J. Mycopathol, Res, 50(1) : 73-76 (2012)

Effect of culture media, temperature, pH and host range on the growth of *Fusarium oxysporum* f.sp.*pisi*

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Received : 11. 08 2011 Accepted : 12. 02. 2011 Published : 30.04.2012

Media, temperature, pH and host range showed significant role on growth and sporulation of *Fusarium oxysporum* f.sp. *pisi*. Out of six culture media, Sabourand Agar Emmons and Potato Dextrose Agar gave best response on the growth of the pathogen with 85.7 mm and 80.2 mm in diameter of colony growth, respectively. The most favourable temperature for growth of the pathogen was found in between 25°C -30°C. Similarly excellent sporulation occurred at pH 6.5, followed by 7.0. The poorest sporulation was found at pH below 5.0 and above pH 7.5. The host range study revealed that *F. o.* f. sp. *pisi* was able to infect pea, gram, lentil, lethyrus, tomato, linseed, sawflower, wheat, barley and mustard during *rabi* season.

Key words: Culture media, temperature, pH, host range, *Fusarium oxysporum* f.sp. *pisi*, pea.

INTRODUCTION

Pisum sativum (L.) Happer popularly known as pea with local name as "matar" is an important pulse as well as vegetable crop in India. The major pea growing states are Uttar Pradesh and Madhya Pradesh which together cover as much as 98.95 per cent of the total area of the country. Recently, there has been continuous increase in area of this crop in Madhya Pradesh, Rajasthan, Maharashtra and Uttar Pradesh (Anonymous, 2004). But the crop sufferes from a number of diseases caused by fungi, bacteria, nematodes, viruses etc. Among the fungal pathogens, Fusarium wilt of pea caused by *Fusarium oxysporum* f.sp. *pisi* (Linford) Synder & Hansen is one of the most important disease. The disease has assumed severe and destructive form from past few years in different ecological conditions. The pathogen shows prominent yellowing and wilting in the lower parts of plant which extend further upwards. The roots show red-brown in colour. The culture media, temperature, pH and host range are important parameters for growth of the pathogen because they affect spore germination, mycelial growth etc. It has also significant role in the distribution of *F.o.* f.sp. *pisi*. Keeping all the point in view, the present study has been undertaken.

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MATERIALS AND METHODS

Isolation of the pathogen

The pathogen was isolated from naturally infected pants collected from Vegetable Research Farm, Chadra Shekhar Azad University of Agricultural and Technology, Kanpur, Uttar Pradesh. The collected plant samples were first washed in tap water to remove dust particle and surface contaminant. Disease portion was then cut into small pieces along with some healthy portions with the help of sterilized blade. The cut pieces were surface sterilized with 0.1% Mercuric chloride ($HgCl_2$) solution under aseptic condition and washed thoroughly 3-4 times with sterilized water to remove the traces of $HgCl_2$. The excess moisture was removed by placing it in the fold of sterilized blotting paper. Then the pieces were transferred with the help of sterilized forceps in Petri-plate which was previously poured with sterilized (2%) PDA medium under laminar flow. The Petri-dish was then incubated at 25 ± 1°C in BOD. The fungus was purified by single spore isolation technique and identified on the basis of description as given by Rangaswamy (2008). Koch's postulates were established through pathogenecity test and the culture was maintained on P.D.A. The stock culture (*F. o.* f.sp. *pisi*) was subcultured after every fortnight and maintained on PDA for further study.

Effect of culture media on the growth of Fusarium oxysporum f. sp. pisi

To assess the role of culture media for the growth of test fungus (*F. o.* f. sp. *pisi*), six different commonly used media viz. Sabourand's Agar Emmons, Potato Dextrose Agar (PDA), King's, Starch agar, Malt extract agar and Rose Bengal agar were used under similar condition of temperature and pH. Exactly 20 ml of each medium was poured in 90 mm sterilized Petri plates and allowed in solidity. A disc of 0.5 mm diameter from actively growing culture of *F. o.* f.sp. *pisi* was placed at the centre of Petri plate. The Petri plates were then incubated at 25 ± 1°C in the BOD. The colony diameter was measured after 7 days of inoculation.

Effect of temperature on growth of Fusarium oxysporum f. sp. pisi

The effect of temperature on linear hyphal growth of *F. o.* f.sp. *pisi* was studied on PDA in 9 cm diameter Petri plates. All the Petri plates were then incubated at nine different temperatures level *viz.* 10°, 15°, 20°, 25°, 30°, 35°, 40°, 45° and 50°C and replicated thricely. The radial growth and sporulation of the test fungus were recorded at seven days of inoculation.

Effect of pH on growth of Fusarium oxysporum f. sp. pisi

The Potato Dextrose Broth (PDB) media with the set of different pH values (from 3.0 to 8.0) was prepared and pH was adjusted by adding appropriate amount of N/10 NaOH or N/10 HCl solution, determination being made with precise pH indicator paper. A small mycelial bit from actively growing culture of *F. o.* f.sp. *pisi* was then placed in conical flask, containing PDB of different pH values. The flasks were then inoculated in BOD incubator at 25 °C ± 1 for 10 days. After 10 days, the mycelial mat was harvested from each flask by collecting the culture filtrate through sterilized filter paper. Three replications were kept for each treatment. The harvested mycelium was kept in hot air oven at 80° C for 48 hrs and weight was measured in milligram.

Host range study

Host range was tested on 10 crops belonging to six different families, namely, Leguminasae (pea, gram, lentil and lathyrus), Poaceae (wheat and barley), Solanaceae (tomato), Linaceae (linseed), Asteraceae (sawflower) and Crucifereae (mustard). Loam soil, which was previously disinfected with 5% solution of formalin, was filled in earthen pots. The inoculums were prepared by growing pure culture of *F. o.* f. sp. *pisi* on sand corn meal for 10 days and inoculation of the soil was done 7 days before sowing of seed by mixing the soil with fungus culture in pots. Control pots were filled with soil without adding inoculums. The healthy and surface sterilized seeds from different crops belonging to different family @ 20 seed per pots were sown in 30 cm diameter earthen pots. The pots were then transferred in glass house and irrigated regularly to maintain sufficient moisture. Triplicate pots were used for each treatment. The pots were kept in glass house and observed critically for seedling emergence and appearance of symptoms on seedling and adult plants up to 60 days of sowing. The percentage of pre-emergence and post-emergence infection was calculated from the number of seeds that failed to emerge and the number of wilted plant, respectively by using following formula:

$$\text{Wilt incidence \% (WI)} = \frac{\text{Number wilted plant/pot}}{\text{Total plant population/pot}}$$

RESULTS AND DISCUSSION

Effect of media on the growth of Fusarium oxysporum f. sp. pisi

The data presented in Table 1 revealed that the radial growth of pathogen was variable on different media. The growth of the fungus was significantly higher on Sobourand's agar Emmons (85.70 mm) followed by PDA (80.20 mm) and Kings agar (75.6 mm) media. The Rose Bengal agar medium supported minimum growth (16.60 mm) of the pathogen. The mycelium was white and profusely branched on all the media. However, hyphal diameter of the pathogen slightly varied in different media. It was also found that sporulation was not found on any of the media. From the table, it was cleared that natural media used during present study were better than synthetic one for the mycelial growth of the pathogen. The present finding is supported by Chaudhery and Singh (2008). They also found that Sobourand's agar was found to be good medium for supporting growth of many species *F.o.* f.sp. *pisi* (De and Dwivedi 2003). They

Table1: Average radial growth and sporulation *Fusarium oxysporum* f. sp. *pisi,* on different culture media

Solid media	Average colony Diameter (mm)	Category	Nature of colony and sporulation
Sabourand's Agar Emmons	85.7	Excellent	White to buff colour colony,compact, velvety growth, profuse sporulation.
P D A Medium	80.2	Excellent	White to buff colour colony, compact, heavy sporulation reverse colours.
King's Medium	75.6	Good	White to light pink colony growth, cottony and heavy sporulation.
Starch Agar Medium	69.1	Good	White, cottony thin reverse
Malt extract Agar medium	35.2	Poor	White, thin, flaky growth restricted reveres white.
Rose Bengal Agar	16.6	Poor	Light pink, compact and heavy sporulation
CD at 5%	4.925		
SE	2.26		

found that growth of *F. o.* f.sp. *pisi* was dependent on abiotic factors.

Effect of temperature on growth of Fusarium oxysporum f. sp. *pisi*

From the Table 2 it was found that the growth of pathogen was least at 10°C but increased significantly with increase in temperature up to 30°C but after it was decreased gradually. The maximum dry weight was obtained at 30°C with 255 mg followed by 243, 217, 185, mg at 25°, 35°, and 20°C, respectively. On the other hand, the excellent sporulation was obtained at 25°C and 30°C. The sporulation was poor at 10°C, 40°C and 45°C. Sharma, *et al.* (2005) reported that the optimum temperature for growth and sporulation of *F. o.* f. sp. *lini* was between 20°C to 25°C.

Table 2 : Mycelium growth of *Fusarium oxysporum* f.sp. *pisi* at different temperature

Temperature (°C)	Dry weight of mycelium (mg)	Sporulation
10	80	Poor
15	120	Fair
20	185	Good
25	243	Excellent
30	255	Excellent
35	217	Good
40	105	Poor
45	24	Poor
50	00	....
CD (5%)	17.79	
SE	8.46	

Effect of pH on growth of Fusarium oxysporum f. sp. *pisi.*

From the Table 3 it was found that the excellent sporulation occurred at pH 6.5 and 7.5, while good at pH 7.5 and 5.5, and fair sporulation were occurred at 5.0 pH level. The lower pH value between

Table 3 : Mycelium growth of *Fusarium oxysporum* f.sp. *pisi* at different pH.

pH	Dry weight of mycelium (mg)	Sporulation
3.0	108.33	Poor
4.0	116.00	Poor
4.5	137.00	Poor
5.0	161.67	Fair
5.5	197.00	Good
6.5	236.67	Excellent
7.0	223.00	Excellent
7.5	176.00	Good
8.0	117.00	Poor
CD (5%)	15.64	....
SE	7.44	

3.0-4.5 and higher pH 8.0 gave least sporulation. It indicated that the fungus grew best on neutral pH (7.0). Sharma *et al.* (2005) also reported that the *F.o.* f. sp. *lini* gave best myceilial growth and sporulation in pH ranges from 5.5 to 6.5.

Host range study

The data presented in Table 4 showed that the pea plant gave superior germination percentage with

Table 4 : Host range study against *Fusarium oxysporum* f. sp. *pisi*

Host Plant	Wilted Plant	% in wilted (Germination)	%wilted all plant sowing
Pea	12	70.58	60.00
Gram	04	23.53	20.00
Lentil	03	17.64	15.00
Lethyrus	02	11.11	10.00
Tomato	02	13.33	10.00
Lineseed	02	12.50	10.00
Sawflower	01	06.66	05.00
Wheat	00	00	00
Barley	00	00	00
Mustard	00	00	00

70.58 % and per cent wilted plant is 60.00, followed by gram as a host in which per cent of germination and per cent of wilted plant are 23.53 and 20.00%, respectively. Among all the host plants under investigation, sawflower showed the poorest performance in germination of seed and per cent wilted plant which are 6.60 and 5.00%, respectively and other host like wheat, barley and mustard exhibited cent per cent germination and not any wilted plant. Hence, it was found that leguminous crops showed excellent example in our host range studies and it is recommended for causing wilt of vegetable pea *(Pisum sativum)*. Naresh *et al.* (2009) also reported that 6 out of 19 plants from different families exhibited reaction with *Bipolaris sorokiniana* causing spot blotch of wheat. These are oat, barley, maize, rice, grass and linseed plant.

It may be concluded from the present finding that growth and sporulation of *F. o.* f. sp. *pisi* are affected by type of media, temperature and pH. The host range study revealed that the pathogen mainly infect leguminous crops.

ACKNOWLEDGEMENT

The authors are grateful to Head, Department of Plant Pathology, Chandra Shekhar Azad University of Agriculture and Technology, Kanpur for providing necessary facilities.

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