

Original Research Article

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Nutritional and Epidemiological Requirements for Growth and Sporulation of *Fusarium oxysporum* f. sp. *lentis* caused Lentil Wilt

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ABSTRACT

Keywords

Lentil, *Fusarium oxysporum* f. sp. *lentis*, Culture media, pH, Temperature, Colony Growth and sporulation, lentil wilt

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Fusarium oxysporum f. sp. *lentis* was isolated from wilt infected lentil plant samples were collected from the field of AICRP on MULLaRP at Agriculture Research Station, Ummedanaj, (Kota). In epidemiological studies, mycelium growth and sporulation by *Fusarium oxysporum* f. sp. *lentis* was significantly influenced with media, temperature and pH level. Maximum colony diameter (90.00 mm) recorded on oat meal agar (Hi-media) medium, which was statistically at par with potato dextrose agar (Hi-media) yielded 88.57 mm colony diameter, Both the media support excellently sporulation by pathogen. After 168 hrs of incubation, significantly higher mycelial growth (90.00 mm) was observed at 30°C, as compared to 25°C produced 87.00 mm colony diameter. A sudden fall in mycelial growth of *Fusarium oxysporum* f. sp. *lentis* was observed in both increasing and decreasing of temperature level. The excellent sporulation density was also observed under microscopic field at temperature 30°C and 25°C. It was found that pathogen grow at all pH levels tested but it was significantly higher at pH 6.0 (90.00 mm) 168 hrs. after incubation when compared with pH 5.0 (86.50 mm) and pH 7.0 (82.50 mm) found favourable pH level for mycelial growth, Excellent sporulation was observed at pH 6.0. Good sporulation was observed at pH 5.0 and 7.0.

Introduction

Lentil (*Lens culinaris* M.) is an edible pulse and belongs to the family Leguminosae. It is a bushy annual plant of the legume family, grown for its lens shaped seeds. Grain of lentil contains 26% protein and 1% fat. It is also rich in vitamins (thiamin and folate) and minerals (Ca, P, Fe and Zn). It is a valuable crop for maintaining soil fertility having a capacity of Nitrogen fixing (Joshi, 1998). Lentil is largely consumed as dal and also use

as food and fodder, the leaves, stems & threshed pods of lentil are important for feeding sheep and goats. In India, lentil is mostly grown in northern plains, central and eastern parts of India. The total area under lentil in India was 14.94 Lakh ha with a total production of 15.06 Lakh tonnes at 1008 kg/ha productivity. In Rajasthan, lentil is grown in Bundi, Kota, Pratapgarh, Bhilwara, Jhalawar and Bhartpur districts covering the total area of 0.31 Lakh hectare, producing 0.43 Lakh tonnes with productivity of 1387

kg/ha, during 2017-18 (Anonymous,2019). Biotic stresses such as fusarium wilt (*Fusarium oxysporum* f. sp. *lentis*), ascochyta blight (*Ascochyta lentis*), stemphylium blight (*Stemphylium botryosum*), anthracnose (*Colletotrichum truncatum*), root rot (*Rhizoctonia solani*), rust (*Uromyces viciaefabae*), white mold (*Sclerotinia sclerotiorum*) and collar rot (*Sclerotium rolfsii*), (Kumar *et al.*, 2013) cause severe yield loss. Among diseases, Fusarium wilt caused by *Fusarium oxysporum* f. sp. *lentis* is one of the major diseases affecting lentil yield all over the world (Bayaa *et al.*, 1998; Khare 1981). Lentil wilt is a serious disease caused by *Fusarium oxysporum* f. sp. *lentis* (*Fol*) and plays major role in reducing lentil yield in India and world (Hamdi and Hassanein, 1996). Fusarium wilt causes yield losses up to 50% in India. Present experiment deals with study of mycelium growth and sporulation under *in-vitro* condition at different culture media, various temperatures and pH level. To carry out the research, eight distinctive culture media *viz.*, Potato Dextrose Agar (Natural), V-8 Juice Agar (Hi-Media), C-Zepak's Agar (Hi-Media), Potato Dextrose Agar (Hi-Media), Oat Meal Agar (Hi-Media), Corn Meal Agar, Lentil Leaf Extract Agar and Lentil Root Extract Agar were used to evaluate for the mycelium growth and sporulation of pathogen. Later suitable temperature and pH were also analyzed for the pathogen growth and sporulation by incubating at different pH level *viz.*, 5.0, 6.0, 7.0, 8.0 and 8.5 and temperatures regimes *viz.*, 10°C, 15°C, 20°C, 25°C, 30°C and 35°C.

Materials and Methods

Collection

Infected plants which showing typical wilting symptoms were collected during the month of January-February, 2019 from the lentil fields of AICRP on MULLaRP at ARS, Ummedganj (Kota). Samples were brought in the

Department of Plant Pathology, College of Agriculture, Ummedganj, Kota for isolation and further studies.

Isolation of fungus

For isolation of the pathogen standard tissue isolation technique followed (Aneja, 2018). The diseased lentil plant showing the wilt symptoms were washed thoroughly with tap water, small pieces (3mm²) from infected roots were cut with the help of sterilized blade. Then these pieces were surface sterilized with 0.1% HgCl₂ solution for one minute. Such pieces were washed thoroughly in sterile distilled water subsequently three times to remove traces of mercuric chloride solution, and then aseptically transferred to sterilized potato dextrose agar (PDA) plates. Petri plate were incubated at 25±1°C for three days for growth of the fungus. Later, the bit of fungal growth was transferred to PDA petri plates. The pure culture of the fungus was obtained by further growing the culture and following hyphal tip culture under aseptic conditions.

Identification of fungus

The pathogen *Fusarium oxysporum* f. sp. *lentis* forms cottony white colonies on petri plates containing PDA. The colonies appeared as pure white mycelial growth were identified by observing the colony against light with the naked eyes and later confirmed sporulation with the help of microscope. On the basis of these characters the pathogen was identified as *Fusarium oxysporum* f. sp. *lentis*. Further, identification of pathogen was confirmed and it is identified by Indian Type Culture Collection, Division of Plant Pathology, IARI, New Delhi as *Fusarium oxysporum* (I. D. No. 11,029.19 & Date- 25/03/2019).

Preparation of Culture Media

Different culture media *viz.*, Potato Dextrose

Agar (Natural), V-8 Juice Agar (Hi-Media), C-Zepak's Agar (Hi-Media), Potato Dextrose Agar (Hi-Media), Oat Meal Agar (Hi-Media), Corn Meal Agar, Lentil Leaf Extract Agar and Lentil Root Extract Agar were prepared to carry out the study.

All the media were sterilized at 121.6°C temperature and 1.05 kg/cm² (15 lbs psi) pressure for 15 min. To carry out study, cool & molten media near about 20-25 ml of each of the medium was poured in each prior Sterilized petriplates. After solidification of media such petriplates were inoculated with 6 mm disc cut from the periphery of actively growing fungal culture grown in petri plates by using sterilized cork borer and incubated at 25 ± 1°C. Each treatment was replicated thrice. Radial growth of colony was recorded by measuring colony diameter in millimetre at 7th days after inoculation and sporulation density per microscopic field at 15th days after inoculation. The data obtained were analysed statistically.

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

Twenty ml of sterilized potato dextrose agar (PDA) was poured in sterilized 90 mm diameter petriplates. Each treatment was repeated four times. The petri plates were inoculated aseptically after solidification of medium by placing 6 mm diameter mycelial disc in the centre, cut aseptically with the help of cork-borer from 5 days old pure culture of *F. oxysporum* f. sp. *lentis*.

These petri plates were incubated at various temperatures range viz., 10, 15, 20, 25, 30, and 35±1°C in B.O.D. incubator. Observations on radial growth and sporulation of fungus were recorded. After 7th days of incubation, observations on radial growth and sporulation

density per microscopic field at 15th days after inoculation. The data obtained were analysed statistically.

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

The set of media having different pH viz., 5.0, 6.0, 7.0, 8.0 and 8.5 were prepared and pH was adjusted by adding appropriate amount N/10 HCl or N/10 NaOH solutions in PDA medium with pH meter. After adjusting pH, the medium was sterilized in an autoclave. After sterilizing media, before cooling it at 45°C temperature, 20-25 ml of media was poured separately in sterilized petri plates aseptically. After solidifying media, 6 mm disc of fungus was cut aseptically with the help of sterilized cork borer and placed in petri-plates and were incubated at 25±1°C temperature in an incubator. For each pH value, four replications were maintained.

Observations on radial growth and sporulation of fungus were recorded. After 7th days of incubation, observations on radial growth and sporulation density per microscopic field at 15th days after inoculation. The data obtained were analysed statistically.

Estimation of sporulation

For estimating the sporulation, after 15th days after incubation period 5 mm disc was cut and suspended in 10 ml of distilled water and shaken well to harvest spores. Number of spores were counted with the help of Haemocytometer.

The results have been expressed as excellent, good, fair, poor, and no sporulation on the basis of following scale are suggested by (Singh, 2015).

Details of expression of sporulation

Sporulation	Represented as	No. of spores/ microscopic field
Excellent	++++	61 & above
Good	+++	41-60
Fair	++	21-40
poor	+	Less than 20
No	-	Nil

Results and Discussion

Effect of different solid culture media on mycelial growth and sporulation of *Fusarium oxysporum* f. sp. *Lentis*

The pathogen was inoculated on different media and observed that maximum colony diameter (90.00mm) recorded on oat meal agar (Hi-media) medium, which was statistically at par with potato dextrose agar (Hi-media) yielded 88.57mm colony diameter but significantly superior as compared to potato dextrose agar (Natural), V-8 juice agar (Hi-Media), lentil Root extract agar, C-zepak's agar (Hi-Media) and corn meal agar were respectively, 86.67, 84.00, 78.40, 69.40 and 37.57mm. Potato dextrose agar (Hi-

media) was at par when compared to potato dextrose agar (Natural). Least colony diameter (19.73 mm) of the test fungus was recorded on lentil leaf extract agar. Similarly, effect on sporulation test fungus sporulated in all medium tried except lentil leaf extract agar and corn meal agar, which not supported for sporulation and also observed scanty mycelial growth. Fungus sporulated excellently in oat meal agar (Hi-media), potato dextrose agar (Hi-media) and potato dextrose agar (Natural). While good sporulation was recorded in V-8 juice agar (Hi-Media) medium. Fair sporulation was observed in lentil root extract agar and C-zepak's agar (Hi-Media) medium according to sporulation scale.

Table.1 *In-vitro* effect of different solid culture medium on mycelial growth and sporulation of *Fusarium oxysporum* f. sp. *Lentis*

Medium Name	Colony diameter in mm (168 HAI) *	Sporulation**
T ₁ = Potato Dextrose Agar (Natural)	86.67	++++
T ₂ = V-8 Juice Agar (Hi-Media)	84.00	+++
T ₃ = C-Zepak's Agar (Hi-Media)	69.40	++
T ₄ = Potato Dextrose Agar (Hi-Media)	88.57	++++
T ₅ = Oat Meal Agar (Hi-Media)	90.00	++++
T ₆ = Corn Meal Agar	37.57	-
T ₇ = Lentil Leaf Extract Agar	19.73	-
T ₈ = Lentil Root Extract Agar	78.40	++
S Em. ± = 0.50	C.D. at 0.05% = 1.50	

*Mean of three replications; HAI = Hours after inoculation

**Categories of sporulation: Excellent (++++) = 61 & above, Good (+++) = 41-60, Fair (++) = 21-40, Poor (+) = Less than 20, No (-) = Nil

Table.2 *In-vitro* effect of different temperature level on growth and sporulation of *Fusarium oxysporum* f. sp. *Lentis*

Temperature (°C)	Colony diameter in mm (168 HAI) *	Sporulation**
T ₁ = 10 °C	21.00	+
T ₂ = 15°C	38.50	+
T ₃ = 20°C	66.50	+++
T ₄ =25°C	87.00	++++
T ₅ =30°C	90.00	++++
T ₆ =35°C	58.50	++
S Em. ± = 0.53		C.D. at 0.05% = 1.59

*Mean of four replications; HAI= Hours after inoculation

**Categories of sporulation: Excellent (++++) = 61 & above, Good (+++) = 41-60, Fair (++) = 21-40, Poor (+) = Less than 20, No (-) = Nil

Table.3 *In-vitro* effect of different pH level on growth and sporulation of *Fusarium oxysporum* f. sp. *lentis*

pH level	Colony diameter in mm (168 HAI) *	Sporulation**
T ₁ = 5.0	86.50	+++
T ₂ = 6.0	90.00	++++
T ₃ = 7.0	82.50	+++
T ₄ = 8.0	76.50	++
T ₅ = 8.5	57.50	+
S Em. ± = 0.52		C.D. at 0.05% = 1.58

*Mean of four replications; HAI=hours after inoculation

**Categories of sporulation: Excellent (++++) = 61 & above, Good (+++) = 41-60, Fair (++) = 21-40, Poor (+) = Less than 20, No (-) = Nil

Plate.4.2 *In-vitro* effect of different solid culture medium on mycelial growth and sporulation of *Fusarium oxysporum* f. sp. *Lentis*



T₁= Potato Dextrose Agar (Natural), T₂= V-8 Juice Agar (Hi-Media), T₃= C-Zepak's Agar (Hi-Media), T₄= Potato Dextrose Agar (Hi-Media), T₅= Oat Meal Agar (Hi-Media), T₆= Corn Meal Agar, T₇= Lentil Leaf Extract Agar, T₈= Lentil Root Extract Agar

Plate.3 *In-vitro* effect of different temperature level on growth and sporulation of *Fusarium oxysporum* f. sp. *Lentis*



T₁ = 10 °C; T₂ = 15 °C; T₃ = 20 °C; T₄ =25 °C; T₅ =30 °C; T₆ =35 °C

Plate.4 *In-vitro* effect of different pH level on growth and sporulation of *Fusarium oxysporum* f. sp. *lentis*



T₁ = 5.0; T₂ = 6.0; T₃ = 7.0; T₄ =8.0; T₅ =8.5

Similar findings were reported by several others workers Khare *et al.*, (1975) reported maximum growth and sporulation of *Fusarium oxysporum* f. sp. *lentis* on PDA, followed by lentil extract and Richard's agar medium. Khan *et al.*, (2011) also reported PDA an excellent medium for growth and sporulation, followed by Richard's agar medium. Singh *et al.*, (2016) studied effect of different solid media and liquid media on

radial growth and sporulation of *Fusarium oxysporum* f. sp. *lentis*. Potato dextrose agar and Richard's agar were the best medium for radial growth and sporulation of *Fusarium oxysporum* f. sp. *lentis*. Kumar *et al.*, (2019) found that fungus grew the best on PDA and Richard's agar media among seven culture media were tested. Poorvasandhya *et al.*, (2020) reported that PDA and Czapek's dox agar media provided maximum mycelia

growth, sporulation, fresh weight and dry mycelial weight of *Fusarium oxysporum* f. sp. *udum*.

In-vitro* effect of different temperature level on growth and sporulation of *Fusarium oxysporum* f. sp. *Lentis

The temperature ranges for growth vary for all microorganisms as well as for host pathogen interactions. After 168 hrs of incubation, significantly higher mycelial growth (90.00mm) was observed at 30°C, as compared to 25°C produced 87.00mm colony diameter. A sudden fall in mycelial growth of *Fusarium oxysporum* f. sp. *lentis* was observed in both increasing and decreasing of temperature level, at 20°C, 35°C 15°C and 10°C significantly decrease mycelial growth of the fungus and yielded colony diameter 66.50mm, 58.50mm, 38.50mm and 21.00mm, respectively. On other hand Excellent sporulation density was observed under microscopic field at temperature 30°C and 25°C, respectively. Good sporulation was recorded at 20°C and Fair sporulation occurred at 35°C temperature. While, poor sporulation was observed at temperature 15°C and 10°C, respectively.

In-vitro* effect of different pH level on growth and sporulation of *Fusarium oxysporum* f. sp. *Lentis

Growth of the test fungus was obtained at all pH levels tested but it was significantly higher at pH 6.0 (90.00 mm) after 168 hrs. of incubation when compared with pH 5.0 (86.50 mm) and pH 7.0 (82.50 mm) found favourable pH level for mycelial growth. Growth of pathogen showed decline in both condition increasing or decreasing the pH level from the pH 6.0. Minimum growth was observed at pH level 8.5 yielded 57.50 mm colony diameter. Effect on sporulation also observed and found that, Excellent

sporulation was observed at pH 6.0. Good sporulation was recorded at pH 5.0 and 7.0, respectively. pH 8.0 supported Fair sporulation while, poor sporulation was observed at pH level 8.5.

Similarly, findings correlated with Chaudhary *et al.*, (2018) reported that growth of *F. udum* was maximum at 30°C after seven days of inoculation, which was reduced drastically below 10°C and above 35°C and the most suitable pH level for growth of fungus was 6.0 and 6.5 with excellent sporulation. Khilare and Rafi (2012) found that growth of *F. oxysporum* was maximum at 30°C (24.7 conidia/μl.) after seven days of inoculation, which was reduced drastically below 15°C and above 35°C and most suitable pH level for growth of fungus was 6.0 and 6.5 with 24.7 conidia/μl. Mohamed *et al.*, (2016) concluded that, growth of *F. oxysporum* was maximum at 25°C (84 mm) followed by 30°C (46 mm) and also observed that, the maximum growth of the fungus was achieved at pH 7 followed by pH 6. Kumar *et al.*, (2019) reported that Growth of *F. verticillioides* was maximum at 27°C after seven days of inoculation, which was reduced drastically below 18°C and above 30°C and also found that, the most suitable pH level for growth of fungus was 6.0 and 6.5 with excellent sporulation and Kumari (2019) reported that 30°C temperature and pH 6. 0 was found optimum for growth and sporulation of *Fusarium oxysporum* f.sp *lentis*.

In conclusion the current experiment was conducted to revealed the suitable pH, temperature and culture media required for the growth of pathogen. The findings and conclusions resulted from the study are here as follows; Among the eight tested culture media, Maximum colony diameter (90.00 mm) recorded on oat meal agar (Hi-media) medium, which was statistically at par with potato dextrose agar (Hi-media) yielded 88.57

mm colony diameter, Both the media support excellently sporulation by pathogen. After 168 hrs. of incubation, significantly higher mycelial growth (90.00 mm) was observed at 30°C, as compared to 25°C produced 87.00 mm colony diameter. A sudden fall in mycelial growth of *Fusarium oxysporum* f. sp. *lentis* was observed in both increasing and decreasing of temperature level. The excellent sporulation density was also observed under microscopic field at temperature 30°C and 25°C. It was found that pathogen grow at all pH levels tested but it was significantly higher at pH 6.0 (90.00 mm) 168 hrs. after incubation when compared with pH 5.0 (86.50 mm) and pH 7.0 (82.50 mm) found favourable pH level for mycelial growth, Excellent sporulation was observed at pH 6.0. Good sporulation was observed at pH 5.0 and 7.0.

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