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To find out the physiological requirement of the *Fusarium oxysporum* f. sp. *pisi* causing wilt of pea

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Abstract
Laboratory studies were conducted to study the effect of different culture media, pH and temperature on radial growth and sporulation of *Fusarium oxysporum* f. sp. *pisi*. The fungus grew the best on PDA media among seven culture media were tested. The most suitable pH level for growth of fungus was 7.0 with excellent sporulation. Growth of *Fusarium oxysporum* f. sp. *pisi* was maximum at 25 ℃ after 168 hours of incubation, which was reduced drastically below 10 ℃ and above 35 ℃.

Keywords: pH, temperature, media, fusarium, pea and wilt

Introduction
Pea (*Pisum sativum*), is the most significant legume crop harvested around the world after soybean, chickpea and groundnut (Foyer *et al*., 2016) [3]. In India, it is mainly cultivated in Himachal Pradesh, Madhya Pradesh, Maharashtra, Rajasthan, Punjab, Haryana and Karnataka. Madhya Pradesh is the second largest pea producer state in India. (MP Horticulture statistics, 2014-15). Jabalpur and Tikamgarh are the largest pea producing district in MP (National Horticulture Mission, 2005). There is number of diseases are responsible for economic loss in pea and wilt is one of the emerging problem in pea growing areas. *Fusarium oxysporum* f. sp. *pisi* is a soil borne pathogen causing wilt in pea. Variation in pH, temperature, incubation period, shaking and inoculums size have great influence on the growth of pathogen (Tyagi and Paudel, 2014; Dubey, 2016) [14, 2]. Present work depicts the role of different media, pH and temperature to understand ecological survival of pathogen which will be helpful in management strategy in the field.

Material and methods
Radial growth of the regular colonies was measured in two directions at right angles with assistance of a linear scale. If there should arise an occurrence of irregular colonies, estimations were recorded at the broadest and tightest diameter and average of two different directions was taken as growth. In every one of the cases radial growth was recorded after 168 hrs of incubation. In case of poisoned food techniques, it was recorded after 72, 120 and 168 hrs of incubation.

Effect of various solid media on growth and sporulation of *Fusarium oxysporum*
The different culture media were prepared according to the standard formulae given by Ricker and Ricker (1936) and Khare *et al*. (1974). Media were evaluated under *in-vitro* condition for growth and sporulation of test pathogen. Solid media constituents were dissolved and agar-agar was added for solidification. The last volume was made up to 1000 ml by adding sterilized water.

Asthana & Hawker's medium: Potassium nitrate 3.50 g, Potassium Dihydrogen phosphate 1.75, Magnesium sulphate 0.75, D-Glucose 5 g, Agar-agar 20 g, Distilled water 1000 ml, pH 7.

Ashby's Agar medium: Mannitol 0.2 g, Sodium chloride 0.2 g, Di potassium phosphate 0.2 g, Magnesium sulphate 0.2 g, Potassium sulphate 0.1 g, Calcium carbonate 5g, Agar-agar 5g, Distilled water 1000 ml, pH 7.

Browns agar medium: Dextrose 2 g, Magnesium sulphate 0.75 g, Tri basic potassium phosphate 1.25 g, Agar-agar 20 g, Distilled water 1000 ml, pH 7.

Coon's agar medium: Sucrose 7.2 g, Potassium Di-phosphate 2.72 g, Dextrose 3.60 g, Potassium nitrate 2.02 g, Magnesium nitrate 1.23 g, Agar-agar 20 g, Distilled water 1000 ml,

Czapex dox agar medium: Potassium chloride 0.5 g, Di potassium hydrogen phosphate 1 g, Magnesium sulphate 0.5 g, Ferrous sulphate 0.01 g, Sodium nitrate 2 g, Sucrose 30 g, Agar-agar 20 g, Distilled water 1000 ml, pH 7.
Richard's agar medium: Potassium nitrate 10 g, Potassium monobasic phosphate 5 g, Ferric chloride 0.02 g, Magnesium sulphate 2.5 g, Sucrose 50 g, Agar-agar 20 g, Distilled water 1000 ml, pH 7.
Potato Dextrose Agar medium: Peeled and sliced potato 200 g, Dextrose 20 g, Agar-agar 20 g, Distilled water 1000 ml.
Solid media were sterilized at 15 Ibs psi. 121.6 °C for 20 minutes in an autoclave. Medium of each flask was poured into 3 Petri-plates @ 20 ml for each plate, allowed to solidify and inoculated with 5 mm disk of 7 days old culture. Plates were incubated at 28±1 °C for 7 days and perceptions were recorded on radial growth after 72, 120 and 168 hours and spore formation following 15 days onwards, respectively.

Effect of temperatures on growth and sporulation of *Fusarium oxysporum*
The examination was performed to figure out the most suitable temperature for mycelial development and sporulation of *Fusarium oxysporum* f. sp. *pisi* under *In-vitro* condition. The sterilized petriplates were poured with potato dextrose agar and inoculated with 5 mm disc of the test microbe of seven days old culture. The inoculated petriplates were incubated at 10, 15, 20, 25, 30, 35, 35 and 40 °C temperature, in triplicates and mycelial development was taken at seven days after inoculation. Haemocytometer was utilized for taking sporulation data.

Effect of pH on growth and sporulation of *Fusarium oxysporum*
The set of various pH *viz*., 5, 5.5, 6, 6.5, 7, 7.5, 8 and 8.5 were ready. For each pH esteem trial was conducted in triplicates. PDA was taken as basal medium. pH of medium was adjusted to needed level by using N/10 HCI or N/10 NaOH. The flasks containing sterilized medium was inoculated with 5 mm mycelium disc and incubated at 28+1 °C. At the gap of 24 hrs, the radial growth was measured till 7 days.

Result
Effect of various solid media
From all the physiological parameter growing media also important for radial growth and sporulation of pathogen and for this following; Ashthana & hawkers agar, Ashby agar, Browns agar, Coons agar, Czapex dox agar, Richards agar and Potato dextrose agar solid media were tested for their effect and data presented in Table 1, graphically presented in Fig 1 and plate 1.
Maximum suitable media for growth was Potato dextrose agar followed by Coons agar and Czapex dox agar with 81.15 mm, 45.81 mm and 37.01 mm radial growth consequently. Minimum growth was recorded in Ashby agar followed by Browns agar and Richards agar with 10.33 mm, 16.66 mm and 27.16 mm radial growth respectively after 168 hours of incubation.
Sporulation was also checked in all solid media, from all excellent sporulation was recorded in Potato dextrose agar. Ashthana & hawkers agar, Coons agar, Czapex dox agar and Richards agar give good result of sporulation. And fair sporulation was recorded in Ashby agar and Browns agar media.

Effect of various temperatures on radial growth and sporulation of *Fusarium oxysporum* f. sp. *pisi*
Table 2 presented the impact of various temperature *viz.* 10 °C, 15 °C, 20 °C, 25 °C, 30 °C, 35 °C and 40 °C is evaluated for the best suitable requirement by pathogen *Fusarium oxysporum* f. sp. *pisi* for growth and sporulation. And the data on radial growth and sporulation at different temperature were graphically illustrated in Fig 2 and Plate 2
Later 168 hours of incubation it was noticed that there was enormous variation in mycelial growth. Maximum was noticed at 25 °C that was 86.68 mm after that at 30 °C, 35 °C and 40 °C that was 82.33 mm, 72.86 mm and 48.30 respectively. Minimum 33.55 mm mycelial growth was recorded at 15 °C and there was no growth observed at 10 °C. Sporulation at various temperatures evaluated and best sporulation was recorded at 25 °C followed by 30 °C, then satisfactory sporulation was recorded at 15 °C, 20 °C and 35 °C. Lowest sporulation was recorded at 40 °C and there was no sporulation at 10 °C.

Effect of various pH on radial growth and sporulation of *Fusarium oxysporum* f. sp. *pisi*
pH is the second physiological parameter required by pathogen for its appropriate growth and sporulation. Various pH *viz.* 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0 and 8.5 were tested for their effect on *Fusarium oxysporum* f. sp. *pisi*. And data presented in Table 2, graphically illustrated in Fig 3 and plate 3.
Maximum radial growth 82.33 mm followed by 69.00 mm and 61.84 mm was recorded in pH value 7.0, 6.5 and 6.0 respectively. And minimum 28.77 mm followed by 36.84 mm in pH value 8.5 and 8.0 consequently after 168 hours of incubation of plates.
Sporulation was also checked for all pH value and excellent result was recorded in 6.5, 7.0, 6.0 and 7.5. And good sporulation was recorded in 8.5 followed by 5.0 and 5.5.

Table 1: Effect of various solid media on radial growth and sporulation of *Fusarium oxysporum* f. sp. *pisi*

Media	Radial growth of pathogen			Sporulation
	72 hours	124 hours	168 hours	
Ashthana & hawkers agar	23.5	34.22	36.54	++
Ashby agar	0	0.00	10.33	+
Browns agar	0	0.00	16.66	+
Coons agar	26.79	35.77	45.81	++
Czapex dox agar	20.36	32.66	37.01	++
Richards agar	14.25	20.21	27.16	++
Potato dextrose agar	35.62	70.33	81.15	++++
SE(m)	0.44	0.73	0.62	
CD (0.5)	1.36	2.24	1.91	

Table 2: Effect of various temperature on radial growth and sporulation of *Fusarium oxysporum* f. sp. *Pisi*

Temperature	Radial growth of pathogen			Sporulation
	72 hours	124 hours	168 hours	
10 °C	0	0	0	-
15 °C	10	18.00	33.55	++
20 °C	14	22.00	45.88	++
25 °C	45	66.00	86.65	++++
30 °C	48	72.00	82.33	+++
35 °C	41	54.00	72.56	++
40 °C	16	23.00	48.30	+
SE(m)	0.56	0.81	1.07	
CD (0.5)	1.73	2.48	3.28	

Table 3: Effect of various pH on radial growth and sporulation of *Fusarium oxysporum* f. sp. *pisi*

pH	Radial growth of pathogen			Sporulation
	72 hours	124 hours	168 hours	
5.0	12.33	24	40.60	++
5.5	23.5	34.00	52.30	++
6.0	30.51	41.50	61.84	+++
6.5	31.88	43.60	69.00	++++
7.0	47.5	50.00	82.33	++++
7.5	21.5	31.00	49.60	+++
8.0	12.3	22.00	36.84	++
8.5	11.2	20.00	28.77	+
SE (m)	0.48	0.64	0.96	
CD (0.5)	1.46	1.94	2.92	

Plate 1: Effect of various solid media on radial growth and sporulation of *Fusarium oxysporum* f. sp. *pisi*

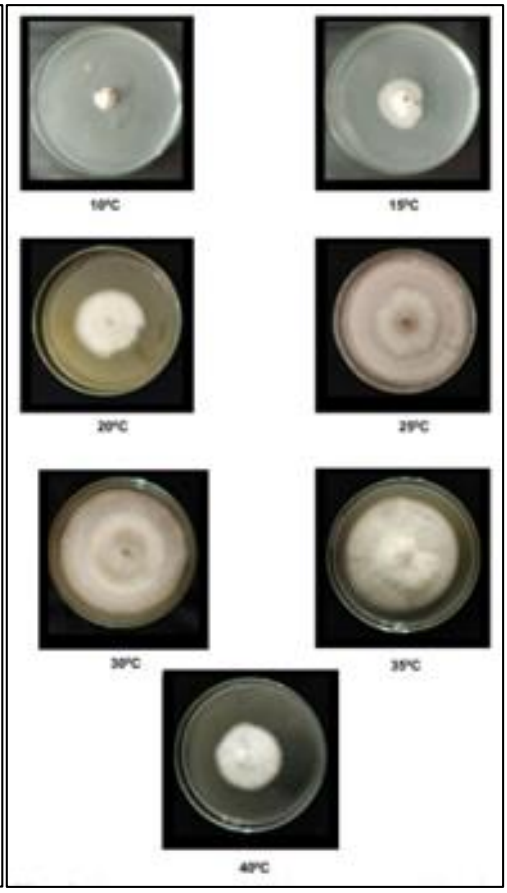

Plate 2: Effect of various temperature on radial growth and sporulation of *Fusarium oxysporum* f. sp. *pisi*

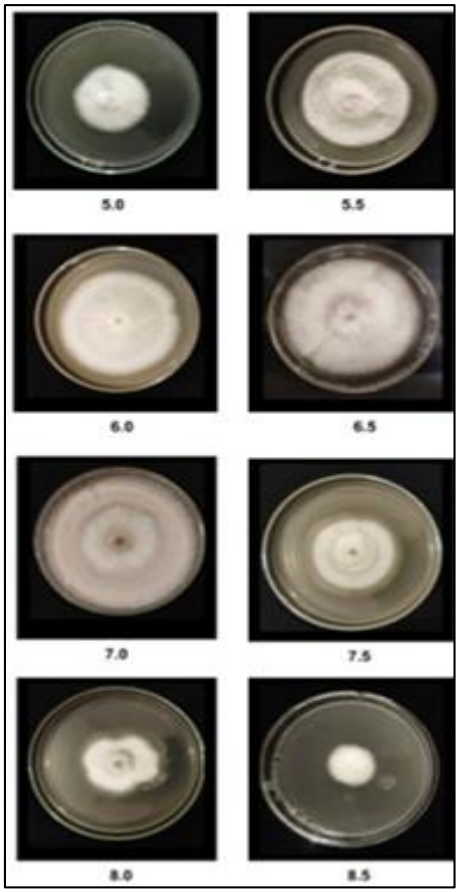

Plate 3: Effect of various pH on radial growth and sporulation of *Fusarium oxysporum* f. sp. *pisi*

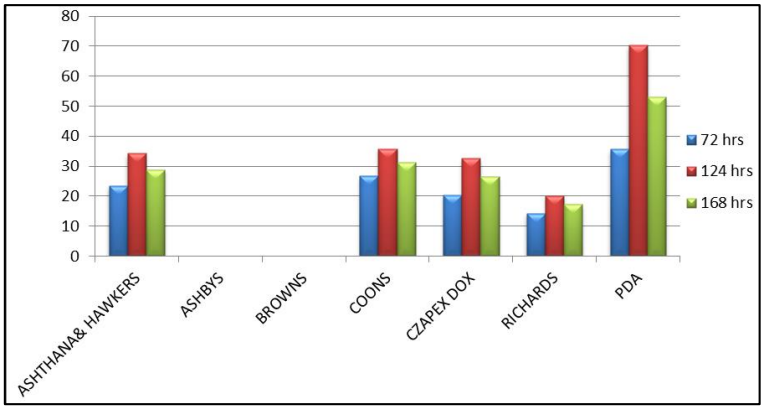

Fig 1: Effect of various solid media on radial growth and sporulation of *Fusarium oxysporum* f. sp. *pisi*

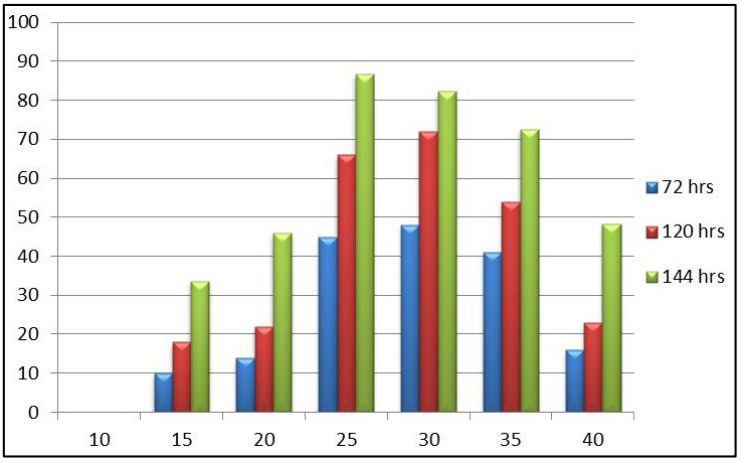

Fig 2: Effect of various temperature on radial growth and sporulation of *Fusarium oxysporum* f. sp. *pisi*

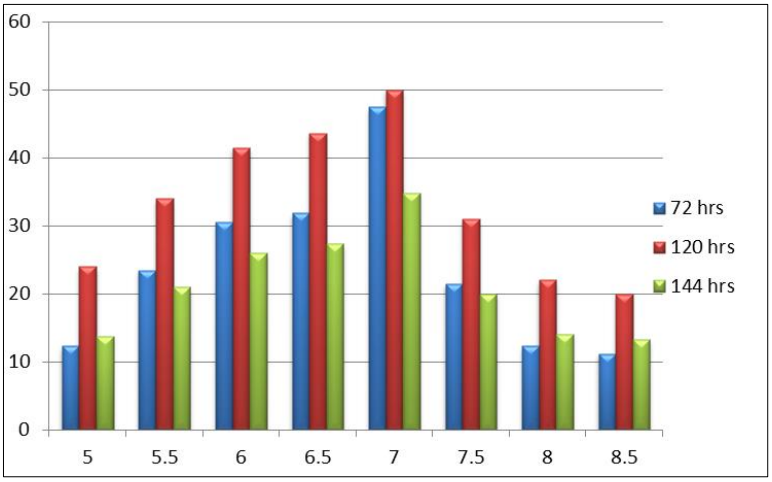

Fig 3: Effect of various pH on radial growth and sporulation of *Fusarium oxysporum* f. sp. *pisi*

Discussion

Effect of various solid media on growth and sporulation of *Fusarium oxysporum* f. sp. *pisi*

Present examinations on different media result that maximum suitable media for growth was Potato dextrose agar (81.15 mm) followed by Coons agar and Czapex dox agar. Similar finding was recorded by Khan *et al*., (2011) [6] and Khilare and Ahmed (2012) [7] when they are working with *Fusarium oxysporum* f. sp. *Ciceri*. Reddy, (2002) [10], Satareddi *et al*. (2003), Kishore (2010) [8] and Gangadhara *et al*. (2010) [4], Yadav *et al*. (2014) [15], Jalander and Gachande (2015) [5] also conclude in their findings that PDA is an excellent media for both mycelial growth and sporulation.

Effect of temperatures on growth and sporulation of *Fusarium oxysporum* f. sp. *pisi*

Temperature in current study found best is 25 °C (86.68 mm) after that at 30 °C, 35 °C respectively. Khilare and Ahmed (2012) [7] studies the effect of temperature on radia growth of the pathogen in between 25 °C-30 °C. Chaudhary *et al* (2014) [1] and Chaudhary *et al*. (2018) [1] in *Fusarium udum* also showed good mycelia growth and sporulation was maximum at 30 °C.

Effect of various pH on radial growth and sporulation of *Fusarium oxysporum* f. sp. *pisi*

pH value was found superior over others for sporulation and mycelial growth was 7.0, 6.5 and 6.0 respectively in present study. In different sp. of fusarium similar findings are reporters by Siddique *et al*. (2012) [12], Chaudhary *et al*. (2014) [1], Tyagi and Paude (2014) [14], Singh *et al*. (2016) and Chaudhary *et al*. (2018) [1] for both mycelial growth and for sporulation.

Conclusion

The auspicious outcome in the current analysis demonstrated that all the readings on growth and sporulation were recorded after 168 hours of incubation and highest suitable media recoded was Potato dextrose agar followed by Coons agar and Czapex dox agar with 81.15 mm, 45.81 mm and 37.01 mm radial growth consequently. It was noticed that there was enormous variation in mycelial growth due to various temperature and 25 °C recorded as maximum suitable temparature as result 86.68 mm after that at 30 °C, 35 °C and 40 °C that was 82.33 mm, 72.86 mm and 48.30 respectively. Maximum radial growth 82.33 mm followed by 69.00 mm and 61.84 mm was recorded in pH value 7.0, 6.5 and 6.0 respectively.

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