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COMPARATIVE ASSESSMENT OF CULTURE MEDIA, PH, AND TEMPERATURE ON THE GROWTH DYNAMICS OF *FUSARIUM OXYSPORUM* F. SP. *LENTIS*, THE CAUSAL AGENT OF LENTIL WILT

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ABSTRACT

Fusarium oxysporum f. sp. *lentis* is a major soil-borne pathogen limiting lentil cultivation in India. The present study evaluated the effects of different pH levels, culture media, and temperatures on the growth and sporulation of *F. oxysporum* f. sp. *lentis*. Among the tested media, lentil seed extract agar supported the highest mycelial growth, whereas maximum sporulation (22.9×10^5 spores ml^{-1}) was recorded on V8 juice agar. A slightly acidic pH (6.0) was most favorable for mycelial growth. The fungus exhibited optimum growth at 30°C after seven days of incubation, while growth was reduced at temperatures below 20°C and above 30°C. These findings provide baseline information on cultural requirements of the pathogen that may assist in further physiological and pathological studies.
Key words **:** *Fusarium*, Lentil, Growth, pH, media, temperature.

Introduction

Among pulses, lentil (*Lens culinaris* Medik, syn. *Lens esculenta* Moench.) is an important crop also known by other names like Masoor, Malka (bold seeded), belonging to the family *Fabaceae*. Lentil is the second most important Rabi pulse crop next to chickpea. Worldwide, lentil has great impact in agriculture because of its high protein content in seeds and straw serve as high value animal feed (Kashem, *et al*., 2014) and have the third highest protein (by weight) level after soyabean and hemp (Chamkhi *et al*., 2022).

It is an economical source of proteins, carbohydrates, minerals and fiber for resource poor people. In India, major lentil producing states are Madhya Pradesh, Uttar Pradesh, Bihar, Uttarakhand and West Bengal. In India Uttar Pradesh has first position in lentil area,production and productivity with 0.49 million ha area, 0.48 million tonnes production and 1014 kg/ha productivity (Anonymous, 2023).

In general lentil crop productivity is very low in Bundelkhand region. There are several constraints for low productivity of lentil in this region are lack of improved package and practice, water deficits and inadaptability of improved varieties low rate of seed replacement, pest and diseases. The biotic stresses have been reported triggering the reduction in the productivity of lentil by 20–25% in India (Maheshwari *et al.*, 2008).

Soil borne diseases such as wilt (*Fusarium oxysporum* f.sp. *lentis*), collar rot (*Sclerotium rolfsii*) and dry root rot (*Rhizoctonia bataticola*) are the major limiting factors in lentil production. Among them, wilt (*Fusarium oxysporum*f.sp. *lentis*) is major devastating disease of lentil in this region. It is the most important seed and soil borne disease. In India 50% yield loss reported from this disease. The disease appears at all the stage but the appearance is prominent at the seedling and the adult stage (Khare 1981; Stoilova and Chavdarov, 2006). Seedling and pre pod stages are very critical, around 100% loss reported, whereas about 67% loss when wilt occurs at flowering and podding stages, while some grain yields are produced when plant infection occur at preharvest stage (Garkoti *et al.*, 2013). Under the favourable condition it may

causes severe damage and complete crop failure (Chaudhary and Kaur, 2002; Chen, *et al*., 2011).

Temperature, water activity, and pH significantly influence fungal growth and development. The type of carbon and nitrogen sources, along with variations in pH, temperature, incubation period, agitation, and inoculum size, also markedly affect pathogen growth. The present study demonstrates the effects of different pH levels, culture media, and temperatures on pathogen development, providing insights into its ecological survival that may be useful for devising effective field management strategies.

Material and Methods

Isolation of Test Pathogen

Lentil plants showing typical wilt symptoms were collected from agriculture Research farm of Banda University of Agriculture & Technology and fungus was isolated by standard tissue isolation method. Sample was washed in the running tap water, and then it was cut into 1-2 cm bits. Bits were surface sterilized in the 2 per cent sodium hypochlorite for 60 seconds then bits were washed twice in the sterilized distilled water under aseptic condition. Bits were again cut into 0.2 cm and then these bits were transferred to PDA plates aseptically, incubated at 27±1^{o}C for 7 days. Based on morphological and cultural characteristics the isolate was identified as *Fusarium oxysporum* f.sp. *lentis*. The culture of as *Fusarium oxysporum* f.sp. *lentis* was sub cultured periodically at an interval of 10 to 15 days and were maintained on PDA medium at 27 ±1^{o}C.

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

To study the effect of pH (in the range of 4.5 to 9.0) on the growth of *Fusarium oxysporum* f.sp. *lentis* on PDA as the basal media was used in the current investigation and pH of the medium was maintained by adding convenient amount of NAOH and HCl. Petri plates containing sterilized PDA medium was inoculated with 5mm hyphal disc and incubated at 27±1^{o}C. Observations for mycelial growth were recorded after 7 days of inoculation. Sporulation was measured by haemocytometer.

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

Effect of seven different temperature viz; 5^{o}C, 10^{o}C, 15^{o}C, 20^{o}C 25^{o}C, 30^{o}C and 35^{o}C on the growth of *Fusarium oxysporum* f.sp. *lentis* was studied with view that these seven temperatures were closer to minimum, optimum and maximum soil temperature normally observed in Bundelkhand region during the crop season. Petri dishes containing sterilized PDA inoculated with 5mm hyphal disc of *Fusarium lentis* were incubated at 5^{o}C, 10^{o}C, 15^{o}C, 20^{o}C 25^{o}C, 30^{o}C and 35^{o}C and observations were recorded at 24 hours interval after up to 7 days.

Effect of different culture media on growth and sporulation of *Fusarium oxysporum* f.sp. *lenti*

Effect of seven different media viz; Potato Dextrose Agar (PDA), Corn Meal Agar (CMA), Oat Meal Agar (OMA), Yeast Malt Extract Agar (YMEA), V8 Juice media, Lentil Seed Extract Agar (LSEA), and Czepex Dox Agar media (CDAM) on growth (radial) and sporulation of *Fusarium oxysporum* f.sp. *lenti* were used in the current investigation. The test pathogen was inoculated by a single disc (5mm) in different culture media then incubated at 27±1^{o}C and observations were taken after the full growth on control (after 7 days). Sporulation was counted using haemocytometer.

Statistical analysis

Each experiment was carried out twice. The data from the laboratory experiment was examined using a completely randomized design (CRD). p≤5 was used to compare the data and analyzed using OPSTAT software.

Result and Discussion

Effect of different pH on the mycelial growth and sporulation of *Foxysporum* f.sp. *lentis*

Effect of different pH (4.5 -9) on mycelial growth of *Fusarium oxysporum* f.sp. *lentis* was evaluated *in vitro* on Potato Dextrose Agar (PDA) medium (Table-1). The results showed that the pathogen (*Fusarium oxysporum* f.sp. *lentis*) grown the over the range of pH (4.5-9). The maximum mycelial growth of pathogen 84.00 mm was recorded at the pH 6.0 followed by 75.86 mm at pH 6.5. However, the lowest mycelial growth of pathogen (43.50 mm) was recorded at pH 4.5 (Fig. and Plate-1). Similar observations were made by Ram (2022) who observed the maximum radial growth of *Fusarium oxysporum* f. sp. *lentis* at pH 6.0 (88.66mm) and pH 6.5 (84.33mm) also found to be favourable. The highly alkaline and acidic pH is not favourable for the best growth and sporulation of *Fusarium oxysporum* f. sp. *lentis*. Chaudhary *et al.* (2018) reported most suitable pH level for growth of fungus was 6.0 and 6.5 with excellentsporulation. The result indicates that the slightly acidic pH (6) to slightly alkaline pH (7.5) is best suited for vegetative growth of the test pathogen. There was a sharp decrement in vegetative growth of the test pathogen as increase or decrease of pH level from 6.

Table 1: Effect of different pH on colony growth of *Fusarium oxysporum* f.sp. *lentis.*

S.No.	pH	Colony diameter (mm) at 7 days
1.	4.5	43.50
2.	5.0	49.50
3.	5.5	73.12
4.	6.0	84.00
5.	6.5	75.86
6.	7.0	74.36
7.	7.5	73.24
8.	8.0	69.24
9.	8.5	66.25
10.	9.0	63.74
	C.D. at 5 %	**1.00**
	C.V.	**1.05**

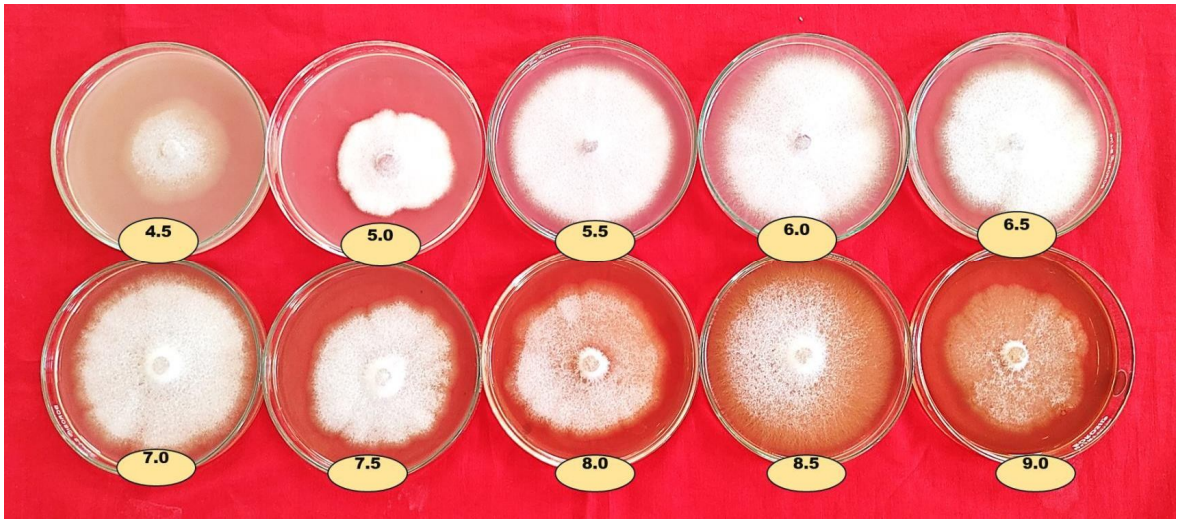

Plate 1: Effect of different pH on colony growth of *Fusarium oxysporum* f.sp. *lentis.*

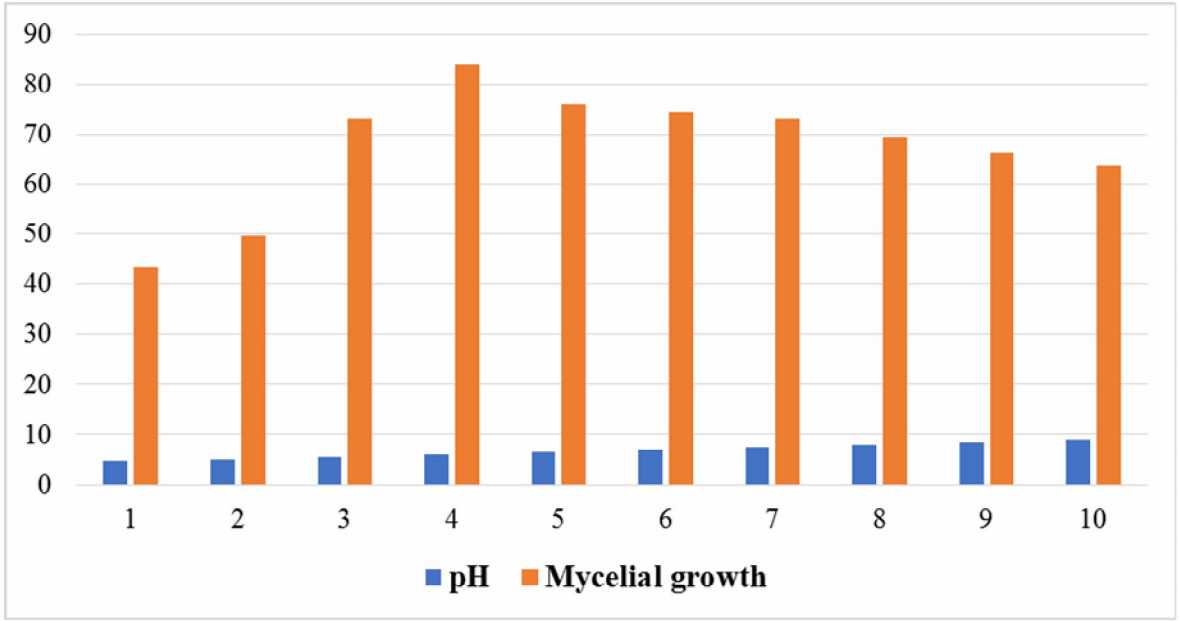

Fig. 1: Effect of different pH on colony growth of *Fusarium oxysporum* f.sp. *lentis.*

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

Effect of different temperature (5-35°C) on mycelial growth of pathogen (*Fusarium oxysporum f.sp. lentis*) *was* tested *in vitro* on PDA medium. The results revealed that the pathogen grew over the range of temperature (15 -30 °C). The maximum mycelial growth of pathogen (90.00 mm) was observed at the temperature 30 °C followed by (87.00 mm) at 25 °C (Table-2). While there was no mycelial growth recorded at 5,10 and 35 °C (Fig. 2 and Plate-2). Imran Khan *et al.* (2011) reported most suitable temperatures level for growth of *Fusarium oxysporum* f. sp. *ciceri* was 30ºC after seven days of inoculation, which was reduced drastically below 15ºC and above 435ºC. These studies are in confirmation with Anjaneya Reddy (2002) who reported that growth of 40 isolates of *F. udum* differed in their temperature requirement which varied from 200C to 350C. The effects of temperature of *F. oxysporum* f. sp. *ciceris* were studied by Landa *et al.* (2001). Chaudhary *et al.* (2018) reported that growth of *F. udum* was maximum at 30ºC after seven days of inoculation. Ram (2022) observed maximum radial growth (84.00mm) of *Fusarium oxysporum* f. sp. *lentis* at at 30 ℃ and next best 78.66mm at 25±2 ℃. The growth and sporulation of the test pathogen was slow when the temperature was increased and decreased at 30 °C. These findings of previous studies support the findings of present investigation.

Table 2: Effect of different temperature on colony growth of *Fusarium oxysporum* f.sp. *lentis.*

S.No	Temperature	Colony diameter (mm) at 7 days
1.	5	0.00
2.	10	0.00
3.	15	37.36
4.	20	70.24
5.	25	87.00
6.	30	90.00
7.	35	0.00
	C.D. at 5 %	0.29
	C.V.	0.55

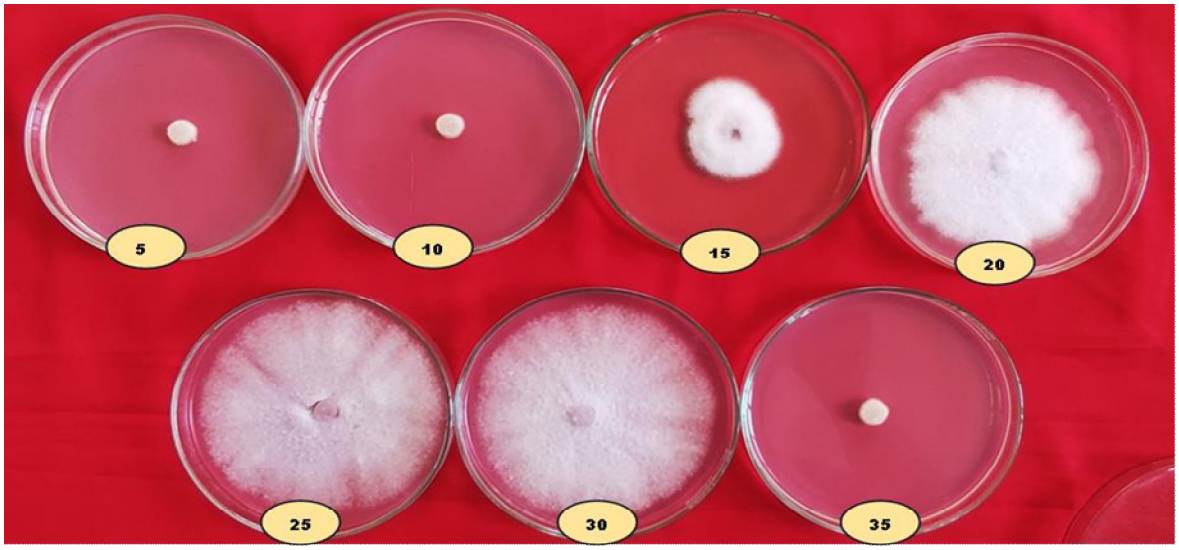

Plate 2: Effect of different temperature on colony growth of *Fusarium oxysporum* f.sp. *lentis.*

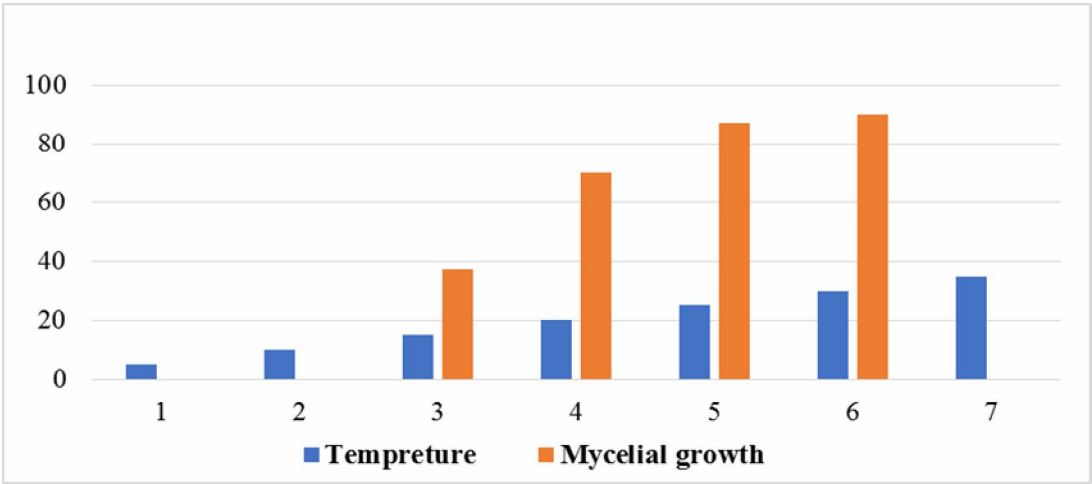

Fig. 2: Effect of different temperature on colony growth of *Fusarium oxysporum* f.sp. *lentis.*

Effect of different media on colony growth and sporulation of *Fusarium oxysporum* f.sp. *lentis.*

Effect of seven different culture media viz., Potato Dextrose Agar (PDA), Corn Meal Agar (CMA), Oat Meal Agar (OMA), Yeast Malt Extract Agar (YMEA), V8 Juice media, Lentil Seed Extract Agar (LSEA), and Czepex Dox Agar media (CDA), was tested for the colony growth and sporulation of pathogen (*Fusarium oxysporum* f.sp. *lentis) in vitro*. The results (Table-3) showed that the maximum mycelial growth of pathogen (80.00 mm) was observed with Lentil Seed Extract Agar (LSEA) medium followed by on PDA (75.50 mm) and Czepex Dox Agar medium (70.60 mm) respectively. While maximum sporulation ($22.9x10^5$ spores /ml) of pathogen was recorded on V8 Juice medium followed by Corn Meal Agar ($21 x10^5$ spores/ml) and on Potato Dextrose Agar medium ($16.2 x10^5$ spores/ml) respectively (fig.3 and plate 3). However, minimum sporulation was recorded on Oat Meal Agar medium ($7.0 x10^5$ spores/ml). These results were accordance with Khare *et al.* (1975) who reported maximum growth of *Fusarium oxysporum* f. sp. *lentis* on PDA followed by Richard's agar. Ingole (1995) also observed maximum growth of *F. udum* on Richard's agar and potato dextrose agar. Anjaneya Reddy (2002) observed maximum growth of *F. udum*on Richard's agar and potato dextrose agar. Gangadhara *et al*. (2010) studied effect of temperature on growth of *F. oxysporum* f. sp. *vanilla* isolates. Imran Khan *et al.* (2011) studied effect of media on *F. oxysporum* f.sp. *ciceris* and found that PDA is best for the growth of different isolates of FOC.

Table 3: Effect of different media on colony growth and sporulation of *Fusarium oxysporum* f.sp. *lentis.*

S.No.	Media	Colony diameter (mm) at 7 days	Sporulation
1.	Lentil Seed Extract Agar	80.00	$8.2 x 10^5$
2.	Potato Dextrose Agar (PDA)	75.50	$16.2 x 10^5$
3.	Czepek Dox Agar Media	70.60	$12.5 x10^5$
4.	Yeast Malt Agar Media	66.00	$16.0x10^5$
5.	V8 Juice media	58.00	$22.9 x10^5$
6.	Oat Meal Agar (OMA)	58.36	$7.0 x10^5$
7.	Corn Meal Agar (CMA)	66.62	$21.0 x10^5$
	C.D. at 5 %	**0.69**	**73.18**
	C.V.	**0.78**	**37.79**

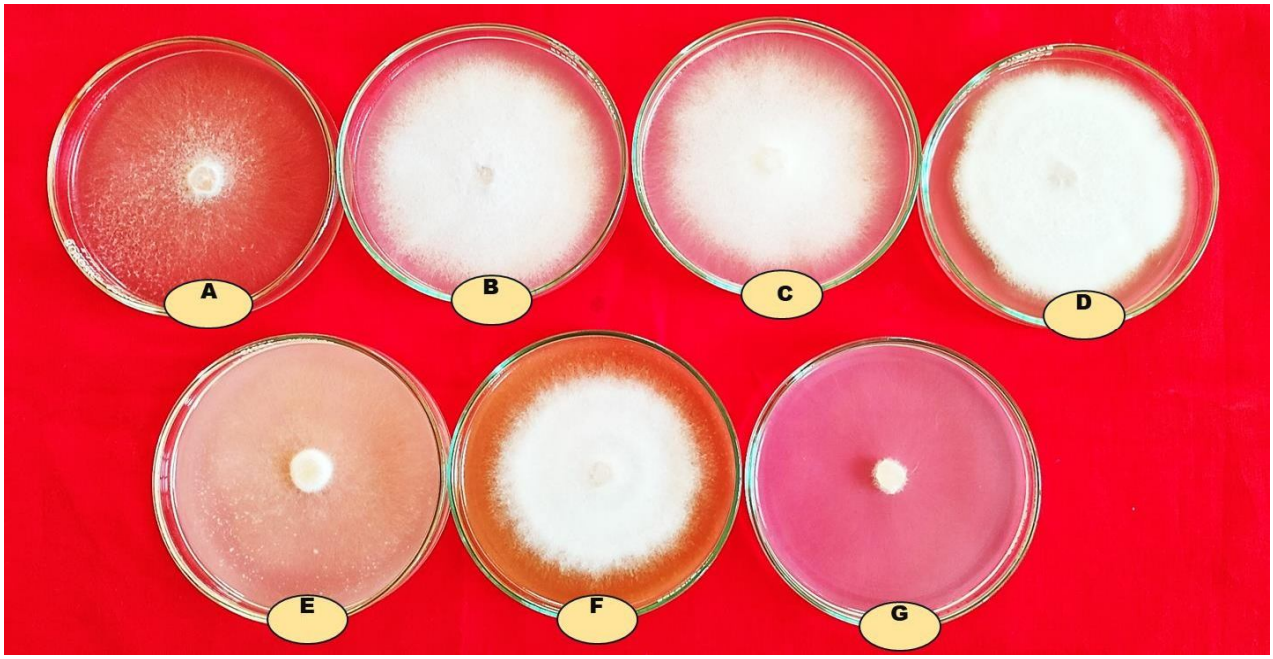

Plate 3: Effect of different media on colony growth and sporulation of *Fusarium oxysporum* f.sp. *lentis.*

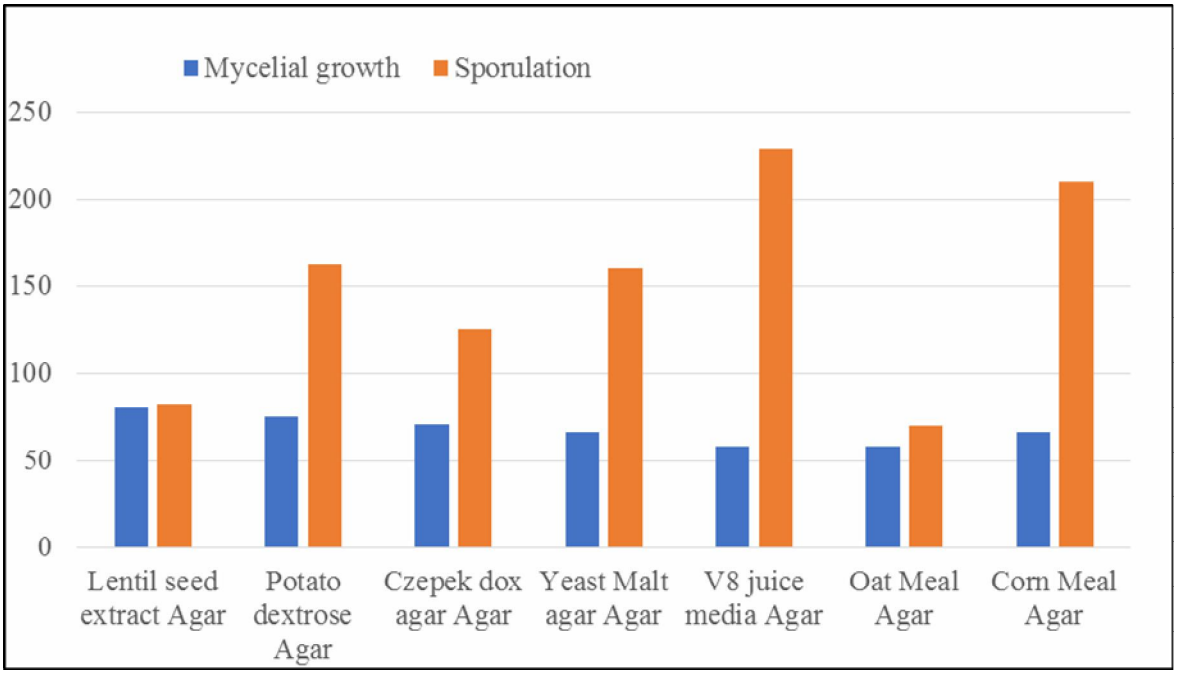

Fig. 3: Effect of different media on colony growth and sporulation of *Fusarium oxysporum* f.sp. *lentis*.

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