



Influence of various Carbon and Nitrogen Sources on Mycelial Growth of *Fusarium oxysporum* f. sp. *lini*

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ABSTRACT: Linseed is the oilseed crop with the greatest omega-3 fatty acid content. It also contains a lot of protein, fiber and 45-50% oil contain. *Fusarium oxysporum* f. sp. *lini* causes Fusarium wilts in linseed. This disease is mostly soil-borne, but it may also be seed-borne. In studying the influence of various carbon and nitrogen sources on mycelial growth of *Fusarium oxysporum* f. sp. *lini* is the need to control for other factors such as temperature, pH, and light intensity, which can also affect fungal growth. Despite these challenges, research on the influence of carbon and nitrogen sources on fungal growth has contributed significantly to our understanding of fungal physiology and metabolism. By identifying the optimal growth conditions for *F. oxysporum* f. sp. *lini*, researchers can improve fungal culture methods and develop more effective strategies for controlling Fusarium wilt disease in linseed plants. In this work, four carbon and seven nitrogen compounds were examined *In vitro* to determine the nutritional requirements of the linseed wilt fungus *Fusarium oxysporum* f. sp. *lini*. Maximum mycelial growth was found in fructose to the influence of different carbon sources on the development of the test pathogen. Fructose followed by D-glucose, Mannitol, and Glucose. All of the carbon sources used was superior to control. Potassium nitrate was determined to be the most suitable nitrogen source for growth of *F. oxysporum* f. sp. *lini*, followed by ammonium acetate. When compared to the control, the total nutrition considerably affected the development of test fungus under *in vitro* condition.

Keywords: *Fusarium oxysporum* f. sp. *lini*, linseed, carbon and nitrogen, mycelial growth.

INTRODUCTION

Linseed (*Linum usitatissimum* L.) is a major Rabi oilseed crop in India and other oil-producing countries. It is said to have originated in south-west Asia and the Mediterranean coastal lands. Linseed oil is rich in alpha-linolenic acid (ALA) and contains about 55% ALA. It serves as a good source of minerals especially, phosphorous (650 mg/100g), magnesium (350- 431 mg/100g), and calcium (236-250 mg/100g) and has very low amount of sodium (27 mg/100g) (Rabetafika *et al.*, 2011). The important linseed growing countries are India, Canada, China, USA, Russia, Egypt and Ethiopia. In India the area of linseed is (0.17 million hectares) with production (0.12 million tonnes) and productivity (671 kg/ha) (Ministry of Agriculture, GOI, 2019-2020). Linseed cake is an excellent manure and animal feed (Kasote, 2013). High-quality fiber is

produced from dual-purpose linseed straw. Linseed is also utilized in the production of paper and polymers (Gill, 1987). Madhya Pradesh has the highest yield and acreage in India, followed by Uttar Pradesh, Maharashtra, Bihar, Rajasthan, Karnataka, and West Bengal. Madhya Pradesh and Uttar Pradesh jointly produce around 70% of national linseed output (Anonymous, 2020). In India, output of this important oil and fiber producing crop is quite low. Among the several variables responsible for affecting its production, infections, particularly those caused by fungus, are thought to be a major one. Wilt, powdery mildew, rust, and Alternaria blight are the major biotic stressors impacting this crop (Kolte and Fitt 1997). Linseed wilt, caused by *Fusarium oxysporum* f. sp. *lini*, is the most damaging of the biotic stressors. Linseed wilt was first documented in India in Madhya Pradesh in 1923. Sattar and Hafiz (1952) reported wilt disease

losses of up to 80% in a linseed crop under favourable conditions. In 2011, Ahmad conducted a study to investigate the nutritional requirements of highly virulent *Fusarium oxysporum* f. sp. *ciceri*, the pathogen responsible for chickpea wilt. The study utilized various nutritional sources, including carbon, nitrogen, phosphates, amino acids, salts, vitamins, oxides, and microelements, to observe the growth response. The results indicated that glucose, potassium nitrate, dipotassium hydrogen orthophosphate, several amino acids (particularly hydroxyproline), and ferric oxide were the most favorable sources for the pathogen's growth. However, magnesium chloride and thiamine were found to be slightly unfavorable for the growth when compared with the control.

Previously Patel (2022) studied on different nitrogenous and carbon sources to know their effect on the mycelial growth of the fungus. Among the N sources evaluated, ammonium chloride was found to be most efficient for mycelial growth promotion. The most preferred carbon source recorded to promote best radial mycelial growth was starch and sucrose. Menge *et al.* (2021) studied the nutritional requirements of fusarium wilt of chickpea by utilizing various carbon and nitrogen sources. The study found that all seven carbon sources were significantly utilized by the pathogen, with Glucose and Dextrose showing maximum growth followed by Lactose. Additionally, among the seven nitrogen sources, Potassium nitrate, Calcium nitrate, and Urea were found to be the best for the growth of the test fungus. To establish the optimum dietary requirements for the development of *Fusarium oxysporum* f.sp. *lini*. For a meaningful impact, the experiment was conducted *in vitro*. As a result, this study was conducted to investigate the influence of various nitrogen and carbon sources on the mycelial growth of *Fusarium oxysporum* f. sp. *lini*.

MATERIAL AND METHOD

The lab experiment was conducted at college of Agriculture JNKVV Jabalpur-482004, Madhya Pradesh.

Various carbon sources on growth of *F. oxysporum* f.sp. *lini*. The effect of several carbon sources on the growth of *F. oxysporum* f. sp. *lini* at 20% was tested *in vitro*. Molten PDA was mixed with the appropriate amount of carbon sources. To make nutrition mixed PDA, 100 ml of PDA was mixed with 20 gram of different carbon sources in a sterile conical flask to achieve a 20% concentration. The nutrient-enriched Potato dextrose agar medium was placed onto Petri dishes and allowed to set. At the centre put a five-mm disc from a ten-day-old culture of *F. oxysporum* f. sp. *lini*. These inoculation plates were incubated aseptically at room temperature ($25\pm 2^\circ\text{C}$). The medium without nutrient sources served as control, three replications were maintained. The mycelial growth was recorded at

3, 5 and 7 days after inoculation. One factor CRD design was used for analysis purpose.

Various nitrogen sources on growth of *F. oxysporum* f. sp. *lini*. The effect of several nitrogen sources on the growth of *F. oxysporum* f. sp. *lini* at 1%, 3%, and 5% was studied under *in vitro* condition. The necessary amount of nitrogen sources was mixed with molten PDA. To make nutritional mixed PDA, 100 ml of PDA was placed in a sterile conical flask and mixed with 1, 3, and 5 gram of different nitrogen sources to produce a concentration of 1, 3, and 5%, respectively. The nutrient-enriched Potato dextrose agar medium was placed onto Petri dishes and allowed to set. At the centre put a five-mm disc from a ten-day-old culture of *F. oxysporum* f. sp. *lini*. These inoculation plates were incubated aseptically at room temperature ($25\pm 2^\circ\text{C}$). The medium without nutrient sources served as control, three replications were maintained. The observations on colony diameter of *F. oxysporum* f.sp. *lini* on different nitrogen sources were recorded 7 days after inoculation. One factor CRD design was used for analysis purpose.

RESULT AND DISCUSSION

Effect of various carbon sources on growth of *Fusarium oxysporum* f. sp. *lini*. Four carbon sources in 20 per cent were amended with Potato dextrose agar medium and the observations on mycelial growth were recorded after 3, 5 and 7 days after inoculation (Table 1 and Plate 1). Result show that maximum mycelial growth was obtained in Fructose (43.50mm). Fructose was followed by Mannital (41.33mm), D-galactose (40.00mm) and Glucose (37.00mm) against control (33.16) after three day of inoculation. For five days after inoculation, maximum mycelial growth was obtained in Fructose (57.66mm). Fructose was followed by D-galactose (55.16mm), Mannital (55.00mm) and Glucose (54.00mm) against control (49.37mm). Seven days after inoculation, maximum mycelial growth was obtained in Fructose (74.83mm). This was followed by D-galactose (70.00mm), Mannital (69.33mm) and Glucose (65.49mm) against control recorded minimum mycelial growth (60.63mm). The current findings are consistent with the findings of previous scientists; Farooq (2005) indicated that all the carbon sources were suitable for the fungus growth. However, Glucose was found to be best carbon source. Among that Glucose and dextrose showed maximum growth followed by lactose particularly Khilare and Ahmed (2011), which discovered that Maltose, Starch, Glucose, Xylose, Lactose, and Fructose were the most beneficial for the development of *Fusarium* spp. Similarly, Reynolds (2015) discovered that glucose and maltose were the optimal carbon sources for *Fusarium oxysporum* f. sp. *lini* growth and sporulation. Vasumathi and Devi (2020) also reported that glucose was found to promote fast growth of *F. oxysporum* in solid media. Menge *et al.* (2021) also work on different

effect of carbon sources on pathogen and show that all the carbon sources were significantly utilized by the pathogen. Similarly, Garuba *et al.* (2022) had earlier reported that *F. oxysporum* had the highest mycelial dry weight in starch and the lowest in fructose carbon

media. Vikram *et al.* (2022) reported among six carbon sources maximum mycelial growth was recorded in sucrose, starch, glucose, mannitol, maltose, and control and minimum was in lactose.

Table 1: Influence of various carbon sources on growth of *Fusarium oxysporum* f. sp. *lini*.

Treatment	Mycelial growth (mm)		
	3DAI	5DAI	6DAI
D-glucose	40.00	55.16	70.00
Glucose	37.00	54.00	65.49
Mannitol	41.33	55.00	69.33
Fructose	43.50	57.66	74.83
Control	33.16	49.37	60.63
CD (5%)	2.69	1.15	2.95
SE(m)	0.84	0.36	0.92

Mean of four replications

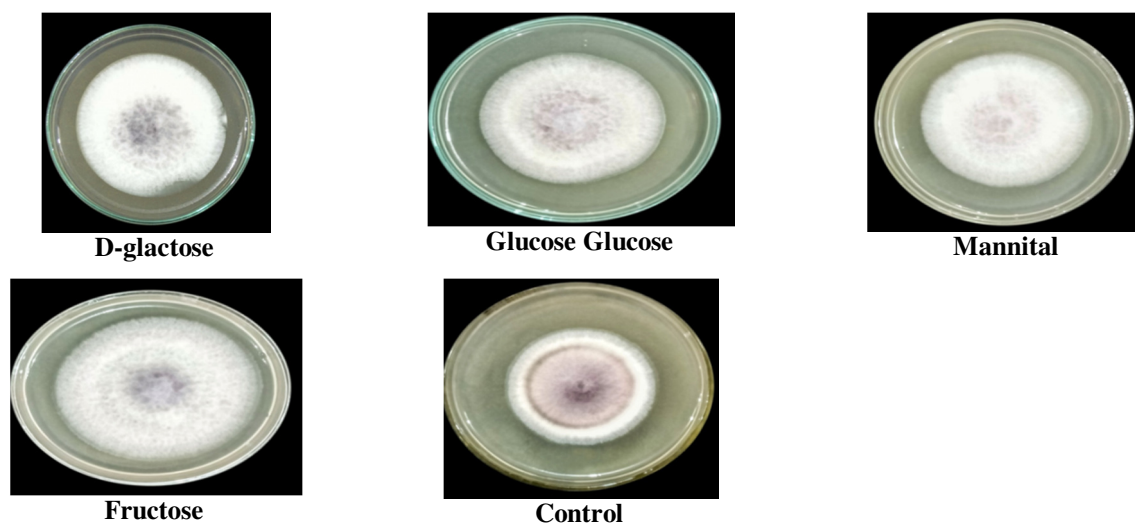


Plate 1: Influence of various carbon sources on growth of *Fusarium oxysporum* f. sp. *lini* at 20 per cent concentration

Effect of various nitrogen sources on growth of *Fusarium oxysporum* f.sp. *lini*. Evaluation of various nitrogen sources on the mycelial growth of *Fusarium oxysporum* f. sp. *lini* in Potato dextrose Agar medium) was conducted at different concentration (1, 3, and 5 %) and observation were recorded after 7 days of inoculation. Result indicated that nitrogen sources significantly enhanced the mycelial growth of test pathogen at all the concentrations after 7 days after inoculation.

At 1 per cent, maximum mycelial growth was obtained in Potassium Nitrate (75.03mm). Potassium Nitrate was followed by Ammonium acetate (71.53mm), Ammonium chloride (67.70mm), Ammonium phosphate (65.06mm), Ammonium nitrate (61.86mm), Sodium nitrate (58.73mm) and Ammonium per sulphate (00.00 mm) after seven days of inoculation (Table 2 and Plate 2). At 3 per cent, maximum mycelial growth was obtained in Potassium Nitrate (78.16mm). This was followed by Ammonium acetate (76.16 mm), Ammonium chloride (71.33mm), Ammonium phosphate (62.30mm), Ammonium nitrate (60.00mm), Singh *et al.*,

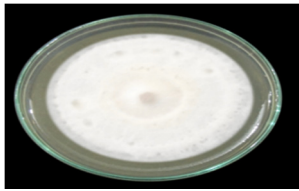
Sodium nitrate (56.50mm) and Ammonium per sulphate (00.00 mm) (Table 2 and Plate 3).

At 5 per cent, maximum mycelial growth was obtained in Potassium Nitrate (90.00mm). This was followed by Ammonium acetate (88.65 mm), Ammonium chloride (73.00mm), Ammonium phosphate (67.00mm), Ammonium nitrate (64.83mm), Sodium nitrate (64.50mm) and Ammonium per sulphate (00.00 mm) (Table 2 and Plate 4).

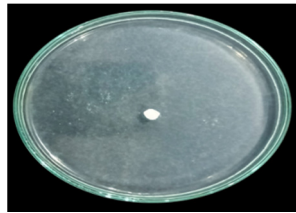
These findings are consistent with those found by Khilare and Ahmed (2011), who reported that calcium nitrate, magnesium nitrate, potassium nitrate, and urea were the most effective to growth, whereas ammonium nitrate and ammonium oxalate were less efficient. Similar results were reported by Reynolds (2015), who discovered that potassium nitrate, calcium nitrate, ammonium lactate, and ammonium phosphate are the greatest nitrogen sources for this fungus. Next in order are as paragine, potassium nitrite, ammonium sulphate, and urea. Patel (2022) also reported that ammonium chloride was found most efficient for mycelial growth promotion. Vasumathi and Devi (2020), reported that

ammonium nitrate was found to be effective for the growth of *F. oxysporum* in solid and liquid media. Menge *et al.* (2021) also work on different effect of nitrogen sources on pathogen and show that all the nitrogen sources were significantly utilized by the pathogen. From the seven nitrogen sources, Potassium

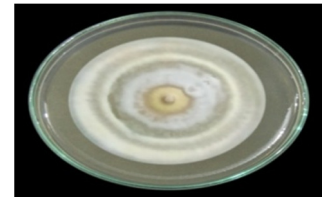
nitrate, Calcium nitrate and urea were best for growth of the test pathogen. Vikram *et al.* (2022) also reported that among six nitrogen sources maximum mycelial growth was recorded in Potassium nitrate and no radial growth was found in urea.



Ammonium acetate



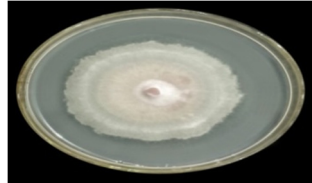
Ammonium per sulphate



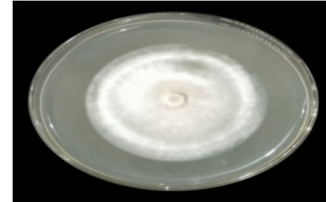
Ammonium chloride



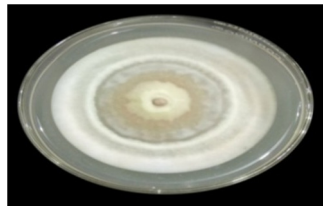
Sodium nitrate



Ammonium phosphate

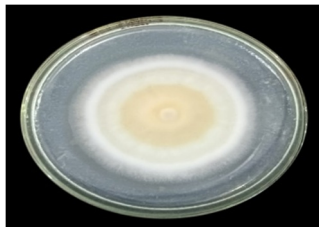


Ammonium nitrate

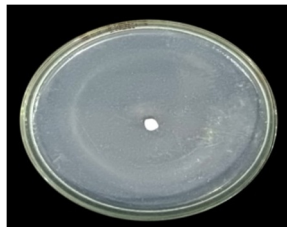


Potassium nitrate

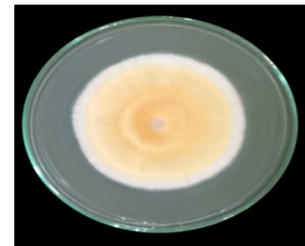
Plate 2: Influence of various nitrogen sources on growth of *Fusarium oxysporum* f. sp. *lini* at 1 per cent.



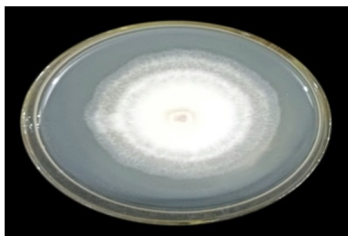
Ammonium acetate



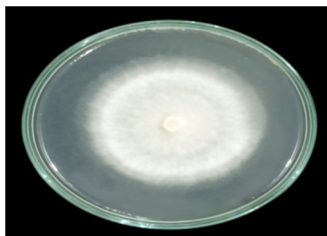
Ammonium per sulphate



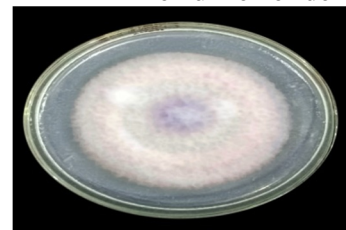
Ammonium chloride



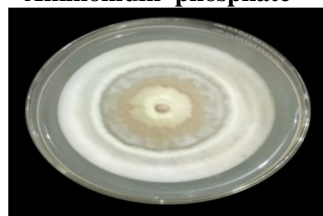
Sodium nitrate



Ammonium phosphate



Ammonium nitrate



Potassium nitrate

Plate 3: Influence of various nitrogen sources on growth of *Fusarium oxysporum* f. sp. *lini* at 3 per cent.

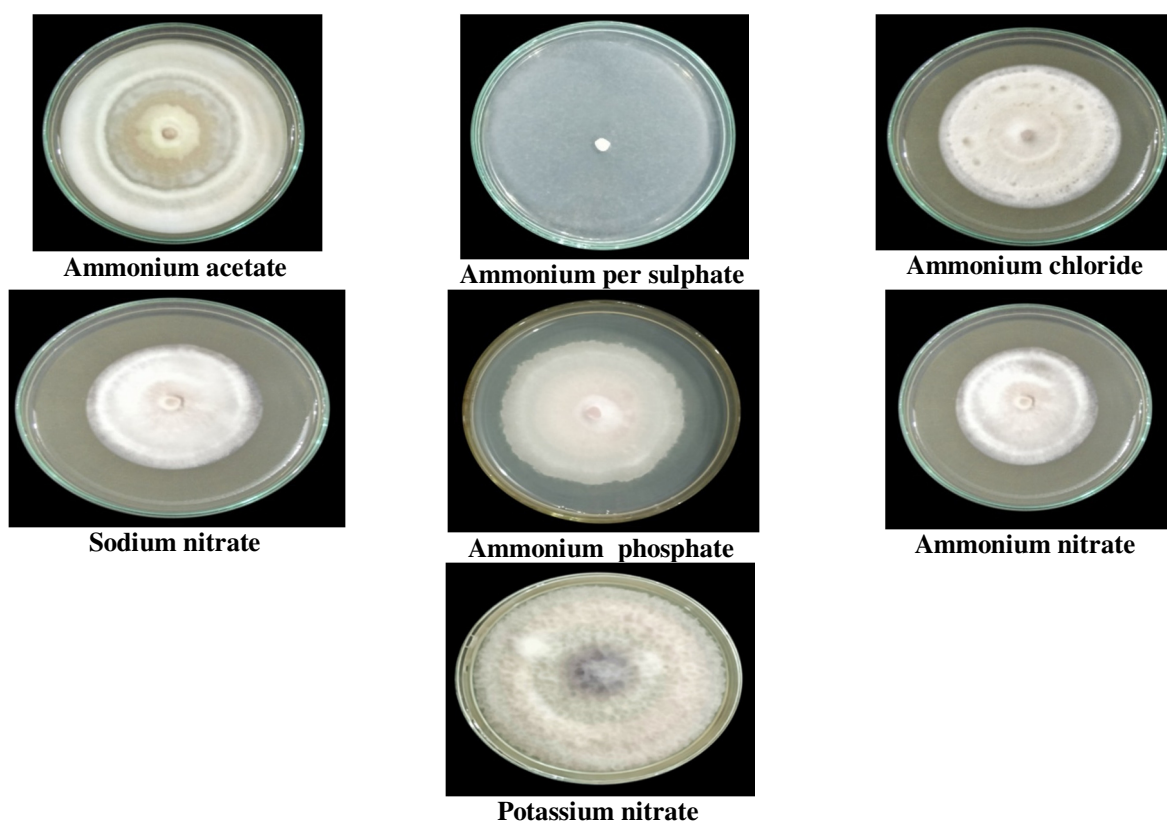


Plate 4: Influence of various nitrogen sources on growth of *Fusarium oxysporum* f. sp. *lini* at 5 per cent.

Table 2: Influence of various nitrogen sources on growth of *Fusarium oxysporum* f. sp. *lini*.

Treatment	Mycelial growth (mm) after 7 days of inoculation		
	1 per cent	3 per cent	5 per cent
Ammonium acetate	71.53	76.16	88.66
Ammonium per sulphate	00.00	00.00	00.00
Ammonium chloride	67.70	71.33	73.00
Sodium nitrate	58.73	56.50	64.50
Ammonium phosphate	65.06	62.30	67.00
Ammonium nitrate	61.86	60.00	64.83
Potassium nitrate	75.03	78.16	90.00
CD (5%)	1.51	1.24	0.76
SE(m)	0.49	3.81	2.33

CONCLUSIONS AND FUTURE SCOPE

The mycelial growth of *Fusarium oxysporum* f. sp. *lini* showed significant variation when different nitrogen and carbon nutritional sources were used. Fructose, as a carbon source, was shown to be favourable for mycelial growth. Potassium nitrate was shown to be the best nitrogen source for mycelial development. The results show which nutrients can increase the virulence of *Fusarium oxysporum* f. sp. *lini*. This study can be further expanded by investigating the effect of different environmental conditions, such as temperature, pH, and light intensity, on the growth and pathogenicity of *F. oxysporum* f. sp. *lini*. Additionally, the study can be

extended to identify potential antifungal agents that can effectively control *Fusarium* wilt disease in linseed plants. Such studies can help in the development of more efficient and sustainable strategies for controlling this devastating disease.

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Conflict of interest. None.

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