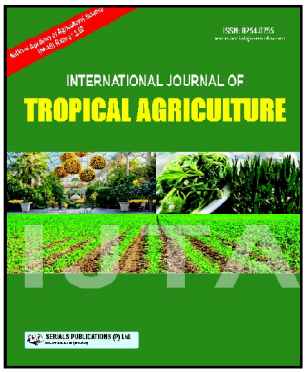

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Study of Carbon and Nitrogen Requirements in the Growth and Sporulation of *F.o.f.sp lycopersici,* the Causative Agent of Wilt of Tomato

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Abstract: From the study of growth and sporulation of *Fusarium oxysporum* f. sp. *lycopersici* in different carbon, nitrogen and C:N ratio it is observed that the fungus prefer Sucrose followed by Fructose, Maltose and Glucose at a concentration 3-4% for its growth and sporulation and found significant at 1% level. Although growth of *F.o.* f. sp. *lycopersici* in different carbon sources was observed from 1% to 10% conc but from 5% to 10% conc growth gradually decreases. In different nitrogen sources, maximum growth of the pathogenic fungus was found in Sodium nitrate at 0.1 to 0.2% concentration followed by Ammonium nitrate, Peptone and Potassium nitrate. So it reveals that the pathogenic fungus utilizes more nitrate nitrogen. In different C : N ratio the maximum mycelia growth of *F.o.* f. sp. *lycopersici* was found in C : N ratio 15 : 1 & 30 : 1 i.e. 588.5mg and 567mg. Among nitrogen concentration 1 gm and 2 gm of nitrogen / litre of media produces higher mycelia growth in respect to varied carbon treatment, than that of any other nitrogen treatment. Sporulation was also found maximum in 15:1 and 30:1 C:N ratio. A significant growth of *F.o.* f. sp. *lycopersici* was observed in different C:N ratio but with increase of concentration of C:N ratio growth of the pathogenic fungus found decreases considerably.

Keywords: *Fusarium oxysporum f.sp lycopersici* , carbon :nitrogen sources, growth , wilt disease.

INTRODUCTION

The nutritional requirement of the pathogenic fungus for disease development is an important aspect. It has been established that Fungi require external carbon and nitrogen sources for germination and infection (Cook & Schroth, 1965; Maurer &

Baker, 1965; Schroth *et. al.*, 1963). Nutritional requirement of most organisms are similar but the quantity needed varies. It is due to the capacity to utilize different sources of inorganic and organic substances to obtain the required elements. Fungi also differ widely in their ability to decompose available nitrogen resources and the ratio of C:N is also important as it primarily determine the amount of microbial growth that can occur and therefore indirectly the amount of decomposition that can take place. So the present aim of the work is to study the effect of different C:N ratio in the growth and sporulation of *F.o.f.sp. lycopersici* that causes wilt disease of tomato.

MATERIALS AND METHOD

(**In carbon sources)** Liquid Czapeks Dox medium devoid of sucrose was taken as the basal medium. Carbon compounds were tested at a concentration equivalent to carbon present in the amount of sucrose (30 g/lit) of the basal medium. Carbon sources taken were Xylose, Lactose, Galactose, Starch, Fructose, Sucrose, Maltose, Glucose and Control was made without any carbon sources. pH of the medium was adjusted to pH 5.6 and sterilized for 20 minutes and inoculated aseptically with 5 mm disc of five days old culture of *F.o.*f. sp. *lycopersici* and incubated for 12 days with three replicates. The dry wt of mycelia of the fungus were recorded and sporulation was expressed in four categories viz poor, moderate, good and excellent.

(In different concentration of carbon) Four carbon sources (Glucose, Fructose, Maltose, and Sucrose) with different concentration viz 1% to 10% were prepared in 100 ml CDA medium along with control plate and radial growth of the fungus were recorded after 7 days.

In nitrogen sources : Liquid Czapek Dox medium devoid of nitrogen was taken as the basal medium and nitrogen sources taken are Potassium nitrate, Ammonium nitrate, Ammonium oxalate, Ammonium sulphate, Phenylalamine and Urea. Concentration of nitrogen was taken 0.2% for each treatment. pH of the media was adjusted to pH 5.6 prior sterilization. A 5 mm disc of *F.o.*f. sp. *lycopersici* was inoculated aseptically in Czapek Dox liquid medium and incubated for 12 days with 3 replicates. Mycelia mats were dried and weighed. For assessing sporulation *F.o.*f. sp. *lycopersici* was grown in different N-sources for 14 days in 10 ml each of the Czapek Dox liquid medium in test tube with 6 replicates. Degree of sporulation was determined and analysed statistically. Spore count / unit area were considered.

(In different concentration of nitrogen sources) Four nitrogen sources viz. Potassium nitrate, Sodium nitrate, Peptone and Ammonium nitrate with different concentration such as 0.0%, 0.1%, 0.2%, 0.3%, 0.4%, 1.0%, 2%, 5% were taken with Czapek Dox medium. A 5 mm disc of *F.o.*f. sp. *lycopersici* was inoculated in 20 ml sterilized media and incubated for 7 days. Average colony diameter of 5 replicates per 7 days were measured (in mm) and recorded.

(In Carbon : Nitrogen ratio) To determine the optimum requirement of C:N ratio for the growth of F.o.f. sp. lycopersici six different concentration of C:N ratio in Czapeks Dox liquid medium were used. The medium was supplemented with Sucrose and Sodium nitrate in order to supply 10, 20, 30, 40, 50, 60 g of carbon and 1, 2, 3, 4 and 5 g of Nitrogen per litre. 100 ml of sterilized medium were inoculated with 5 mm disc of *F.o.*f. sp. *lycopersici* under aseptic condition. The colonies are incubated for 12 days with 5 replicates. Mycelia mat were weighed.

RESULTS AND DISCUSSION

Mycelial growth of *Fusarium oxysporum* f. sp. *lycopersici* was recorded maximum in Sucrose (Av. 405.13 mg) with high sporulation as compared with fructose (Av 296.67 mg) followed by maltose, glucose and lowest in xylose (Av. 99 mg) (Table 1)

Table I
Effect of Carbon sources on the growth & sporulation of *Fusarium oxysporum* f. sp. *lycopersici*

Carbon Sources	*Mycelial Growth (mg) (Mean ± SD)*	** Sporulation*
Maltose	243.45±12.552	++
Galactose	193.53±9.495	++
Xylose	99±1.414	+
Starch	153.63±12.234	+
Sucrose	405.13±18.637	++++
Fructose	296.67±12.472	+++
Glucose	203.33±12.472	+
Control	22.33±2.054	+
SEd(±)	11.89	
CD (P = 0.05)	25.21	
CD (P = 0.01)	34.73	

* + = Poor, ++ = Moderate, +++ = Good, ++++ = Excellent

The growth of the colony of *Fusarium oxysporum f. sp. Lycopersici* was found from 1 to 3% level of sucrose and gradually decreases with increase of concentration in 4 to 10% level.

In case of glucose growth of the fungus was found upto 2%,3% and 4% level and after that growth decreases. In maltose and fructose growth was found upto 4%level and after that with increase of concentration its growth decreases. (Table II)

In *Fusarium* wilt of coriander and wilt of *Solanum khasianum* the growth of the pathogenic fungus was found best in sucrose followed by fructose (Mathur, 1963, Borah, 1990). In *Fusarium oxysporum f. sp.cumini* Patel and Prasad (1963), showed similar results in case of glucose, starch and galactose. Growth of the pathogenic fungus is different on various carbon sources.

The ability of the fungus to utilize certain simple form directly or on its power to convert the complex carbon compound into simpler ones vary with different organisms as well as virulence of the pathogen (White, 1927, Tandon, 1967) and others.

Good response on fructose has been reported (Lilly and Barnett, 1953) and present result showed that glucose has moderate effect on the growth of pathogenic fungus. Poor growth was observed in lactose and xylose. Jurihar and Mehta , 1977. Tandon, 1967, White, 1927 reported lactose as a poor source of carbon for the mycelial growth in *F.moniliforme, F. solani* and *F, lycopersici.*

The growth of *F.o.* f.sp. *lycopersici* in different nitrogen sources was recorded maximum in Sodium nitrate (Av 583.2mg), Ammonium nitrate and Peptone (Av 490mg &Av 480mg). Sporulation was found more in Sodium nitrate, Ammonium nitrate and Potassium nitrate and recorded $11.2^{L}/cm^{3}$, $9.1^{L}/cm^{3}$ and $8^{L}/cm^{3}$. . A significant growth & sporulation of *F.o.* f. sp. *lycopersici* was found in nitrogen sources viz. Sodium nitrate, Ammonium nitrate, Potassium nitrate and Peptone than other nitrogen sources. The pH of the filtrate was found increased from 5.6 to 5.9, 6.5 and 6.3 in media containing Ammonium nitrate, Sodium nitrate and Potassium nitrate as nitrogen sources. . The growth of *F.o.* f. sp. *lycopersici* was found more in Sodium nitrate at 0.2% concentration and recorded 56mm (Table IV), minimum mycelia growth of the pathogenic fungus was found in Potassium nitrate at 0.1% and 5% concentration i.e. 22mm & 20mm (Table IV). Mycelia growth of *F.o.* f. sp. *lycopersici* was found from 0.1% to 5% concentration of nitrogen sources and are significant but a gradual decrease in growth of *F.o.* f. sp. *lycopersici* was observed from 0.3% to 5% concentration of nitrogen. Minimum mycelia growth was found in Phenylalamine (156.53mg) and Urea (90.3mg). Sporulation also found good in Ammonium oxalate $8.4^{L}/cm^{3}$ and low in Phenylalamine i.e. $2^{L}/cm^{3}$

In Phenylalamine, Urea and Ammonium sulphate the pH of the filtrates was decreased to pH

Table II
Growth of *Fusarium oxysporum* f. sp. *lycopersici* in different concentration of Carbon sources (Growth in mm)

Carbon sources	*1% M±SD*	*2% M±SD*	*3% M±SD*	*4% M±SD*	*5% M±SD*	*6% M±SD*	*7% M±SD*	*8% M±SD*	*9% M±SD*	*10% M±SD*
Glucose	39.7±1.56	51±0.816	61±0.5	50±0	47.7±4.84	40.2±0.16	36±4.32	29.7±3.82	28.2±2.97	20±0.82
Sucrose	48.4±4.04	53.4±2.539	67.7±4.18	54±1.63	49.7±2.04	47.2±3.80	40±0.81	36.4±0.08	33.4±2.26	27±3.55
Maltose	42±3.62	48±4.242	54±3.26	62±1.63	49.8±2.04	43.2±2.44	36±4.96	30±2.82	28±2.82	14±4.32
Fructose	44±3.62	48.8±3.432	50.2±4.08	65±0.77	55±0.81	45.2±0.28	38±4.32	33.2±4.16	30.2±4.16	20±4.08
SEd(±)	N.S.	N.S.	2.10	0.894	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
CD(P = 0.05)	N.S.	N.S.	4.575	1.948	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
CD (P = 0.01)	N.S.	N.S.	6.415	2.731	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.

Table III
Effect of Nitrogen sources on the growth & sporulation of Fusarium oxysporum f. sp. lycopersici.

Nitrogen Sources	*Mycelialgrowth M±SD(in mg)*	*Final pH of the filtrate*	*Sporulation per unit area*	*Sporulation lakhs per cm³.*
Potassium nitrate	461.67±3.858	6.3	500	8
Sodium nitrate	583.2±2.861	6.5	700	11.2
Urea	90.3±7.80	5.0	238	3.8
Ammonium sulphate	200±8.286	4.2	180	2.8
Ammonium oxalate	214±1.632	5.0	530	8.4
Peptone	480±4.082	5.8	442	7.0
Ammonium nitrate	490±3.265	5.9	575	9.1
Phenylalamine	156.53±0.410	4.5	130	2
Control	20±0	5.6	125	2
SEd (±)	4.740		5.136	
CD (P = 0.05)	10.060		8.649	
CD (P = 0.01)	13.840		12.440	

Table IV
Growth of *Fusarium oxysporum* f. sp. *lycopersici* in different concentration of Nitrogen sources(growth in mm.)

Nitrogen sources	*Concentration of N Sources %*							
M±SD	*0.0*	*0.1*	*0.2*	*0.3*	*0.4*	*1.0*	*2.0*	*5.0*
Sodium nitrate	12±0	42±1.632	56±1.632	55.33±1.247	50.0±0	47.22.039	40±0	32±0.816
Ammonium nitrate	12±0	49±0	54±1.414	50±0	48±3.741	46±0.816	35±3.559	30±0.816
Potassium nitrate	12±0	22±1.414	42±1.414	40±0	381.632	30.±0.816	29±2.160	20±0.816
Peptone	12±0	404.082	38±1.632	34±0.816	28±2.828	25±3.559	22±1.414	20±0
Sed(±)	N.S.	2.107	1.312	0.849	2.549	1.530	1.643	0.666
CD (P = 0.05)		5.155	3.210	2.077	6.237	3.743	4.020	1.629
CD (P = 0.01)		7.810	4.863	3.147	9.449	5.671	6.090	2.468

4.5, 5.0 & 4.2 For growth and sporulation of *F. o.* f. sp. *cubense*, *F. moniliformae* and *F. vasinfectum* nitrogen sources are essential (Rai & Jha 1989), and Pai (1953), Hawker (1971). In different C : N ratio the growth of *F.o.* f. sp. *lycopersici* are found different (Table V). Maximum mycelia growth of *F.o.* f. sp. *lycopersici* was found in C : N ratio 15 : 1 & 30 : 1 i.e. 588.5mg and 567mg. Among nitrogen concentration 1 gm and 2 gm of nitrogen Besides various microelements *F. fructigenum* require a large amount of macroelements such as Carbon and Nitrogen which was also observed by (Walker & Foster 1946; Sadasivan, 1951; Lilly & Barnett, 1951; Cook, 1937; Fisher (1935), Hawker, 1971); Scheffer & Walker1953, Goswami & Islam 2000) in different *Fusarium* sp.Utilization of different compounds as nutrition of fungi was reported by different workers (Brown, 1925; Robbins, 1937). Subramanian & Pai (1953) reported that for the growth and sporulation of *Fusarium moniliforme* and *Fusarium vasinfectum* nitrogen sources are essential. (Brown, 1923) reported that carbohydrate has an effect on production of conidia in *F. fructigenum* and also noted the maximum sporulation of *Fusarium* sp at a concentration of nutrients level which makes the dividing point between staling and non-staling type of growth(Horne & Mitter 1927). For growth and sporulation of *Fusarium moniliforme*, Fructose and Asparagin are the best carbon and nitrogen sources as reported by Jurihar & Mehta (1977). Tandon (1967) have shown that potassium nitrate to be superior to Ammonium nitrate for large number of imperfect fungi. The function of carbohydrate and nitrogen nutrients in the establishment of *Fusarium solani* f. sp. *phaseoli* as a pathogen was pointed out by Toussoun *et al.*, (1960) and Cochrane *et al.* (1963). A significant growth of *F.o.* f. sp. *lycopersici* was observed in different C:N ratio but with increase of concentration of C:N ratio growth of the pathogenic fungus found decreases considerably From above results (Table I-V) Carbon is required in greater quantities than any other essential elements by the fungi. Similarly, nitrogen is needed by fungi to synthesize protoplasm and in the absence of adequate nitrogen fungi will be considerably impaired to their ability to decompose available carbohydrate.The change of growth pattern in association with different carbon sources and nitrogen sources observed during the experiment and the C/N ratio may also play an important role in the optimum growth of the fungus (Cochrane 1958). In

Table V
Effect of C : N ratio on the growth and sporulation of *Fusarium oxysporum* f. sp. *lycopersici* (mycelial growth in mgs)

Sodium nitrate g/ lit	*Sucrose concentration (g / litre)*											
	10 g		*20 g*		*30 g*		*40 g*		*50 g*		*60 g*	
	M±SD	*sp#*	*M±SD*	*sp#*	*M±SD*	*sp#*	*M±SD*	*sp#*	*M±SD*	*sp#*	*M±SD*	*sp#*
1	320.25±1.08	+	342.25±2.27	+++	567±7.81	++++	487.75±4.60	+++	468.5±1.65	++	330.75±3.11	++
2	327.75±1.08	+	364.74±6.53	+++	588.5±4.97	++++	503±4.12	++	489.5±7.39	+++	3444.527	+
3	299±1.73	++	336.5±4.03	++	531.25±6.37	+++	474±8.24	++	467±6.40	++	253.75±5.309	+
4	228±4.94	++	322±2.44	++	517.8±2.17	+++	459±2.23	++	438.5±1.65	++	215.53.27	+
5	197±4.12	++	319±1.08	++	508.75±1.92	++	447.25±4.20	++	434.75±3.41	++	2673.0	-
Sed(±)	2.576		2.976		4.340		4.392		4.035		3.391	
CD(P = 0.05)	5.656		6.481		9.378		9.570		8.792		7.388	
CD(P = 0.01)	7.930		9.091		13.148		13.417		12.320		10.359	

the second and fourth experiment increase in carbon con^{c} in the medium as well as nitrogen con^{c} without a proportionate increase in nitrogen or carbon may not encourage greater uptake of carbon or nitrogen. In the fifth experimentt the C/N ratio plays an important role in the growth and sporulation of the pathogenic fungus. In different C/N ratio only in 15: 1 & 30: 1 the growth was recorded maximum. In this present preliminary work, Carbon and Nitrogen requirements in the growth and sporulation of *F.o.f.sp lycopersici* has been studied. Beside these nutritional requirements, soil factor also plays a significant role in the growth of pathogenic fungus. So variation in the type of soil, soil pH, soil carbon in relation to disease development must be considered to understand the physiology of the fungus.

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