The Pharma Innovation Journal 2022; 11(6): 1043-1047

www.ThePharmaJournal.com

The Pharma Innovation

**ISSN (E): 2277-7695**
**ISSN (P): 2349-8242**
**NAAS Rating: 5.23**
TPI 2022; 11(6): 1043-1047

www.thepharmajournal.com
Received: 18-03-2022
Accepted: 29-04-2022

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# Effect of different pH, temperature and media on growth and sporulation of *Fusarium oxysporum* f. sp. *lentis*, causing wilt of lentil

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**Abstract**
Lentil is one of the major rabi pulse crop grown in India. It is a rich source of protein, minerals and vitamins for human nutrition and straw is also valued animal feed. The maximum radial growth (86.33mm) at seven days with maximum mycelial dry weight (326.11mg) at twenty-one days after inoculation at 25±2 ℃ was observed. The next best culture media were oat meal agar and Richard's agar which showed 80.33mm and 75.66mm radial growth and 295.20mg and 288.10mg of dry weight. Lentil meal agar was the least effective, showing 37.00mm of radial growth and 186.90mg of mycelial dry weight. This fungus sporulates in all test media, but excellent sporulation was recorded in potato dextrose agar and Richard's agar media. The maximum radial growth at pH 6.0 (88.66mm) and pH 6.5 (84.33mm) were also found to be favorable. The highly alkaline and acidic pH is not favourable for the best growth and sporulation of *Fusarium oxysporum* f. sp. *lentis*. Maximum radial growth was also found to be favourable 84.00mm at 30 ℃ and next best 78.66mm at 25±2 ℃. The growth and sporulation of the test pathogen is slow when the temperature is increased and decreased at 30 °C.

**Keywords:** *Fusarium oxysporum* f. sp. *lentis*, lentil wilt, pH, temperature and culture media

## Introduction
Lentil (*Lens culinaris* Medikus) was among the first crops domesticated and has become an important food legume crop in the farming and food systems of many countries globally. Its seeds are a rich source of protein, minerals, and vitamins for human nutrition, and the straw is valuable for animal feed. Its ability for nitrogen and carbon sequestration improves soil nutrient status, which in turn provides sustainability in production systems Sarker and Erskine (2006) [15]. It is a Rabi pulse crop (sown from October to November) in terms of area under cultivation of 1.32 million hectares, production of 1.18 million tonnes and yield of 894 kg/hectare in India (Anonymous, 2020) [3]. The crop suffers from many serious diseases, caused by a variety of fungi, bacteria, viruses, and nematodes. Wilt, root rot complex, collar rot, rust, ascochyta blight, and downy mildew are among the most common and potentially destructive diseases, according to Agrawal and Prasad (1997) [1]. *Fusarium oxysporum* f. sp. *lentis*, the severe causative agent of lentil wilt has been appearing in severe form for a long time and is causing a major problem in lentil growing parts of the country. Infections not only lower output, but they also degrade seed quality. Chaudhary, *et al.* (2010) [5] reported for the first time a national lentil wilt-root rot incidence at the crucial reproductive stage and their associated pathogens. The main pathogens found associated with plant mortality at this stage were *Fusarium oxysporum* f. sp. *lentis* (62.0%), *Rhizoctonia bataticola* (25.2%), and *Sclerotium rolfsii* (9.8%). Choudhary and Kumar (2016) [6] Fusarium wilt has been reported to cause 100% yield losses if it affects the crop in the seedling stage. Wilt incidences of 50 to 78 percent have been reported in some fields in Madhya Pradesh Khare (1981) [10] and Agrawal *et al*. (1991) [2]. In the present study, an experiment has been given on the role of different culture media, pH, and temperature in the ecological survival of the pathogen, which will be helpful in management of disease incidence and yield against *Fusarium oxysporum* f. sp. *lentis*.

## Material and Methods
### Collection of the samples and isolation of causal pathogen
Fresh diseased plants were collected from the field of different location of Hamirpur district. Tissue isolation technique was followed after through surface sterilization of root pieces (2-3 mm size) with 0.1 per cent mercuric chloride solution for a minute.

After this, the treated pieces were thoroughly washed thrice in sterile distilled water to remove mercuric chloride from the treated pieces. These pieces were transferred in petri plates containing solidified potato dextrose agar medium. The inoculated plates were then incubated at 25±2 ℃ temperature. The fungal growth appearing on the root pieces was examined and purified by following the single hyphal tip cut methods (Rangaswami, 1958) [14]. *Fusarium oxysporum* f. sp. *lentis* was identified based on the spores and conidiophores morphology.

**Effect of different culture media on radial growth of *Fusarium oxysporum* f. sp. *lentis***

Different culture media *viz*., Potato dextrose agar, Richard's agar, Czapek-dox agar, Brown's agar, Lentil leaf agar, Lentil meal agar and Oat meal agar were prepared for further study. All culture media were sterilized for 20 minutes at 121.6 °C and 15 lbs psi were cooled and melted, then poured into 90mm sterilized petri plates for solidification. A five-mm mycelial disc was cut through the sterilized cork borer of the test pathogen and placed carefully on 90mm diameter petri plates containing solidified PDA medium. The inoculated petri plates were incubated at 25±2℃ for 7 days. Each culture media is replicated three times. After seven days, regularly observed for fungal radial growth and sporulation, they were recorded.

**Effect of different pH value on radial growth of *Fusarium oxysporum* f. sp. *lentis***

There were eight different pH values ranging from 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0 and 8.5 were prepared and pH was adjusted by using a pH meter and adding the appropriate amount of N/10 HCl or N/10 NaOH solution in PDA medium. After adjusted pH values, the medium was sterilized with the help of an autoclave. A five-mm mycelial disc was cut through the sterilized cork borer of the test pathogen. This propagule was placed carefully on 90mm diameter petri plates containing solidified PDA medium and inoculated petri plates were incubated at 25±2℃ in a BOD incubator. Each experiment was replicated three times. After seven days, regularly observed for fungal radial growth and sporulation were recorded. The data obtained was analyzed statistically.

**Effect of different temperature on radial growth of *Fusarium oxysporum* f. sp. *lentis***

Potato Dextrose Agar medium was poured into 90mm diameter sterilized petri plates containing 20ml of medium in each plate. A five-mm mycelial disc was cut through the sterilized cork borer of the test pathogen and placed carefully on 90mm diameter petri plates containing solidified PDA medium. Inoculated petri plates were incubated at various temperature ranges, *viz*., 10, 15, 20, 25, 30, 35 and 40±2℃ in the BOD incubator. Each experiment was replicated three times. After seven days regularly observed for fungal radial growth and sporulation, were recorded. The data obtained was analyzed statistically.

**Result and Discussion**

**Effect of different culture media on radial growth, dry weight and sporulation of *Fusarium oxysporum* f. sp. *lentis***

The result presented in Table 1 and its corresponding histogram (Fig. 1) reveals that observation of radial growth showed that all the culture media were significantly effective. Among then incorporation of potato dextrose agar was found significantly superior over all the medium, resulting in maximum radial growth (86.33mm) at seven days with maximum mycelial dry weight (326.11mg) at twenty-one days after inoculation at 25±2℃ was observed. The next best culture media were oat meal agar and Richard's agar which showed 80.33mm and 75.66mm radial growth and 295.20mg and 288.10mg of dry weight, followed by Czapek's agar, Brown's agar and lentil leaf agar, respectively. They were statistically at par with each other. Lentil meal agar was the least effective, showing 37.00mm of radial growth and 186.90mg of mycelial dry weight. This fungus sporulates in all test media, but excellent sporulation was recorded in potato dextrose agar and Richard's agar media. In Richard's medium, there was a good of sporulation showed. Our finding is close agreement with the finding of the Singh, and Kumar (2016) [17] observed that PDA medium produced the best mycelial growth with an isolate of *Fusarium oxysporum f. sp. lentis*, and Patra and Biswas (2017) [13], Jalander and Gachande (2015) [9] and Gangadhara, *et al*. (2010) [8] reported similar results, that PDA and Richard's agar media supported best mycelial growth with an isolate of *Fusarium oxysporum f. sp. ciceri, Fusarium udum and Fusarium oxysporum f. sp. vanillae*.

**Effect of different pH values on radial growth and sporulation of *Fusarium oxysporum* f. sp. *lentis***

The result is obtained from Table 2 and its corresponding histogram (Fig. 2). The radial growth of the test pathogen ranged from 52.66 mm to 88.66 mm seven days after inoculation on PDA medium at different pH values. The maximum radial growth at pH 6.0 (88.66) and pH 6.5 (84.33) were also found to be favorable, followed by 70.0 mm at pH 5.5, 73.33mm at pH 7.0, 70.66mm at pH 5.0, 68.66mm at pH 7.5 and 62.33mm at pH 8.0 level, respectively. They were statistically at par with each other. The level of pH 8.5 was the least effective, showing 52.66mm of radial growth. In pH 6.0 and 6.5 respectively, excellent sporulation was observed. The highly alkaline and acidic pH is not favourable for the best growth and sporulation of *Fusarium oxysporum* f. sp. *lentis*. Similar results were also reported by Tyagi and Paudel (2014) [18], found that pH level 6.0 is the optimum pH for the growth as well as sporulation of the fungus which was found best at pH level 4.5. Further increases in the pH level showed a retarding effect on growth and sporulation. Pal *et al.* (2019) [12] reported the maximum growth of *Fusarium oxysporum* f. sp. *lini* at pH 5.5. Khilare and Rafi (2012) [11] reported the best growth of *F. oxysporum* f. sp. *ciceri* at pH 6.0 and 6.5 and maximum growth at 30℃ after seven days of inoculation, which was drastically reduced below 15℃ and above 35℃.

**Effect of different temperature on radial growth and sporulation of *Fusarium oxysporum* f. sp. *lentis***

The result is obtained from Table 3 and its corresponding histogram (Fig. 3). The test pathogen's radial growth varied from 22.33 mm to 84.00 mm seven days after inoculation on PDA medium at various temperature levels. Maximum radial growth was also found to be favourable 84.00mm at 30 ℃ and next best 78.66mm at 25±2 ℃ followed by 68.66 mm at 20 ℃, 66.66mm at 35 ℃, 43.33mm at 15 ℃ and 42.00mm at 40±2℃, respectively. They were statistically at par with each other. The 10±2 ℃ teperature was the least effective, with radial growth of 22.33mm was observed. Excellent

sporulation was showed at temperature 30 and 25±2 ℃, respectively. The growth and sporulation of the test pathogen is slow when the temperature is increased and decreased at 30 °C. In the similar reported that optimum temperature for the growth and spore production of *Fusarium udum in vitro* was within range (25-30 °C) Desai, *et al.* (2016) [7] and Chaudhary, *et al.* (2018) [4]. Scott *et al*. (2010) [16] found that radial growth rates of *F. oxysporum* f. sp. *lactucae* were observed to increase from 10°C up to an apparent maximum near 25 °C.

**Table 1:** Effect of different culture media on radial growth, dry weight and sporulation of *Fusarium oxysporum* f. sp. *Lentis*

| S.N. | Name of the medium | Agar media | | Broth medium | |
|---|---|---|---|---|---|
| | | Colony diameter (mm)* | Sporulation | Dry mycelium weight (mg)* | Sporulation ** |
| 1. | Potato dextrose agar | 86.33 | ++++ | 326.11 | ++++ |
| 2. | Richard's agar | 75.66 | ++++ | 288.10 | ++++ |
| 3. | Czapek's agar | 70.33 | +++ | 280.80 | +++ |
| 4. | Brown's agar | 61.00 | ++ | 245.16 | ++ |
| 5. | Lentil leaf agar | 40.66 | + | 210.56 | ++ |
| 6. | Lentil meal agar | 37.00 | + | 186.90 | ++ |
| 7. | Oat meal agar | 80.33 | +++ | 295.20 | +++ |
| CD at 5% | | 3.38 | | 5.20 | |
| S.Em | | 1.10 | | 1.70 | |

*Average of three replication
** Categories of sporulation: Excellent (++++), Good (+++), Fair (++) and Poor (+).

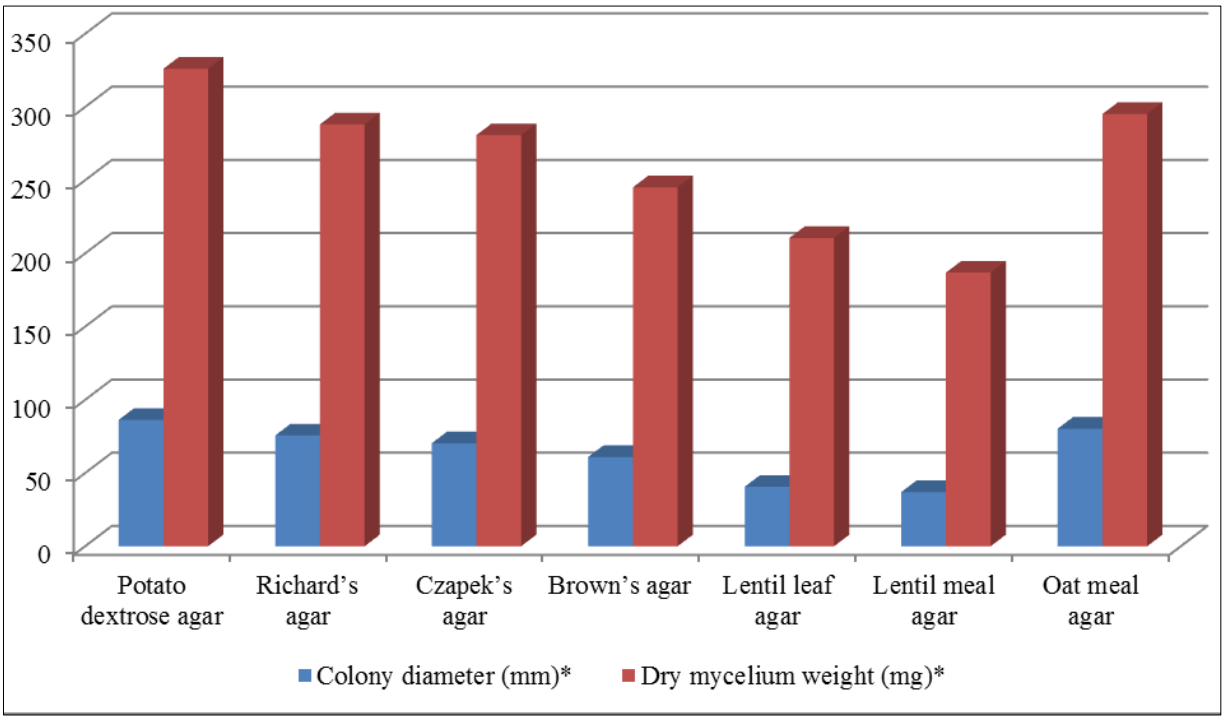


**Fig 1:** Effect of different culture media on radial growth and dry weight of *Fusarium oxysporum* f. sp. *lentis*

**Table 2:** Effect of different pH values on radial growth and sporulation of *Fusarium oxysporum* f. sp. *Lentis*

| S.N. | pH values | Colony diameter (mm)* | Sporulation ** |
|---|---|---|---|
| 1. | 5.0 | 70.66 | ++ |
| 2. | 5.5 | 76.00 | ++ |
| 3. | 6.0 | 88.66 | ++++ |
| 4. | 6.5 | 84.33 | ++++ |
| 5. | 7.0 | 73.33 | +++ |
| 6. | 7.5 | 68.66 | ++ |
| 7. | 8.0 | 62.33 | ++ |
| 8. | 8.5 | 52.66 | + |
| CD at 5% | | 4.87 | |
| S.Em | | 1.61 | |

*Average of three replication
** Categories of sporulation: Excellent (++++), Good (+++), Fair (++) and Poor (+).

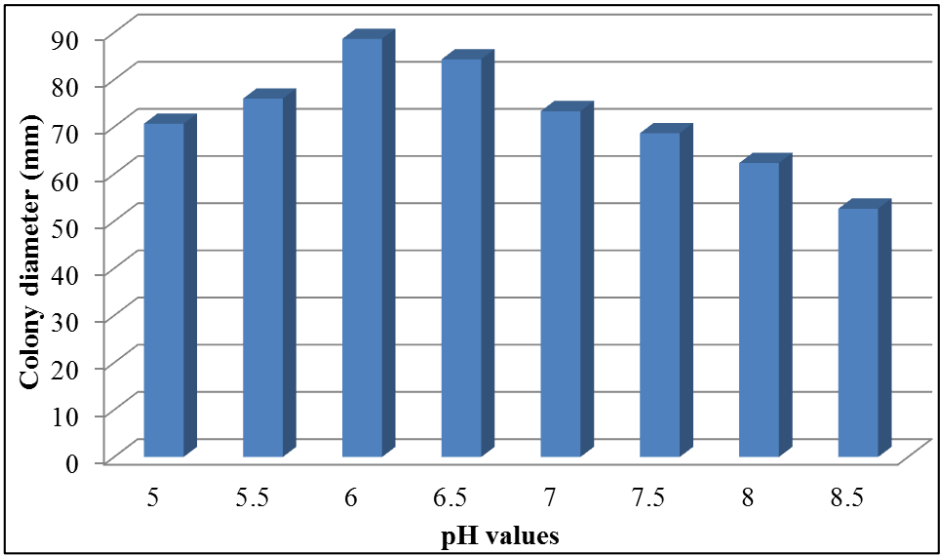


**Fig 2:** Effect of different pH values on radial growth of *Fusarium oxysporum* f. sp. *lentis.*

**Table 3:** Effect of different temperature on radial growth and sporulation of *Fusarium oxysporum* f. sp. *Lentis*

| S.N. | Temperature ℃ | Colony diameter (mm)* | Sporulation** |
|---|---|---|---|
| 1. | 10 | 22.33 | + |
| 2. | 15 | 43.33 | + |
| 3. | 20 | 68.66 | +++ |
| 4. | 25 | 78.66 | ++++ |
| 5. | 30 | 84.00 | ++++ |
| 6. | 35 | 66.66 | +++ |
| 7. | 40 | 42.00 | + |
| CD at 5% | | 4.15 | |
| S.Em | | 1.35 | |

*Average of three replication

** Categories of sporulation: Excellent (++++), Good (+++), Fair (++) and Poor (+).

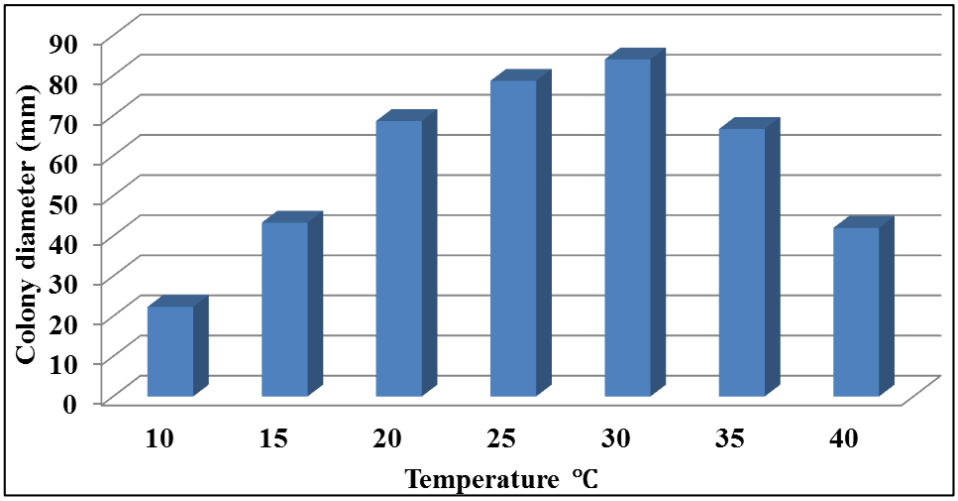


**Fig 3:** Effect of different temperature on radial growth of *Fusarium oxysporum* f. sp. *lentis*

## Conclusion

In conclusion, the present study demonstrated that potato dextrose agar and Richard's agar culture media with pH values of 6.0 and 6.5 and temperatures of 25 and 30 °C were found optimum for mycelial growth and sporulation of *Fusarium oxysporum* f. sp. *lentis*, which causes wilt disease of lentils. These experiments to be helpful for management of wilt incidence and improved yield.

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