °C and less was observed at 15, 35°C in both Czapek (Dox) agar and PDA (Fig. 3).

Effect of temperature on dry mycelial weight of *F. solani*

Table 2 indicated that temperature had significant role on the dry mycelial weight of *F. solani*. The maximum dry mycelial weight (439.67 mg) was recorded at 25 °C which was significantly superior to all other treatments. There was no growth at 40 and 45 °C.

Table 2: Effect of temperature on growth of *Fusarium solani* (F-2) in Potato Dextrose Agar and Potato Dextrose Broth

Temperature (°C)	Radial growth (mm) PDA	Dry mycelial weight (mg) PDB
15	70.00 (8.47)*	185.67
20	90.00 (9.54)	375.33
25	90.00 (9.54)	439.67
30	90.00 (9.54)	389.67
35	73.22 (8.61)	281.67
40	0.00 (1.00)	-
45	0.00 (1.00)	-
S.Em. ±	0.02	0.68
CD at 1%	0.10	2.86

* $\sqrt{X+1}$ transformed values

These results are in agreement with the findings of Khan *et al.* (2011) in *Fusarium oxysporum* and *Fusarium.* sp. *ciceri.* Temperature of 25 to 30 °C was optimum for the growth of pathogen where all the metabolic activities will trigger and maximum growth can be achieved. In both liquid and solid media, pathogen growth was excellent at this temperature range.

Effect of hydrogen ion concentration (pH) on the growth of *F. solani* (F-2)

Different fungal pathogens require a particular hydrogen ion concentration (pH) for their growth and development. The pH of the media changes the growth rate of the different pathogens. In the present investigation an effort was made to know the optimum hydrogen ion concentration required for the *Fusarium solani*. The results revealed that the effect of different pH levels on the growth of the *F. solani* was found significant (Table 3).

Table 3: Effect of hydrogen ion concentration (pH) on the growth of *F. solani* (F-2) in PDB

pH	Dry mycelial weight (mg)	Sporulation
3	221.33	(+)*
4	396.33	(+)
5	476.00	(+++)
6	497.33	(++++)
7	490.67	(+++)
8	448.33	(+)
9	399.33	(+)
10	354.00	(+)
11	178.33	(+)
S.Em.±	0.80	
CD at 1%	3.26	

* Sporulation	Conidia /microscopic field
++++	>40
+++	30-40
++	20-30
+	<20

Significantly maximum dry mycelial weight of *F. solani* was observed at pH 6.0 (497.33 mg) and which was superior to all other treatments. Followed by pH 7 (490.67 mg) and the minimum dry mycelial weight was recorded at pH 11 (178.33 mg). The decrease in dry mycelial weight was recorded above pH 8. Maximum sporulation was observed at pH 6 and was followed by pH 7 and 5. Both lower and higher pH reduced the sporulation. These findings are in conformity with the findings of Jaruhar and Prasad (2011) [13] in lentil wilt caused by *F. oxysporum* and *Fusarium* sp. *lentis.*

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