International Journal of Basic and Applied Biology (IJBAB)
Print ISSN: 2349 – 5820; Online ISSN: 2349 – 5839 ; Volume 2, Number 1; October, 2014 pp. 103 – 106

http://www.krishisanskriti.org/ijbab.html

Effect of different pH on the growth and sporulation of Fusarium oxysporum: The causal organism of wilt disease of Tomato

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Abstract: *In-vitro* studies were conducted to check the effect of pH on the growth and sporulation of *Fusarium oxysporum.* After two weeks incubation of culture in Potato dextrose broth, It was observed that pH level 6.0 is the optimum pH for the growth as well as sporulation of the fungus. However chlamydospore sporulation was found best in the pH level 4.5. Further increases in the pH level showed retarding effect on growth and sporulation.

1. INTRODUCTION

The genus *Fusarium* comprises a diverse array of fungi, members of which are phytopathogenic to a wide range of plants under diverse environmental conditions. Phytopathogenic *Fusarium* fungi cause several diseases of small-grain cereals, including seedling blight and foot rot, fusarium head blight (FHB) (also known as 'scab' or ear blight) and ear rot of maize (Parry et al., 1995). The *Fusarium* species *Fusarium graminearum* (teleomorph *Gibberella zeae*), *F. culmorum*, *F. poae*, *F. avenaceum* (teleomorph *G. avenacea*) and *Microdochium nivale* (formerly known as *Fusarium nivale*, teleomorph *Monographella nivalis*) are common pathogens.

F. oxysporum f.sp. *ciceri* is a soil borne root pathogen colonizing xylem vessels, blocking them and causing wilting (Bateman *et al* .,1996) and causes a serious wilt disease in tomato which is an important crop. The extent of the damage to the crop due to the disease ranges from 20-24% annually, (Saxena and Johansen, 1990 and Ali, 2007). Studies indicate that *Fusarium spp* grow at different pH levels for growth and sporulation (Souramma and Singh, 2004; Groenewald, 2005). The pathogen is soil born and infects the host during seedling stage through root and blocks the vascular system (Vasudeva and Srinivasan, 1952). The disease is important in dry and warm season. Nene and Reddy (1987) repoted that actual yield loss is about to 10 to 12 percent globally.

Fusarium wilt caused by *Fusarium oxysporum* alternate teleomorph, (*Gibberella fusikori)* is an important biotic constraint in tomato production in Indian subcontinent. In India alone, the loss due to this disease was estimated to be US $71 million per year and the percentage of disease incidence varies from 5.3 to 22.6%. Identification and detection of pathogenic *Fusarium* sp. was traditionally based on either symptom on the host or culture dependent isolation of the pathogen from affected host tissue. Hallsworth and Magan (1996) detailed the impact that changes in pH had effect on growth and accumulation of endogenous sugar alcohols and trehalose in conidia, with the aim of improving ecological competence.

Booth (1971) reported that *Fusarium oxysporum* produces three types of asexual spores: microconidia, macroconidia and chlamydospores. According to Messiaen and Cassini (1981), there is a good deal of variation in spore morphology within the species, even within a specialised form or race, with respect to shape and size of macroconidia and the proportion of microconidia to macroconidia. Certain isolates do not produce any macroconidia (Messiaen and Cassini 1981). *F. Oxysporum* exhibits varying cultural morphology on (potato dextrose agar) PDA (Smith *et. al.* 1988). The aerial mycelium first appears white and may then change to a variety of colors, ranging from violet to dark purple, according to the strain of *F. oxysporum.*

The objectives of the study were to better understand the effect of different pH condition as well as sporulation and morphological studies.

2. MATERIAL AND METHOD

2.1 Isolation, Screening and Identification

Fusarium oxysporum was isolated from Agricultural fields and identified according to Foschino *et al,* 2004, The stock culture was maintained on a Potato Dextrose Agar (PDA) slant. From the culture of the isolates, pure culture was obtained using 10^{-5} decimal level dilution plate technique. For inoculum preparation, the fungus was initially grown at 28°C on a PDA plate for 7 days. A 0.8 cm^2 plug from the outer zone of the colony was punched with a sterile well cutter and transferred to 100 mL Potato Dextrose Broth (PDB) medium in a 250 mL

Erlenmeyer flask and grown at 28°C under basal conditions (static) or on a rotary shaker at 200 rpm for 7 days.

2.2 Effect of different pH on the growth/sporulation of fungi;

There were 10 different pH level ranging from 2.0 to 6.5 with a difference of 0.5 were prepared by using pH meter by using either N/10 HCl or NaOH before autoclaving. 100 mL of the medium was taken in 250 mL conical flask and five replicate sets were used in each case. The solution was autoclaved at 15 psi for 15 minutes. The inoculation was done with 3 mL of the fungus broth culture from a margin of 12 days old colony growing in Potato Dextrose (PD) broth medium. Flasks were then inoculated at 26ºC ± 2ºC for two weeks and kept at 200 rpm on a rotary shaker. The growth as dried mycelium weight as well as sporulation was determined separately in the similar manner as mentioned above.

2.3 Dry mycelium weight

The culture broth was centrifuged at 14,000 rpm for 20 min and the supernatant fluid was filtered through a filter paper (Whatman No.1). The mycelial biomass yield was estimated by washing with deionized water and dried at 50°C for 48 h. The mean dry weight of the mycelium was determined as described by Prasad and Chaudhary (1966).

2.4 Spore count

The number of spores was counted using known depth of Haemocytometer slide (0.01 cm) using the formula: Where,
Number of spores / 100 mL = V/NX100
N = Average number of spores per square of the four corner square of haemocytometer counted.
V = Volume of haemocytometer (0.256 x10-5) cc
Length of the spores was measued by calibrated ocular micrometer under compound microscope (10 x 45 x of magnification).

3. RESULT AND DISCUSSION

Table 1: Effect of different pH on the growth and sporulation of *Fusarium oxysporum*

S. No	pH range	Dry wt. in mg.	Spores in millions /100mL medium*			Size of spores in µ*		
			Macro conidia	Micro conidia	Chlamydo spores	Macro conidia	Micro conidia	Chlamydo spores
1	2.0	27.00	0.47	2.42	0.39	18.20	6.40	3.89
2	2.5	43.00	0.61	3.43	2.58	26.70	6.80	4.90
3	3.0	48.88	1.31	4.61	3.04	28.86	10.08	6.18
4	3.5	63.00	1.42	6.42	4.20	28.90	8.90	6.38
5	4.0	84.00	1.61	8.37	4.71	30.20	9.86	7.82
6	4.5	98.00	1.98	11.81	6.03	30.80	9.80	8.50
7	5.0	128.00	2.12	13.68	3.16	34.20	11.70	6.50
8	5.5	163.00	2.43	49.40	1.73	36.30	12.88	8.20
9	6.0	206.00	4.32	84.03	1.19	34.20	14.90	8.30
10	6.5	240.00	5.06	122.4	1.51	38.10	18.18	8.50
11	7.0	180.0	2.43	116.01	0.32	24.81	6.62	4.88
12	7.5	132.00	0.41	88.08	0.10	18.22	4.12	2.06

3.1 Dry mycelium weight of the fungus at different pH

Mean of the dry mycelium weight of the fungus on different pH levels is calculated shown in Table 1. Results showed that *Fusarium oxysporum* grew maximum in pH 6.5.(240.00 mg)

At high acidic range of pH, fungi showed very poor growth of mycelium. Mycelial mat accumulation increased with increase in pH but declined after pH 6.0.(Jaruhar and Prasad,2011)

3.2 Effect of pH on sporulation

Maximum sporulation of the macroconidia and microconidia was observed at pH 6.5. (5.06 and 122.4 spore/100 mL of medium respectively) and minimum sporulation occurred in pH 2.0(0.47 and 2.42 spore/100 mL of medium respectively). However maximum production of Chlamydospores were observed at pH 4.5.(6.03 spore/100 mL of medium). Length of macroconidia and microconidia were the maximum at pH 6.5(38.10 and 18.18 respectivaly) and thereafter it started to decline. Diameter of chlamydospores increased with increase in pH level but after reaching to neutral pH it starts declining.

Influence of various pH levels on the growth and sporulation of many fungi have been studied under in vitro condition. Kishore *et al*. (2009); Souramma and Singh (2004); Groenewald (2005); also found pH 5.5 to 7.0 to be the best for growth and sporulation of *Fusarium oxysporum* f. sp. lini (Belley). Chaudhary (1971) and Prasad *et al*. (1992) reported 6.0 pH level as the best for the growth and sporulation of *Fusarium moniliforme v subglutinanse*. Mix (1933) found that pH range from 4 to 8 showed good growth for *Phyllosticta solitaria*. Wilson (1946) observed acid soil (pH 4.2) support growth of *Fusarium spp*. where as a pH near neutrality prevents growth. Srobar (1978) found pH 6 to be the most suitable for the growth of all species while a highly acidic medium was unsuitable for sporulation of all species causing fusarioses disease in wheat. Jat and Goyal (1978) found that growth and sporulation of *Claviceps microcephala* to be optimum at pH 7.5 and 6.0 respectively. This indicates that unusual acidity badly hampered the growth and sporulation of the fungus.

On the other hand sporulation of chlamydospores at more acidic range indicates that this spore is characteristically different from other two spores and more tolerable to stressed condition than the macro and micro conidia. (Jaruhar and Prasad,2011)

4. CONCLUSION

Fungi have the ability to produce a number of secondary metabolites, typically dependent on environmental factors ranging from nutrient concentrations to light, pH and temperature etc (Pradeep and Pradeep,2013). In this study, different pH strongly influenced the growth and sporulation by *Fusarium oxysporum*. Optimum temperature required for maximum production of spores and maximum biomass was pH 6.5.

5. ACKNOWLEDGEMENT

Thanks are due to Dr. Neelam Garg, Head of Department Microbiology, Kurukshetra University, Kurukshetra (Haryana) and special thanks to Dr. Anita Tyagi And Dr. Satya Deo Tyagi for providing their continuous guidance and support throughout the study.

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