Effect of Nutritional Sources on the Growth of Fusarium Oxysporum F. Sp. Capsici Causing Wilt Disease to Chilli (Capsicum Annuum L.)

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Abstract
Chilli is a valuable crop grown globally for its significant nutritional and medicinal benefits as well as its commercial importance. However, Fusarium wilt disease is a major economic constraints in its production. It has emerged as a significant biotic threats to profitable cultivation of chilli crop. Therefore, present study was undertaken with the objectives viz. effect of carbon, nitrogen sources and vitamins on the growth of Fusarium oxysporum f. sp. capsici isolates i.e. sensitive, resistant and resistant mutant. The nutritional requirement of targeted pathogens was carried out by using various carbon and nitrogen sources and vitamins. All the eleven carbon sources were significantly utilized by the pathogen. Among that dextrose showed a maximum growth to sensitive, cyclodextrine to resistance and dextrose and xylose to resistant mutant. Succinic acid showed a less effect and control the mycelial growth of all isolates. From the ten different nitrogen sources urea, ammonium nitrate and sodium nitrate were best for the growth of tested isolates of F. oxysporum while silver nitrate and cobalt nitrate completely inhibited the mycelial growth of all isolates. Among the six different vitamins ascorbic acid was suitable for the growth of all isolated of tested pathogen at 0.1% concentration while folic acid showed a less growth at 0.3% concentration.

Keywords: Capsicum annuum L., Fusarium wilt, carbon sources, Nitrogen sources, vitamins.

Introduction:
Chilli (Capsicum annuum L.) is a commercially and economically important vegetable cum spice crop cultivated in almost all over tropical and sub-tropical regions in the world. Chilli is a perennial crop belonging to family solanaceae. It is originated from south and Central America but in India it was introduced by the Portuguese in the 19^{th} century in Shimla hills. Regions to region chilli has a various name from their size, shape and colour. In India different varieties of chillies are cultivated for vegetables, pickles, condiments and spice viz. Kashmiri, Guntur, Byadgi, Sankeshwari, Bhut Jolokia, Jwala and Kanthari.
Green chillies are known to be a rich source of antioxidants and vitamins viz. A, C and K, minerals like (potassium, phosphorus, magnesium, iron, manganese and copper), protein and carbohydrate. They are also a good source of dietary fiber and capsaicin compound which is responsible for their heat and offers various health benefits. It is a digestive stimulant and cure for rheumatic troubles. Chillies have been used

since long for pain relief as they are known to inhibit pain messengers. Extracts of chilli peppers are used for alleviating the pain of arthritis, headaches, burns and neuralgia. It is also claimed that they have the power to boost immune system and lower cholesterol. (Source: https://agmarknet.gov.in)
India is the leading country in producing and consuming the chilli crop in world. In India chilli has grown for two purpose, one for vegetable i.e. green chilli and second for spices i.e. red chilli. The area under chilli cultivation is about 1.96 lakh hectares with a production of 20.60 lakh tonnes and the productivity is 4109kg/ha during the year 2022-2023 (https://angrau.ac.in; https://pjtsau.edu.in). Day by day the chilli production is increases due to their economic value. Though the chilli crop is profitable but sometime the production and productivity of this crop on farmer's field is poor. There are several factors are responsible for the low productivity. Among them one of the major factors is diseases caused due to the biotic agents i.e. fungus and bacterial. The major diseases of chilli crop is caused by fungal pathogens viz. F. oxysporum f. sp. capsici, C. capsici, C. gloeosporioides, Rhizoctonia solani, Phytophthora capsici, Leveillula taurica and Pythium aphanidermatum.
Among these pathogens F. oxysporum f. sp. capsici is a severe fungal pathogens on chilli crop causing Fusarium wilt disease to chilli fruit. This is the major economic constrain in chilli production reducing productivity, quality and the market value of chilli globally. Basically Fusarium is a soil borne fungus. Once the farm is infected with Fusarium oxysporum pathogen, they may survive in the soil for many years. Fusarium wilt is an important disease in dry and warm season. Fusarium wilt is the most predominant disease which is losses around 10-50 percent chilli crop production in the world and 10-80 percent in India.[1] The present work depicts the role of various nutritional sources on the growth of F. oxysporum f. sp. capsici in survival to develop the cultural disease management practices in agriculture.

Materials and Methods:

Fungal Isolation:

Chilli plants exhibiting wilt disease symptoms caused by Fusarium oxysporum were collected during a roving survey conducted in chilli-growing regions of Maharashtra state. Wilted chilli fruits were surface sterilized by 1 percent $HgCl_2$ solution. Sterilized chilli fruits were cut longitudinally by sterile sharp blade and placed on Potato dextrose agar (PDA) media in a petriplates. Associated fungus was isolated from growing edge young mycelium and recultured its on same media in new petriplates to obtained pure culture along with macro, micro and Chlamydospores for identification and its pathogenicity was tested on chilli fruit. For further studies sensitive and resistant isolates of F. oxysporum was obtained through their sensitivity tested against biopesticide from tested fifteen isolates and resistant mutant was obtained through mutagenic treatment (SP, UV, EMS and SA) and their sensitivity against biopesticide. These isolated of F. oxysporum were obtained through the sensitivity which was tested against biopesticide.[10] Each isolates of F. oxysporum f. sp. capsici were performed their growth against various nutritional sources. Cultures of the same isolates on PDA slants were stored at 4^0C for further use.

Effect of various nutritional sources:

Various nutritional elements effect were studied on the mycelial growth of F. oxysporum f. sp. capsici. Altogether eleven carbon sources viz. Maltose, sucrose, xylose, lactose, starch, dextrose, Galactose, fructose, cyclodextrine, cellulose and succinic acid; ten nitrogen sources viz. ammonium nitrate, urea, sodium nitrate, silver nitrate, aluminium nitrate, barium nitrate, calcium nitrate, cobalt bitrate, peptone and potassium nitrate at 0.1, 0.5 and 1.0 percent concentration and six vitamins viz. ascorbic acid, folic acid, niacin, pyridoxine, riboflavin and thiamine at 0.1 and 0.3 percent concentration of each elements were incorporated into potato dextrose agar (PDA) medium and it was poured into sterile petriplates. Five mm disc from 7 days old culture of sensitive, resistant and resistant mutant isolates of F. oxysporum f. sp. capsici was used for the inoculation of same plates. The inoculated plates were incubated at 27±2^{0}C temperature for seven days. The linear growth of Fusarium oxysporum was measured on each nutritional sources and it's compared with control i.e. without nutritional sources.

Results:

1) Effect of carbon sources:

Elven different carbon sources were used for the growth and sporulation of sensitive, resistant and resistant mutant isolates of F. oxysporum and they was showed a significant effect on their growth and sporulation and it is depicted in table1 and fig.1. The results indicated that the growth of F. oxysporum sensitive isolate was maximum in dextrose (88.33mm) and minimum in succinic acid (13.33mm) while in other carbon sources the growth was recorded in between dextrose and succinic acid. Resistant isolate showed the highest growth in cyclodextrine (89.33mm) and least growth succinic acid (16.42mm) while the other growth range was recorded in between cyclodextrine and succinic acid. In resistant mutant isolate the maximum growth was showed in dextrose and xylose (89.67mm) followed by sucrose (89.00mm), fructose (88.67mm), lactose (88.33mm), maltose (88.00mm), starch (87.33mm), cyclodextrine (84.33mm), cellulose (82.67mm), galactose (81.33mm) and succinic acid (20.00mm) as compared with control (46.67mm) without a carbon source.

2) Effect of nitrogen sources:

Ten different nitrogen sources were used for this study and the results of same are shown in table. 2 and fig. 2. It was seen that the nitrogen sources showed a significant variation in the growth of sensitive, resistant and resistant mutant isolates of F. oxysporum. The highest mycelial growth of F. oxysporum was recorded in urea for sensitive isolates (90.00mm); ammonium nitrate, urea and sodium nitrate for resistant isolate (90.00mm) and urea for resistant mutant (90.00mm) at 0.1% concentration. Followed by this maximum mycelial growth was observed in ammonium nitrate to sensitive isolate (86.67mm); ammonium nitrate and sodium nitrate to resistant and resistant mutant isolate (90.00mm) at the rate of 0.5% concentration. The maximum mycelial growth of sensitive isolate was noted in sodium nitrate (86.00mm); resistant and resistant mutant (90.00mm) in ammonium nitrate and sodium nitrate at 1.0 % concentration of F. oxysporum when compared with without nitrogen source.

3) Effect of vitamins:

A total of six vitamin sources were used for the growth and sporulation of F. oxysporum (Table. 3). It was noted that the maximum growth of F. oxysporum was observed in ascorbic acid in all isolates i.e. sensitive, resistant and resistant mutant (42.00mm, 44.67mm, 47.67mm) at 0.1% concentration followed by niacin, riboflavin, thiamine, pyridoxine and folic acid. At the higher concentration i.e. 0.3% of ascorbic acid shows more favourable growth to sensitive, resistant and resistant mutant (39.33mm, 42.00mm and 47.00mm) as compared with control. Pyridoxine and folic acid showed least growth as compared to control. Ascorbic acid were most favourable vitamin to all isolates for their growth and sporulation at both concentration.

Discussion:

Different types of fungi on different plants have different nutrient requirements. Because depending on each tree the deficiency of nutrients in that fungus is slightly more or

Table 1. : Effect of different Carbon Sources on the growth of F. oxysporum on PDA Plates.

Sr. No.	Carbon Sources	Diameter of the mycelial growth in mm		
		Sensitive	Resistant	Resistant Mutant
1	Maltose	85.67	85.67	88.00
2	Sucrose	88.00	86.67	89.00
3	Xylose	87.00	86.67	89.67
4	Lactose	85.00	85.00	88.33
5	Starch	82.67	85.67	87.33
6	Dextrose	88.33	86.67	89.67
7	Galactose	77.67	76.00	81.33
8	Fructose	84.33	86.33	88.67
9	Cyclodextrine	82.33	89.33	84.33
10	Cellulose	78.00	82.67	82.67
11	Succinic Acid	13.33	16.42	20.00
12	Control	39.33	43.00	46.67
	SE	6.2576	7.1121	5.8463
	CD at 0.05%	13.6353	15.4972	12.7390
	0.01%	19.1169	21.7272	17.8604

* Value are the mean of three replicates.

less. Carbon, nitrogen and vitamins are the essential components for the fungus which are play fundamental role in their growth, development and reproductive process. In the present investigation revealed that among the tested nutritive elements dextrose, xylose, sucrose, cyclodextrine, cellulose and Galactose from carbon; urea, ammonium nitrate and sodium nitrated from nitrogen sources and ascorbic acid from vitamins were the most favorable for the growth of Fusarium oxysporum f. sp. capsici. Similar results were made by some researcher like as Farooq (2005) stated that all carbon sources were suitable for the fungus growth.[7] Patel (2020) were tested the nine different Carbone sources and ten different nitrogenous (N) sources on PDA medium to know their effect on the mycelial growth rate of Fusarium solani NVS671. Among the carbon sources they evaluated that to promote the best radial mycelial growth was Starch (7.96±0.05cm) and Sucrose (7.93±0.05cm). The most preferred N sources recorded for the best radial mycelial growth of Fusarium solani NVS671 was in Ammonium chloride

Table 2. : Effect of different Nitrogen Sources on Mycelial growth of F. oxysporum in vitro.

Sr. No.	Nitrogen Sources	Conc. %	Diameter of Mycelial growth (in mm)		
			Sensitive	Resistant	Resistant Mutant
1	Ammonium Nitrate	0.1	85.67	90.00	88.33
		0.5	86.67	90.00	90.00
		1.0	81.33	90.00	90.00
2	Urea	0.1	90.00	90.00	90.00
		0.5	50.67	49.67	50.33
		1.0	34.33	36.00	36.00
3		0.1	75.00	90.00	86.00

	Sodium Nitrate	0.5	83.67	90.00	90.00
		1.0	86.00	90.00	90.00
4	Silver Nitrate	0.1	0.00	0.00	0.00
		0.5	0.00	0.00	0.00
		1.0	0.00	0.00	0.00
5	Aluminium Nitrate	0.1	43.67	47.00	50.33
		0.5	35.33	40.00	45.67
		1.0	32.67	35.67	38.67
6	Barium Nitrate	0.1	35.33	38.00	41.00
		0.5	38.33	40.33	43.33
		1.0	43.00	45.00	50.00
7	Clacium Nitrate	0.1	38.67	47.67	58.33
		0.5	35.33	42.33	61.00
		1.0	32.00	40.67	65.00
8	Cobalt Nitrate	0.1	0.00	0.00	0.00
		0.5	0.00	0.00	0.00
		1.0	0.00	0.00	0.00
9	Peptone	0.1	58.00	62.00	64.33
		0.5	56.00	67.00	69.33
		1.0	58.33	68.67	73.00
10	Potassium Nitrate	0.1	35.67	45.00	56.00
		0.5	32.00	39.67	59.00
		1.0	28.00	33.67	65.33
11	Control		42.33	47.33	48.33
	SE		4.3164	4.4006	3.7039
	CD at 0.05%		9.6169	9.8045	8.2522
	0.01%		13.6786	13.9455	11.7376

* Value are the mean of three replicates.

Table 3. : Effect of vitamins on the growth of F. oxysporum in Vitro.

Sr. No.	Sources of Vitamins	Conc. %	Diameter of Mycelial growth (in mm)		
			Sensitive	Resistant	Resistant Mutant
1	Ascorbic acid	0.1	42.00	44.67	47.67
		0.3	39.33	42.00	47.00
2	Folic acid	0.1	28.33	26.33	33.33
		0.3	24.67	26.33	30.00
3	Niacin	0.1	40.33	42.67	43.67
		0.3	32.67	34.33	41.00
4	Pyridoxine	0.1	25.33	33.00	35.67

		0.3	26.67	30.00	32.00
5	Riboflavin	0.1	39.33	43.33	43.67
		0.3	38.33	41.00	45.00
6	Thiamine	0.1	29.33	35.00	36.67
		0.3	27.33	31.00	33.00
7	Control		35.67	40.67	44.00
	SE		1.7656	1.8193	1.7169
	CD at 0.05%		4.3204	4.4518	4.2012
	0.01%		6.5450	6.7441	6.3645

* Value are the mean of three replicates.

(7.96±0.11cm), Ammonium nitrate (7.7±0.1cm) and Ammonium sulphate (7.3±0.1cm).[14] Menge et al., (2021) noted that among the seven carbon sources Glucose and Dextrose showed a maximum growth followed by Lactose and from the nitrogen sources Potassium nitrate, Calcium nitrate and Urea were to showed a best for the growth of Fusarium oxysporum f. sp. capsici.[12]
Similarly Garuba et al., (2022) was reported as earlier that the F. oxysporum had shown the highest mycelial dry weight in Starch and lowest in Fructose carbon media.[9] Singh et al., (2023) reported the effect of 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. The maximum mycelial growth of the fungus was found in carbon sources in Fructose and in nitrogen sources in Potassium nitrate.[16] Buttar et al. (2024) noted that sucrose supported

Fig. 1. Effect of various carbon sources on the mycelial growth of F. oxysporum.

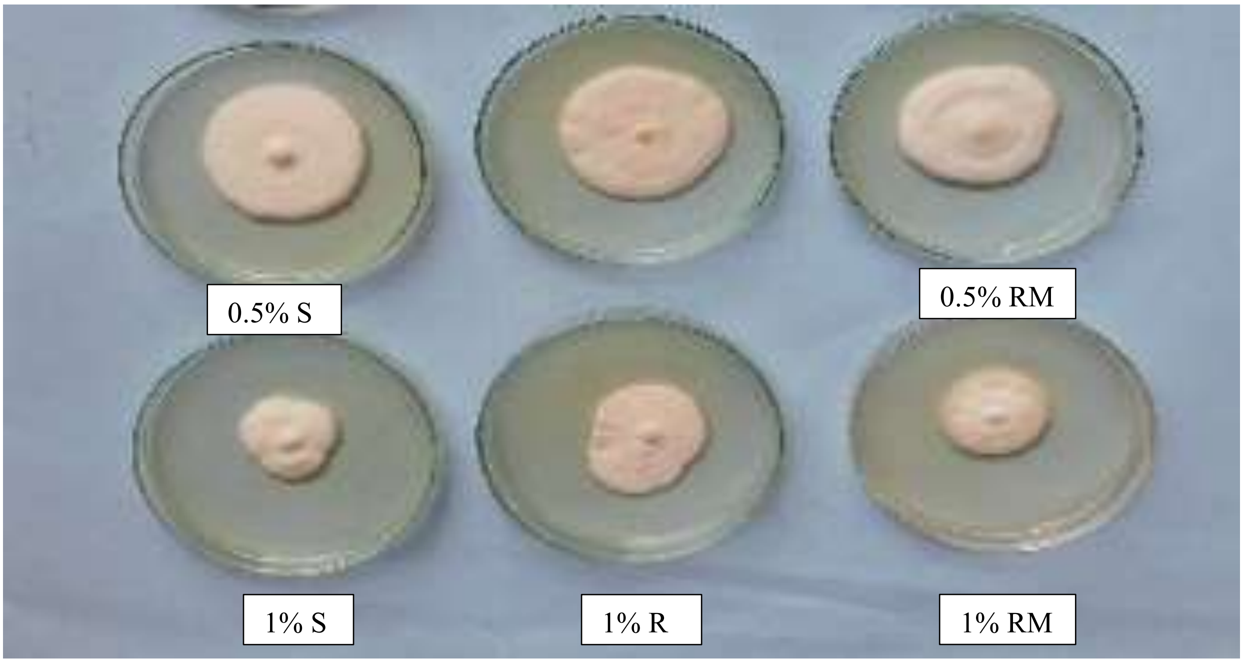

Fig. 2. Effect of Urea at different concentrations on F. oxysporum mycelium growth.

maximum growth and sporulation of both F. equiseti and F. chlamydosporum followed by other simple sugars like dextrose and maltose. Starch was least effective, while citric acid did not support growth, indicating the importance of readily metabolizable carbon sources while regarding the nitrogen sources potassium nitrate was the most effective nitrogen source for both F. equiseti and F. chlamydosporum resulting in maximum mycelial growth and sporulation, while ammonium nitrate and ammonium chloride were least effective.[3]

However in vitamin Bhargava and Tandon (1963) were studied the effect of four vitamins on the mycelial growth and reproduction of Fusarium solani, Botryodiplodia annane and Macrophomina phaseoli. They found that no marked influence of external supply of vitamins on the growth of these pathogens because they was self-sufficient with vitamins but they recorded as a slight variations on sporulation.[2] Similarly deficiencies of vitamin biotin were recorded by Meyer and Maraite (1967) for the pathogen Fusarium oxysporum f. sp. melonis [13] and by Friend (1968) for F. laterifium.[8] Prasad (1972) revealed the effect of vitamins on sporulation of F. oxysporum and F. moniliforme v. subglutinansm. He reported that the selective vitamins Thiamine, Biotin, Inositol was accelerating to macro-conidial production in F. moniligorme.[15] The effect of eight water-soluble vitamins was studied on germination, germ-tube extension, growth and sporulation of F. oxysporum f. sp. vasinfectum by EL-Abyad and Ramadan (1979).[6] Among the utilized vitamins they noted, the fungus appeared to be highly sensitive to Thiamine and Pyridoxine, moderately sensitive to Inositol and Pantothenate and least affected by Folic acid.

Khilare and Ahmed (2011) used seven vitamins on the growth of Fusarium oxysporum f. sp. ciceri causing a wilt in chickpea. They noted that all the tested vitamins to showed a less mycelial growth of pathogen when they was compared with control. Folic acid was found to be highly ineffective amongst all vitamins.[11] Cho et al. (2013) found that the thiamine dilauryl sulfate (TDS) nanoparticles effectively inhibit the mycelial growth and spore germination of Fusarium oxysporum, demonstrating superior efficacy compared to conventional pesticides.[4] Dr. Jaruhar (2024) reported that Pyridoxine hydrochloride and riboflavin significantly enhanced the mycelial growth of Fusarium oxysporum f. sp. lentis, particularly in older cultures, while D-biotin and thiamine showed no effect. In contrast, nicotinic acid, followed by folic acid and p-aminobenzoic acid, strongly inhibited fungal growth, with inhibition increasing over time.[5]

Conclusion:

Different nutritional sources significantly affect the fungal growth. So significant variation was observed in the mycelial growth of F. oxysporum f. sp. capsici when different carbon, nitrogen and vitamin sources were used. The availability of carbon, nitrogen and vitamin sources are to determine the growth rates, biomass and cellular processes like sporulation, reproduction and metabolism. In carbon sources dextrose was optimal for sensitive isolates, cyclodextrine for resistant ones and dextrose and xylose for resistant mutant to be a favorable for the healthy mycelial growth. Urea for sensitive and resistant mutant, ammonium nitrate, urea and sodium nitrate for resistant at 0.1% concentration; ammonium nitrate to sensitive, ammonium nitrate and sodium nitrate to resistant and resistant mutant at 0.5%; sodium nitrate to sensitive, ammonium and sodium nitrate to resistant and resistant mutant was shown to be the best nitrogen sources at 1% concentration for mycelial development. While ascorbic acid was the best vitamin for the growth and sporulation in all isolates of F. oxysporum.

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