I.J.S.N., VOL. 2(3) 2011: 524 - 528 ISSN 2229 – 6441

INTERNATIONAL JOURNAL OF SCIENCE AND NATURE

www.scienceandnature.org

EFFECT OF NUTRITIONAL SOURCES ON THE GROWTH OF *Fusarium oxysporum* f. sp. *ciceri* CAUSING CHICKPEA WILT

[1]Khilare, V.C. and [2]Rafi Ahmed

[1]Botany Research Centre, Vasantrao Naik College, CIDCO, Aurangabad 431 003;

[2]Department of Botany, Maharashtra College, 246-A, Jehangir Boman Marg, Mumbai-400 008

ABSTRACT

In order to know the nutrition of highly virulent *Fusarium oxysporum* f. sp. *ciceri* causing chickpea wilt; different nutritional sources like carbon, nitrogen, phosphates, amino acids, salts, vitamins, oxides and micro elements were used to observe the growth response. Amongst different nutritional sources; glucose, potassium nitrate, di-potassium hydrogen orthophosphate, many amino acids (especially hydroxyproline), ferric oxide were most favorable for the growth of the pathogen. However, magnesium chloride and thiamine were slightly unfavorable for the growth when compared with control. The pathogen responded to a wide range of nutritional sources with considerable variation in its utilization.

KEY WORDS: *Fusarium oxysporum* f.sp. *ciceri*, nutritional sources, growth

INTRODUCTION

Chickpea (*Cicer arietinum* L.) is a rich source of high-quality protein. India is the largest producer of chickpea in terms of the area and production in world. Chickpea wilt is an important fungal disease caused by *Fusarium oxysporum* f. sp. *ciceri* is a major constrain to chickpea production globally. The disease is important in dry and warm season. Although actual yield loss is estimated to be 10 to 12 percent globally (Nene and Reddy, 1987). Generally chickpea is cultivated as a rain fed crop in Maharashtra State and yield losses caused by the Fusarial wilt amounted to 10 to 15 percent (Khilare, *et al*. 2009). Present work depicts the role of different nutritional factors on the growth of *F. oxysporum* f.sp. *ciceri* in survival to develop cultural disease management practices. The object of the present investigation was to know suitable nutritional sources favoring the mycelia growth.

MATERIALS AND METHODS

Fungal isolation

Wilted chickpea plants were collected from 24 chickpea cultivating districts of Maharashtra State. Wilted roots were surface sterilized by 70 percent ethanol and 1 percent $HgCl_2$ solution. Sterilized roots were cut longitudinally by sterile sharp blade and placed on Czapeks Dox agar (CZA) and associated fungus was isolated from growing edge young mycelium and re-cultured on same media to obtain pure conidia. Conidial culture of fungus was obtained by serial dilution method and identified as *F. oxysporum* f.sp. *ciceri* (and herein after FOC) and its pathogenicity tested on chickpea cultivar, JG-62. Further studies were performed with 24 isolates of FOC. Cultures on CZA slants were stored at 4°C for use.

Virulence analysis

A total of 24 isolates of FOC were examined for their virulence analysis. It was confirmed by soil inoculation method in pot (Haware and Nene, 1982). Surface-sterilized plastic pots (0.1% mercuric chloride) filled with 2 kg sterilized soil (three subsequent sterilizations at 1.1 kg/cm2 for 1 h for 3 days), inoculated with the 14-day-old culture of the pathogen. The sterilized soil inoculated with FOC. The highly susceptible variety of chickpea JG-62 was used in all the experiments. All these pots were then watered lightly and kept in glasshouse for further recording of observations as percent seed germination, seedling mortality, etc. The observation on pre- and post-emerge were recorded up to four weeks and highly virulent (> 80 to 100% wilt) isolate was selected for nutritional studies.

Effect of different nutritional sources

To study the effect of different nutritional elements on the growth of FOC; altogether eight sources like carbon, nitrogen, phosphates, amino acids, salts, vitamins, oxides and micro elements were checked on the Czapek dox agar medium. The different sources were replaced in place of original one in Czapek dox agar medium. The medium was poured in Perti plates. Five mm disc from 10 days old culture of highly virulent FOC isolates was used for inoculation. The inoculated plates were incubated at 27±1ºC. The colony diameter on different nutritional sources was measured at 10 days after inoculation and the results are presented as follows.

RESULTS

Effect of carbon sources

A total of nine carbon sources were used at the concentration of 20 percent. The results are shown in Table 1. It was observed that maltose, starch, glucose, xylose, lactose and fructose were most favorable (growth >85 mm) for the growth of this pathogen. Galactose was found to be unfavorable when compared with control. Growth is totally absent in absence of carbon sources. The growth habits related to carbohydrate utilization are unique.

TABLE 1. Effect of different carbon sources on the growth of highly virulent FOC

Sr. No.	Carbon sources	FOC Growth (mm)
1	D-fructose	85.5
2	D-galactose	65.7
3	D-xylose	86.6
4	Lactose	86.6
5	Mannitol	83.1
6	Maltose	89.5
7	Starch	87.5
8	Sucrose	76.6
9	D-glucose	87.1
10	Control	00.0
	Mean	74.8
	C.D. (P= 0.05)	18.4
	(P= 0.01)	26.5

TABLE 2. Effect of different nitrogen sources on the growth of highly virulent FOC

Sr. No.	Nitrogen sources	FOC Growth (mm)
1	Ammonium nitrate	57.6
2	Ammonium oxalate	45.3
3	Ammonium sulphate	36.1
4	Calcium nitrate	85.9
5	Magnesium nitrate	86.4
6	Potassium nitrate	88.2
7	Sodium nitrate	76.6
8	Sodium nitrite	72.7
9	Silver nitrate	38.4
10	Urea	87.5
11	Control	87.3
	Mean	69.3
	C.D. (P= 0.05)	13.4
	(P= 0.01)	19.1

TABLE 3. Effect of different phosphate sources on the growth of highly virulent FOC

Sr. No.	Phosphate sources	FOC Growth (mm)
1	Di-potassium hydrogen orthophosphate	89.1
2	Ammonium phosphate	78.2
3	Potassium dihydrogen phosphate	77.6
4	Sodium dihydrogen phosphate	88.6
5	Calcium phosphate	82.2
6	Control	74.6
	Mean	81.7
	C.D. (P= 0.05)	5.7
	(P= 0.01)	9.0

TABLE 4. Effect of different amino acids on the growth of highly virulent FOC

Sr. No.	Amino acids	FOC Growth (mm)
1	Alanine	76.2
2	Butyric acids	77.3
3	L-Arginine	78.2
4	DL-Aspartic acid	76.6
5	L-Cysteine	80.2
6	L-Cystine	84.6
7	DL-Dopa	86.6
8	L-Glutamic acid	83.7
9	Glycine	86.2
10	L-Histidine	83.1
11	L-Hydroxyproline	87.5
12	L-Leucine	81.5
13	DL-iso-Leucine	81.7
14	DL-Nor-leucine	80.8
15	L-Lysine	80.8
16	DL-Methionine	80.2
17	L-Ornithine	82.6
18	DL-B phenylalanine	81.9
19	L-Proline	83.3
20	DL-Serine	84.8
21	DL-Threonine	85.1
22	DL-Tryptophan	81.5
23	L-Tyrosine	82.6
24	DL-Valine	83.4
25	Control	79.6
	Mean	82.0
	C.D. (P= 0.05)	1.1
	(P= 0.01)	1.6

Effect of nitrogen sources

The results are given in Table 2 with 10 different nitrogen sources. It was seen that calcium nitrate, magnesium nitrate, potassium nitrate and urea were most favorable for the growth (growth >87.5 mm), whereas magnesium nitrate, calcium nitrate were also proved to be effective (growth >85 mm). Ammonium sulphate, silver nitrate and ammonium oxalate were less effective (growth 36.1 to 45.3mm). All these sources were studied at 0.3 percent concentration.

Effect of phosphate sources

A total of 5 phosphate sources were used at the concentration of 0.1 percent. It was noted that Di-potassium hydrogen orthophosphate, sodium dihydrogen phosphate showed higher growth as (growth >88.6). All other phosphate sources were also favorable when compared with control (Table 3).

Effect of amino acids sources

Altogether 24 amino acids were incorporated in the medium at the concentration 0.05 percent. The results are presented in Table 4**.** It was seen that L-Hydroxyproline, DL-Dopa , Glycine, DL-Threonine, L-Cystine, DL-Serine, L-Glutamic acid, DL-Valine, L- Proline, L-Tyrosine and L-Ornithine highly supported the growth (82.6 to 87.5 mm) of pathogen when compared with control. However, Alanine, Butryic acid, L-Arginine and DL-Aspartic acid were showed weak growth response when compared with control.

TABLE 5. Effect of different salts on the growth of highly virulent FOC

Sr. No.	Salt sources	FOC Growth (mm)
1	Aluminum chloride	77.5
2	Ammonium chloride	67.7
3	Barium chloride	75.1
4	Calcium chloride	82.2
5	Ferric chloride	76.8
6	Magnesium chloride	85.1
7	Potassium chloride	74.4
8	Zinc chloride	78.2
9	Sodium chloride	31.3
10	Control	87.4
	Mean	73.6
	C.D. (P= 0.05)	10.7
	(P= 0.01)	15.4

TABLE 6. Effect of different vitamins on the growth of highly virulent FOC

Sr. No.	Vitamin sources	FOC Growth (mm)
1	L- Ascorbic acid	60.8
2	D-Biotin	72.6
3	Folic acid	55.1
4	Inositol	84.5
5	Riboflavin	66.4
6	Thiamine	85.3
7	Niacin	63.6
8	Control	90.0
	Mean	72.3
	C.D. (P= 0.05)	10.1
	(P= 0.01)	14.9

Effect of different salts

A total of 9 salts in the form of chloride were used in the medium at 0.05 percent. Result in the Table 5 indicated that magnesium chloride, calcium chloride showed higher growth than other salts but both are unfavorable when compared with control. It was also noted that sodium chloride retards the growth of FOC in medium. It was clearly observed that presence of salts in medium was ineffective for pathogen growth.

Effect of different vitamins

A total of 7 vitamins were used in this study (Table 6). It was noted that all the vitamins showed less growth of pathogen when compared with control. Folic acid was found to be highly ineffective amongst vitamins and showed 55.2 mm growth when compared with control (90.0). Rest vitamins were also least effective.

Effect of different oxides

A total of 7 sources of oxides were used in the medium at the concentration 0.05 percent and the results are presented in Table 7. It was noted that molybdenum oxide and ferric oxide showed higher growth than that of control. Mercuric oxide and calcium oxide was less favorable when compared with other oxide sources. However, mercuric oxide and calcium oxide could be used as seed dressing fungicides.

TABLE 7. Effect of different oxides on the growth of highly virulent FOC

Sr. No.	Oxide sources	FOC Growth (mm)
1	Aluminium oxide	66.8
2	Calcium oxide	29.7
3	Molybdenum oxide	85.7
4	Zinc oxide	76.8
5	Mercuric oxide	22.6
6	Ferric oxide	83.9
7	Magnesium oxide	75.3
8	Control	78.8
	Mean	65.0
	C.D. (P= 0.05)	19.3
	(P= 0.01)	28.5

TABLE 8. Effect of different micro elements on the growth of highly virulent FOC

Sr. No.	Micro elements	FOC Growth (mm)
1	Cobaltous sulphate	33.6
2	Copper sulphate	58.2
3	Ferrous sulphate	55.9
4	Magnesium sulphate	90.0
5	Manganese sulphate	89.1
6	Sodium sulphate	88.7
7	Zinc sulphate	54.8
8	Potassium sulphate	73.5
9	Nickel sulphate	16.6
10	Control	78.8
	Mean	63.9
	C.D. (P= 0.05)	16.8
	(P= 0.01)	24.2

Effect of different micro elements

Cobaltous sulphate, copper sulphate, ferrous sulphate, magnesium sulphate, manganese sulphate, sodium sulphate, zinc sulphate, potassium sulphate and nickel sulphate were used at the concentration 0.01 percent. The results are presented in Table 8. It was interesting to see that magnesium sulphate, manganese sulphate and sodium sulphate showed increase in the growth of pathogen when compared with control. Nickel sulphate, cobaltous sulphate, zinc sulphate, copper sulphate and ferrous sulphate were found to be inhibitory for the growth of FOC.

DISCUSSION

Carbon is the most important and essential component of fungal cell required for their growth and development. The present investigation revealed the growth of *Fusarium oxysporum* f.sp. *ciceri* on the various carbon sources tested, maltose, starch, glucose, xylose, lactose and fructose were most favorable. Similar observations were made by Sowmya (1993) amongst different carbon sources tested against *F. oxysporum* f.sp. *cubense,* glucose was the best carbon source for isolates I & II. Glucose, xylose and D-galacturonic acid, carboxymethyl cellulose, xylan and pectin were used by Steinberg *et al.,* (1999) to study *F.*

oxysporum pathogenic on tomato**.** Carbon utilization was best on sucrose followed by maltose, starch, glucose, fructose and cellulose successively by *F. oxysporum* causing cotton wilt (Naim and Sharoubeem, 1963). The fungus may convert certain forms of complex carbon compounds into simple form, which may be readily metabolized (Bais *et al*., 1970). Fructose, mannose and galactose are needed for the growth of *F. solani* (Schuerger, *et al*., 1993). Desai *et al.* (1994) recorded that, the carbon sources supported better growth of race one, except maltose and succinic acid which supported good growth of race three of *F. oxysporum* f .sp. *ciceri.* Nitrogen is an important component required for protein synthesis and other vital functions. The study revealed that the maximum growth of the pathogen was observed in calcium nitrate, magnesium nitrate, potassium nitrate and urea. whereas magnesium nitrate, calcium nitrate were also proved to be effective. The results are in agreement with Naim and Sharoubeem (1963) regarding use of ammonium nitrate to *F. oxysporum* causing cotton wilt. Out of the 10 nitrogen compounds tested against *F. oxysporum* f.sp. *elaeidis*; good growth and sporulation were recorded on sodium, ammonium and potassium nitrates, peptone and DL-leucine (Oritsejafor, 1986**).** While moderate growth of the fungus was recorded on ammonium sulphate, calcium nitrate, L-asparagine and DL-aspartic acid, sporulation in these compounds was poor and ammonium sulphate induced chlamydospore formation (Naim and Sharoubeem, 1963). The results are in agreement with the reports of Bhatnagar *et.al.,* (1968) in case of *F. oxysporum* f.sp. *aurentifoliae* which showed good growth on D-leucine and aspargine. Patel (1990) reported that aspargine supported maximum mycelial growth of *F. solani.* High nitrogen levels, which decreased disease severity, increased the protein content in leaf tissues. Of 17 amino acids only proline content increased with increasing nitrogen supply (Sarhan *et al*. 1982). Growth of *F. solani*, *F. avenaceum*, and *F. oxysporum* on an agar medium minus K. salts was retarded; P deficiency prevented sporulation. $KaNO_3$ and Ca $(NO_3)_2$ were most favourable, while NH_4Cl and $(NH_4)_2SO_4$ inhibited growth (Korobeinikova, 1960). Prasad (1972) studied the effect of vitamins on sporulation in *F. oxysporum* and *F. moniliforme* v. *subglutinans*m. Thiamine, biotin, inositol are selective in accelerating macro-conidial production in *F. moniliforme.*The effect of eight water-soluble vitamins on germination, germ-tube extension, growth, and sporulation of *F. oxysporum* f.sp. *vasinfectum* was studied by El-Abyadm and Ramadan (1979). Among the vitamins used, the fungus appeared to be highly sensitive to thiamine and pyridoxine, moderately sensitive to inositol and pantothenate, and least affected by folic acid. Role of oxides could be attributed to killing the fungus by heat or mineral ash arising from soil burning. Sun and Huang (1985) worked with Fusarial wilt in sandy soil and used mineral ash composed of calcium oxide. This results are similar with our work. *F. oxysporum* var. nicotianae grew well with sucrose and ammonium nitrate, potassium, phosphorus, magnesium, sulfur and calcium. In addition to the micronutrients; iron, zinc, copper, manganese, and molybdenum **(**Steinberg, 1950).

ACKNOWLEDGEMENT

The authors are grateful to University Grants Commission, New Delhi for financial assistance under major research project (F.No.31-171/2005 (SR) dated 31/03/2006).

REFERENCES

Bais, B.S., Singh, S.B. and Singh, D.V. (1970) Effect of different carbon and nitrogen sources on the growth and sporulation of *Curvularia pallescens*. *Indian Phytopath*ology, **23**: 511–7

Bhatnagar, C. C., Prasad, N. and Mathur, R. L. (1968) Nitrogen requirement of *Fusarium oxysporum* f.sp. *aurentifoliae* Bhat and Prasad. *Indian Phytopathology*, **21**: 237 – 239.

Desai, S., Nene, N. L., and Ramachandra Reddy, A. G., (1994) Races of *Fusarium oxysporum* causing wilt in chickpea. *Indian Journal of Mycology and Plant Pathology*, **24** : 120 – 127.

El-Abyadm, M. S. and Ramadan, Z. M. (1979) Vitamin requirements of *Fusarium oxysporum* f. sp. *vasinfectum.* Zentralbl Bakteriol Naturwiss.**134**:412-420.

Haware, M. P. and Nene, Y.L. (1982) Races of *Fusarium oxysporum* f. sp. *ciceri*. *Plant Disease* **66:** 809–810

Khilare, V.C., Rafi Ahmed, S.S. Chavan and Kohire, O.D. (2009) Management of *Fusarium oxysporum* f. sp. *ciceri* by different fungicides. *Bioinfolet*, **6**: 41 – 43.

Korobeinikova, Mme A. V. (1960) The effect of nutrition, temperature, humidity, and pH on the development of some species of the genus *Fusarium*. *Trudy Inst. Biol. ural'sk. Fil. Akad. Nauk SSSR*, **15**: 71-81

Naim, M.S. and Sharoubeem, H. H. (1963) Carbon and nitrogen requirements of *Fusarium oxysporum* causing cotton wilt. *Mycopathologia,* **22:** 59-64

Nene, Y.L. and Reddy, M.V. (1987) Chickpea disease and their control. In: Saxena M.C., Singh K.B. (eds): The Chickpea. III Viral Diseases. Wallingford, Oxon, CAB International, ICARDA: 233–270.

Oritsejafor, J.J. (1986) Carbon and nitrogen nutrition in relation to growth and sporulation of *Fusarium oxysporum* f.sp. *elaeidis. Transactions of the British Mycological Society*, **87**: 519-524

Patel, S. T. (1990) Studies on some aspects of wilt of chickpea. Ph.D., thesis submitted to University of Agricultural Sciences, Dharwad, pp 15-125

Prasad, M. (1972) Comparative studies on the effect of vitamins on sporulation in *Fusarium oxysporum* Schlecht. ex. Fr. and *Fusarium moniliforme* v. *subglutinans* Wr. & Rg. *Mycopathologia,* **46:**367-372.

Sarhan, A.R.T., Barna, B. and Kiraly, Z. (1982) Effect of nitrogen nutrition on *Fusarium* wilt of tomato plants. *Annals of Applied Biology*, **101:** 245–250

Schuerger, A. C., Mitchell, D. J., Kaplan, D.T. (1993) Influence of carbon source on attachment and germination of macroconidia of *Fusarium solani* f. sp. *phaseoli* on roots of *Vigna radiate* grown in hydroponic nutrient solution. *Plant Disease,* **83**:1171–1177.

Sowmya, G. S. (1993) Studies on panama disease of banana caused by *Fusarium oxysporum* f.sp. *ciceri M.Sc., (Agri) thesis* submitted to University of Agricultural Sciences, Bangalore. pp 120.

Steinberg, C., Whipps, J.M., Wood, D.A., Fenlon, J. and Alabouvette**, C.** (1999) Effects of nutritional sources on growth of one non-pathogenic strain and four strains of *Fusarium oxysporum* pathogenic on tomato. *Mycological Research*, **103**: 1210-1216

Steinberg, R.A. (1950) Growth on synthetic nutrient solutions of some fungi pathogenic to tobacco. *American Journal of Botany*, **37:** 711-714

Sun, S. K., and Huang, J. W. (1985) Formulated soil amendment for controlling Fusarium wilt and other soilborne diseases. *Plant Disease*, **69:**917-920.