Physiological Studies of *Fusarium oxysporum* Causing Root Rot of Mulberry in Maharashtra

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ABSTRACT
A Scientific Investigation of mulberry root rot was conducted in 18 districts of Maharashtra State to assess disease incidence in farmers' fields. The disease incidence was recorded based on the number of infected or dead plants showing typical root rot symptoms. The pathogen was isolated from infected mulberry roots and identified as *Fusarium oxysporum* through morphological characteristics. Physiological studies were carried out to determine the nutritional requirements of the pathogen using Czapek's Dox agar medium supplemented with different carbon, nitrogen, phosphate, amino acids, vitamins, salts, oxides, and trace elements. Among carbon sources, D-glucose, maltose, mannitol, D-xylose, and lactose supported maximum growth, while D-galactose was least favourable. Calcium nitrate, potassium nitrate, magnesium nitrate, and urea were most effective nitrogen sources, whereas ammonium salts were less supportive. Di-potassium hydrogen orthophosphate and calcium phosphate enhanced growth among phosphate sources. Several amino acids, including L-cysteine, L-glutamic acid, glycine, and L-lysine, promoted abundant growth. Inositol and thiamine were found to be the most favourable vitamins, while others such as folic acid and niacin were less effective. Magnesium chloride showed growth comparable to control among salts. Molybdenum oxide and ferric oxide were stimulatory among oxides, while magnesium sulphate, manganese sulphate, and sodium sulphate enhanced growth among trace elements. These findings highlight the wide physiological adaptability of *F. oxysporum* and provide insights into its nutritional ecology, which may be useful in devising management strategies for mulberry root rot disease.

Keywords: *Fusarium oxysporum*, mulberry root rot, Czapek's Dox, temperature, pH, media interaction

INTRODUCTION

Mulberry belongs to the family (Moraceae). It is a fast-growing deciduous plant grown extensively for feeding the silkworms. Mulberry silk is mainly produced in Karnataka. Mulberry is grown under different types of soil and varied climatic conditions, from temperate to tropical regions. Due to the perineal nature of the crop and repeated harvesting of mulberry leaves, the soil nutrients get depleted, and the plant succumbs to many soil-borne diseases. Many pathogens, including fungi, bacteria, viruses, and nematodes, cause mulberry diseases. The data from a survey conducted in central mulberry-growing districts of southern Karnataka revealed that mulberry root rot is initiated by these fungal pathogens, namely Fusarium solani, Lasiodiplodia theobromae, and Macrophomina phaseolina. Mulberry root rot is reported both in nurseries and established gardens in different types of spacing, various soil and climatic conditions. Once the pathogen sporulates extensively, it becomes very difficult to manage. Keeping this in view, the present investigation was carried out on the different physiological characteristics of the root rot pathogens. (Baloch, A *et al.* 2018) Silk has been one of the fascinating materials in the life of man even since its discovery by the Chinese people and is aptly referred to as the queen of textiles. Sericulture, a gamut of activities necessary for silk production, was earlier confined to a few locations in India. It later developed as an industry under the patronage of Tipu Sultan and flourished in British India. With an increase in demand for silk, more and more mulberry plantations were taken up in recent years to rear more silkworms. Further promotion of sericulture by governmental agencies as a subsidiary to agriculture for improvement of rural economy has increased the area under mulberry cultivation substantially. In sericulture, production of high-quality mulberry leaves for feeding silkworms to produce cocoons of high standard is of prime importance. In India about 80% of the rural masses are engaged in agriculture which constitutes their main occupation. It forms the backbone of the Indian economy. The present status reveals that about 415 million hectares of land in India is under agricultural practices. In a tropical country like ours where the agricultural activities are season bound; sericulture can be practiced throughout the year. Keeping in view this advantage, sericulture as a cottage industry offering relatively higher returns for the modest investment thus helping the farmers in improving their socio- economic condition, is highly suitable. A large segment of

the farming community in India therefore depends for their livelihood on sericulture. Besides, millions of people are engaged in other activities related to the sericulture industry.

Silk industry provides employment to approximately 72.5 lakh persons, most of whom are small and marginal farmers. India is the second largest producer of silk and silk fabrics, next to China, with 14.57 percent share in global raw silk production. The world production of raw silk as of 2010 is 1,40,051 MT, China produces 1,15,000 MT being the first in the world. India produces 20,410 MT as a second stake holder (CSB, 2011). Out of this mulberry silk dominated with around 87% share. Mulberry sericulture in India is practiced in traditional states like Karnataka, Andhra Pradesh, Tamil Nadu, West Bengal and Jammu and Kashmir, besides some non-traditional states like Kerala, Maharashtra, Rajasthan, Orissa and Assam.

The silk production depends on the quantity and quality of mulberry leaves. Therefore, it was estimated that about 60% of the cost of cocoon production goes towards mulberry leaf production (Yokoyama ,1962, Anonymous, 1997). Mulberry diseases are a manifestation of the complex interactions of host, pathogen and environment. Diseases have always been a major limiting factor for mulberry cultivation (Sastry, 1984). The diseases of mulberry, which are prevalent under the different agro-climatic conditions of India. Diseases of the root system pore more serious problems than foliar diseases during mulberry cultivation. Root knot, root rot and nursery diseases affect the crop to a greater extent. Root rot was reported to be causing extensive damage to the mulberry plant. *Fusarium oxysporum, F.solani, Macrophomina phaseolina* and *Botryodiplodia theobromae* were reported to cause the root rot diseases in mulberry in different parts of India (Sydow and Butler, 1916). Root rot symptoms caused by *F. oxysporum* appear as sudden withering of plants and defoliation of leaves starting from bottom of the branch and progressing upward. The below ground symptoms include decaying of roots and bark which turn black due fungal spores/mycelia on severity the entire root system gets decayed resulting in the death of the plants. Affected plants after pruning, either fail to sprout or the sprouted plants bear small and pale-yellow leaves with rough surface. The severely affected plants lose the hold in the soil and can be easily uprooted. The yield losses caused by the root rot disease amounted to 10–15 % in India. Now-a-day's integrated disease management of various crop diseases has been advocated in order to avoid chemical application. Biological control is an important practice in Integrated Disease Management (IDM), which has relied heavily on pesticides, and is no longer applicable in many cases due to the lack of reliable control alternatives. Therefore, an alternative approach of biological control of plant pathogens has been recommended in recent years by trends in agriculture towards greater sustainability. So, to avoid heavy infection of root rot, rhizosphere microorganisms are found to be very helpful as biocontrol agents and hence selected in this study. In the present study the survey of mulberry root rot was done in 18 districts of Maharashtra State.

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In the present study survey on the incidence and intensity of root rot diseases in mulberry and to find out the predisposing factors responsible for outbreak of diseases under different agro climatic conditions in India. In this study the abundance and frequency of beneficial/ harmful/ saprophytic microflora in rhizosphere and rhizoplane habitats of healthy and diseased mulberry gardens was selected. The virulent *F. oxysporum* was studied on different media and noted again variation in the growth of isolates. The predisposing factors like temperature, pH, humidity and water potential were also studied against the pathogen to evaluate the survival ability. The research was similar with the findings of Khamari et al. (2018) who conducted an experiment to study influence of physiological parameters like temperature, pH and light period on growth and sporuation of *M. phaseolina* It was found that, the pathogen grew well at slightly acidic pH 6.5 (285.8 mg) followed by neutral pH of 7 (278.0 mg).

MATERIALS AND METHODS

Studies on Mulberry rhizosphere

This was studied by soil dilution plate count method as suggested by Hiltner (1904). Soil samples were collected from mulberry fields to analyze the rhizosphere and rhizoplane microflora. Samples then were brought to the laboratory in polythene bags. The dried soil samples were finally powdered, sieved and used for isolation of microorganisms. The microflora viz. bacteria, fungi, and actinomycetes of the collected soil samples were isolated by following serial dilution plate technique (Waksman 1922) by using Martins Rose Bengal agar medium.

The composition of the MRB medium was Dextrose - 10 gms,

Peptone - 5 gms, KH_2PO_4 - 1 gms,

$MgSO_4$ - 0.5 gms, Rose Bengal - Trace,

Streptomycin – 0.03 gms, Agar-Agar – 20 gms Distilled water -1000 ml

Survey of mulberry root rot

A survey of mulberry root rot was carried out in the farmer's fields in Maharashtra State. Altogether 18 fields from 18 districts were surveyed and disease incidents were recorded. The disease incidence was determined on the basis of the

number of infected / dead plants in the mulberry garden due to root rot disease, by careful examination.

Isolation of *Fusarium oxysporum*

Isolation of the pathogen was done by cutting infected roots of mulberry plant samples. The infected bits were placed on Martins Rose Bengal agar in Petri dish. The plates were incubated in the laboratory at temperature 28 ± 1^{O}C. After one week the *Fusarium* spp. were isolated and transferred to potato dextrose agar slants and maintained for further studies. Identification of isolate was done by comparing the microscopic characteristic according to Subramanian (1971) and other literature.

Physiology of *F. oxysporum*

In order to know the nutrition of virulent *F. oxysporum* causing root rot of mulberry. The effect of carbon, nitrogen, phosphates, amino acids, salts, vitamins, oxides and trace elements was observed for the growth on the Czpek's Dox agar medium. Different sources were added in the medium and inoculated with *F. oxysporum*, growth was observed after a week and the results are presented as follows.

Effect of carbon sources:

A total of 9 carbon sources were used at the concentration of 20%. It was observed that D-maltose, D-xylose, lactose, mannitol, maltose and D-glucose were most favourable(>80mm) for the growth of this pathogen. D-galactose was found to be unfavourable (<70mm) when compared with other carbon sources. Growth is totally absent in absence of carbon sources. The growth habits related to carbohydrate utilization are unique.

Effect of nitrogen sources:

It was seen that Calcium nitrate, magnesium nitrate, potassium nitrate and urea were most favorable for the growth (>80mm), whereas ammonium nitrate, ammonium oxalate, ammonium sulphate, sodium nitrate and sodium nitrite, silver nitrate were less effective (<50mm). All sources were studied at 0.3% concentration

Effect of phosphate sources:

A total of 5 phosphate sources were used at the concentration of 0.1%. It was noted that Di-potassium hydrogen orthophosphate, sodium dihydrogen phosphate and calcium phosphate showed higher growth (>75mm) as compared with other sources.

Effect of Amino acid sources:

The 24 amino acids were incorporated in the medium at the concentration of 0.05%. It was seen that L-cysteine, L-cystine, DL-Dopa, L-glutamic acid, glycine, L-histidine, L- hydroxyproline, L-leucine, DL- isoleucine, DL-Nor-leucine, L-lysine, DL-methionine, L- ornithine, DL-B phenylalanine, L-proline, DL-serine, DL- threonine, DL- tryptophan, L- tyrosine and DL- valine highly supported the growth (>70mm)of the pathogen when compared with other amino acids.

Effect of different vitamins:

A total of 7 vitamins were used in this study. It was noted that inositol and thiamine were most favourable (>80mm) for the growth of this pathogen. Folic acid, L-ascorbic acid, niacin, riboflavin and D-biotin was found to be unfavourable (>70mm) for the growth of this pathogen, when compared with control.

Effect of different salts:

A total of 9 salts in the form of chloride were used in the medium at 0.05%. Indicated that only magnesium chloride alone showed close similar growth (81.5 mm) when compared with control (81.9 mm). Rest of all the salts like aluminium chloride, barium chloride, calcium chloride, ferric chloride, potassium chloride and zinc chloride showed slightly unfavourable to the growth of pathogen when compared with control.

Effect of different oxides:

A total of 7 sources of oxides were used in the medium at the concentration of 0.05%. It was noted that molybdenum oxide and ferric oxide showed higher growth than that of control. Calcium oxide, mercuric oxide was less favorable when compared with other oxide sources.

Effect of different trace elements:

Cobaltous sulphate, copper sulphate, ferrous sulphate, magnesium sulphate, manganese sulphate, sodium sulphate, zinc sulphate and nickel sulphate were used at the concentration 0.01%. It was interesting to see that magnesium sulphate, manganese sulphate and sodium sulphate showed an increase in the growth of pathogens when compared with control. Zinc sulphate, cobaltous sulphates, copper sulphate and ferrous sulphate were found to be inhibitory for the growth of *F. oxysporium*.

Effect of different carbon sources on the growth of *F. oxysporum*

Sr. No.	Carbon sources	*F. oxysporum* growth (mm)
		HV*
1	D-fructose	80.55
2	D-galactose	60.77
3	D-xylose	81.66
4	Lactose	81.66
5	Mannitol	78.12
6	Maltose	84.55
7	Starch	82.55
8	Sucrose	71.66
9	D-glucose	82.12
10	Control	00.00
	Mean	60.77
	C.D. (P=0.05)	6.43

* Virulent

Effect of different nitrogen sources on the growth of *F. oxysporum*

Sr. No.	Nitrogen sources	*F. oxysporum growth* (mm)
		HV*
1	Ammonium nitrate	52.66
2	Ammonium oxalate	40.33
3	Ammonium sulphate	31.11
4	Calcium nitrate	80.99
5	Magnesium nitrate	81.44
6	Potassium nitrate	83.22
7	Sodium nitrate	71.66
8	Sodium nitrite	67.77
9	Silver nitrate	33.44
10	Urea	82.55
11	Control	77.00
	Mean	31.11
	C.D. (P= 0.05)	17.27

* Virulent

Effect of different phosphates on the growth of *F. oxysporum*

Sr. No.	Phosphate sources	F. oxysporum growth (mm)
		HV*
1	Di-potassium hydrogen	84.11
2	Ammonium phosphate	73.22
3	Potassium dihydrogen phosphate	72.68
4	Sodium dihydrogen phosphate	83.67
5	Calcium phosphate	77.22
6	Control	69.64
	Mean	72.68
	C.D. (P= 0.05)	6.32

* Virulent

Effect of different amino acids on the growth of *F. oxysporum*

Sr. No.	Amino acids sources	*F. oxysporum* growth (mm)
		HV*
1	Alanine	72.12
2	DL,2-Amino-n-butyric acids	74.43
3	L-Arginine	75.42
4	DL-Aspartic acid	72.86
5	L-Cysteine	76.52
6	L-Cystine	81.46
7	DL-Dopa	82.56
8	L-Glutamic acid	80.58
9	Glycine	82.20
10	L-Histidine	80.62
11	L-Hydroxyproline	83.20
12	L-Leucine	78.55
13	DL-iso-Leucine	78.56
14	DL-Nor-leucine	76.89
15	L-Lysine	76.50
16	DL-Methionine	74.22
17	L-Ornithine	79.66
18	DL-B phenylalanine	78.50
19	L-Proline	80.33
20	DL-Serine	79.20
21	DL-Threonine	82.10
22	DL-Tryptophan	77.55
23	L-Tyrosine	78.56
24	DL-Valine	79.48
25	Control	75.56
	Mean	72.12
	C.D. (P= 0.05)	13.99

* Virulent

Effect of different vitamins on the growth of *F. oxysporum*

Sr.No.	Vitamin sources	F. oxysporum growth (mm)
		HV*
1	L- Ascorbic acid	58.80
2	D-Biotin	69.46
3	Folic acid	53.12
4	Inositol	80.58
5	Riboflavin	64.86
6	Thiamine	81.30
7	Niacin	60.56
8	Control	84.10
	Mean	53.12
	C.D. (P= 0.05)	10.5

* Virulent

Effect of different salts on the growth of *F. oxysporum*

Sr.No.	Salts sources	*F. oxysporum* growth (mm)
		HV*
1	Aluminum chloride	71.40
2	Ammonium chloride	62.88
3	Barium chloride	72.10
4	Calcium chloride	78.82
5	Ferric chloride	72.80
6	Magnesium chloride	81.50
7	Potassium chloride	71.44
8	Zinc chloride	75.20
9	Sodium chloride	28.63
10	Control	81.92
	Mean	28.63
	C.D. (P= 0.05)	13.51

* Virulent

Effect of different oxides on the growth of *F. oxysporum*

Sr. No.	Oxides sources	*F. oxysporum* growth (mm)
		HV*
1	Aluminium oxide	61.80
2	Calcium oxide	27.56
3	Molybdenum oxide	81.60
4	Zinc oxide	74.80
5	Mercuric oxide	20.62
6	Ferric oxide	80.90
7	Magnesium oxide	72.66
8	Control	73.80
	Mean	20.62
	C.D. (P= 0.05)	24.73

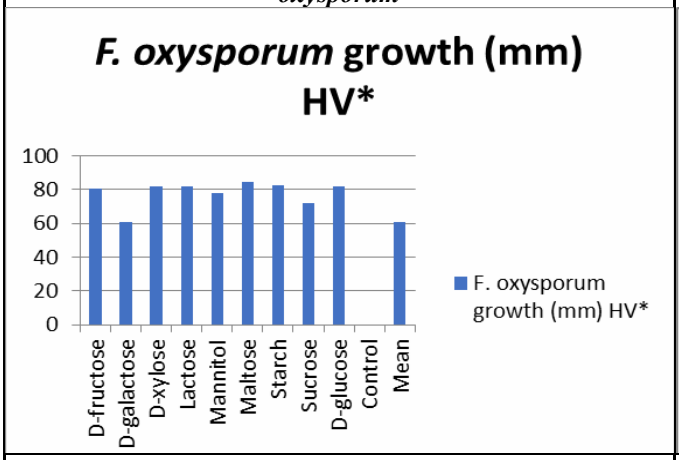
* Virulent

Effect of different trace elements on the growth of *F. oxysporum*

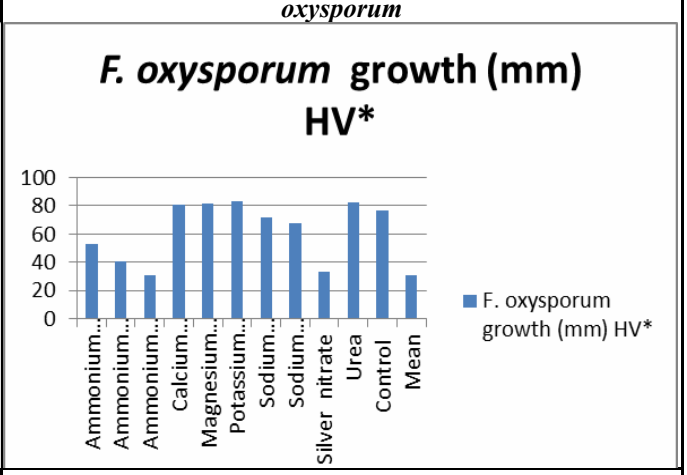
Sr.No.	Trace elements	F. oxysporum growth (mm)
		HV*
1	Cobaltous sulphate	33.66
2	Copper sulphate	54.20
3	Ferrous sulphate	52.94
4	Magnesium sulphate	87.66
5	Manganese sulphate	85.10
6	Sodium sulphate	85.70
7	Zinc sulphate	51.76
8	Potassium sulphate	70.56
9	Nickel sulphate	14.60
10	Control	74.80
	Mean	14.60
	C.D. (P= 0.05)	22.14

* Virulent

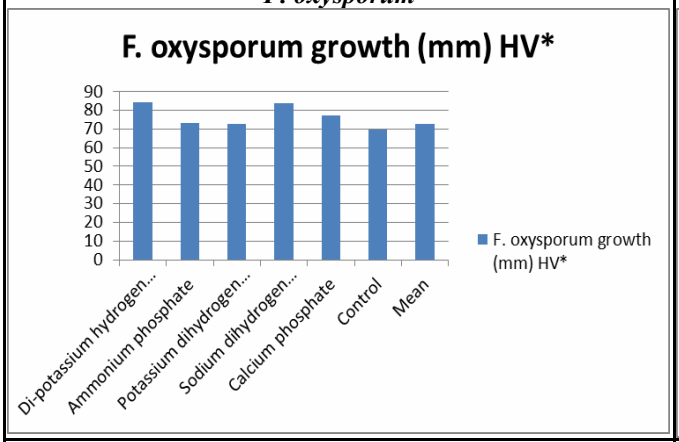
Effect of different carbon sources on the growth of *F. oxysporum*



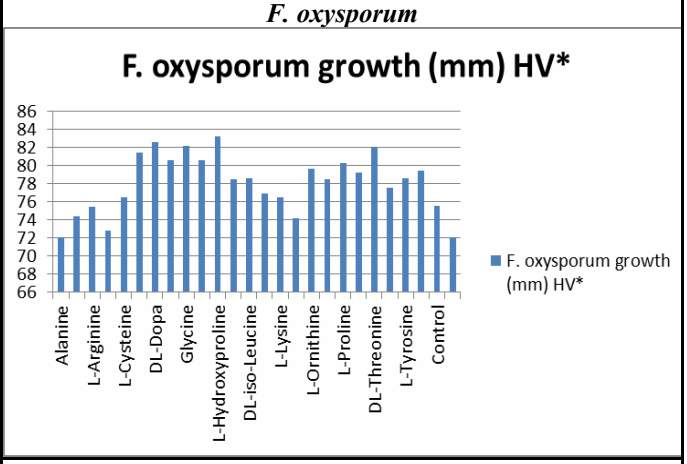
Effect of different nitrogen sources on the growth of *F. oxysporum*



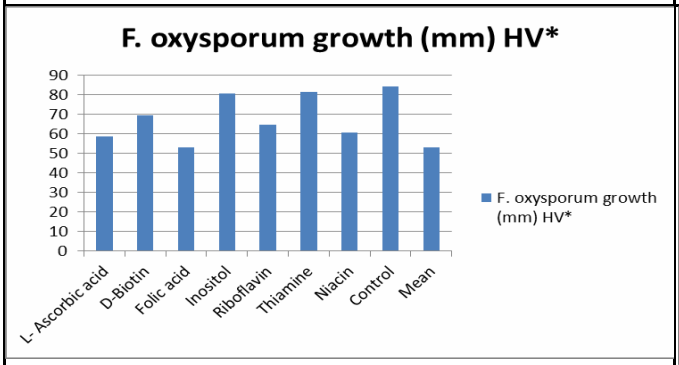
Effect of different phosphates on the growth of *F. oxysporum*



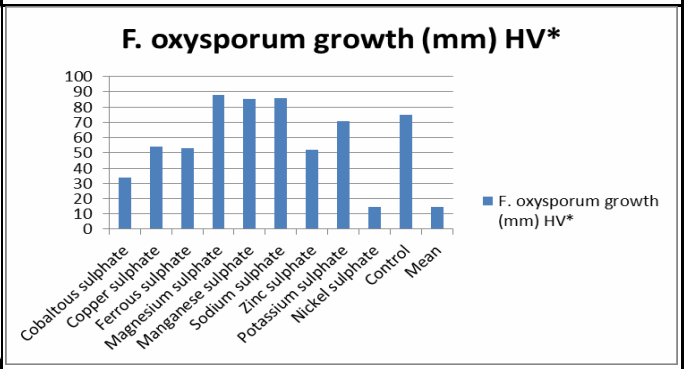
Effect of different amino acids on the growth of *F. oxysporum*



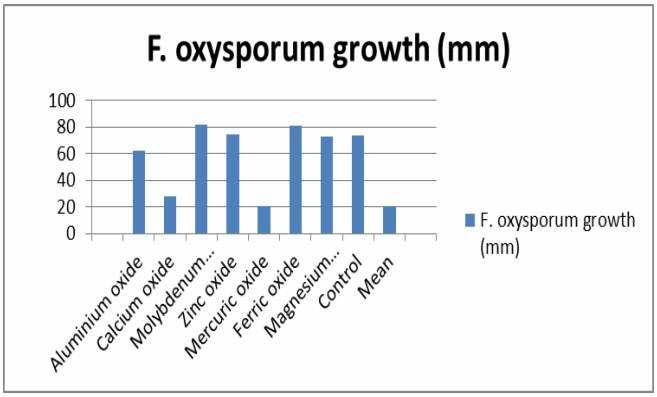
Effect of different vitamins on the growth of *F. oxysporum*



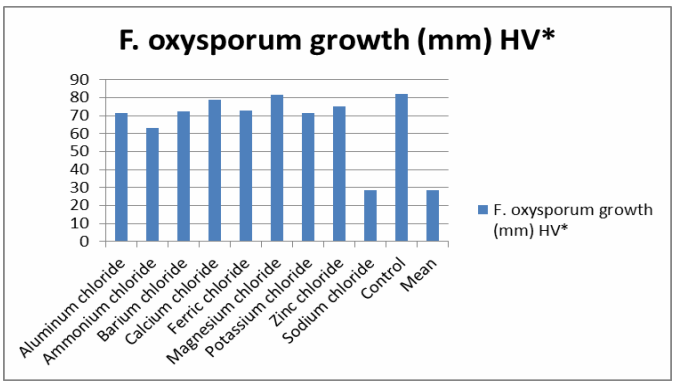
Effect of different salts on the growth of *F. oxysporum*



Effect of different oxides on the growth of *F. oxysporum*



Effect of different trace elements on the growth of *F. oxysporum*



RESULTS AND DISCUSSION

The growth of these isolates was studied in different media. Some isolates from different places showed higher growth on Czapek's Dox agar medium. Similarly, the effects of temperature and pH were examined on the survival of the isolates. We found that a temperature of 300 °C and a pH range of 5 to 6.5 was highly favourable for the growth of *F. oxysporum*. Each isolate had the ability to utilize the specific substrates for its growth and multiplication. Czapex Dox agar medium is favourable for the growth of *F. oxysporum.* Minimum growth was observed in Ashby's agar medium. Different media showed marked variation in radial mycelium growth. The interaction of different isolates and media also exhibited significance, indicating that each isolate had a distinct ability to utilize the ingredients in the media. The size of conidia is variable in different districts. The mean size of macroconidia was 17.07 × 3.75 µm; however, in microconidia, it was 8.64 × 3.14 µm. The results confirm those studied among 21 Indian isolates of *F. oxysporum F*.sp. *ciceri.* The most suitable pH levels for the growth of the fungus were 5.0 and 6.0.

The effects of pH are in confirmation with the findings of Moore (1924), who reported that two strains of *F. coeruleum could* tolerate a pH range of 3.0 to 11.0. Studies conducted on *F. oxysporum* f. sp. *nivium have indicated* that, as the pH decreases or increases from the optimum, the rate of growth gradually decreases. Studies have shown the effect of pH levels on the development of *F. oxysporum* f. sp. *vanillae isolates*. The fungus exhibited optimal growth at a pH of 5.0. The least growth was observed among all the isolates at a pH of 9.0. The results showed an optimum pH for the growth of *F. oxysporum f.* sp. *ciceri* to range from 6.5 to 7.0.

Studies indicated that *F. solani* isolates grew well at a higher temperature of 280. The fungus grew at a temperature range of 10–350 °C. However, the growth of the fungus was drastically reduced below 150 °C and started to decline above 300 °C, eventually reaching zero at 400 °C, as these temperatures did not favour the growth of the fungus. It was observed that at 250 °C and 300 °C, the fungus attained maximum growth of 76.8 mm and 85.4 mm, respectively, while at 250 °C, it reached

59.3 mm after seven days of inoculation. The soil temperature relationship indicated that a suitable temperature for the development of chickpea wilt is 25-30 °C. Gupta *et al.* (1986) reported similar findings regarding temperature requirements for this fungus. These studies confirm Anjaneya Reddy's (2002) findings, who noted that the growth of 40 isolates of *F. udum* differed in their temperature requirements, which varied from 20 °C to 350 °C. The effects of temperature on *F. oxysporum* f. sp. *ciceris* were studied. They found that disease development was greater at 25°C compared with 20°C and 30°C. The effect of temperature on *Fusarium* wilt of lettuce (*Lactuca sativa*), caused by *F. oxysporum* f. sp. *lactucae*, was observed to increase from 10°C up to an apparent maximum near 25°C. The ideal temperature was 300 °C, and a pH range of 6.0 to 7.0 was found to be suitable for growth in laboratory conditions. The role of temperature was reported in *F. oxysporum* f. sp. *vanillae*. Maximum growth occurred at 250 °C after seven days of inoculation, which was drastically reduced below 150 °C and showed zero growth at 40 °C. This work aimed to study the effect of temperature on ensuring the elimination of *F. oxysporum* f.sp. *ciceri.* Results showed the *F. oxysporum* f.sp. f *ciceri* grew highest at 300.

A temperature of 300 °C was most favourable for the growth of biocontrol agents used, including *Verticillium chlamydosporium, Trichoderma harzianum*, and *T. pseudokoningii*. However, pH 7.5, humidity of 90 per cent, and water potential of 0.1 per cent were highly favourable for these isolates for sporulation in soil, providing further control. The physiology of virulent *F. oxysporum* was studied by applying various nutritional sources. The effects of carbon, nitrogen, phosphates, amino acids, salts, vitamins, and oxides were investigated. Among the different sources, maltose, potassium nitrate, dipotassium hydrophosphate, hydroxyproline, thiamine, magnesium chloride, molybdenum oxide, and magnesium sulphate were most favourable for the growth of this pathogen.

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