IJPS INTERNATIONAL JOURNAL OF PLANT SCIENCES
e ISSN-0976-593X Volume **11** | Issue 2 | July, 2016 | 213-217

DOI: 10.15740/HAS/IJPS/11.2/213-217
Visit us - *www.researchjournal.co.in*

RESEARCH ARTICLE

Nutritional and physiological studies of *Fusarium oxysporum* f. sp. *ciceri* (Padwick) Snyder and Hansen causing wilt of chickpea

■ **D.S. THAWARE, O.D. KOHIRE, V.M. GHOLVE, S.S. WAGH AND A.A. CHAVAN**

SUMMARY

Nutritional and physiological requirements of *Fusarium oxysporum* f. sp. *ciceri* were studied using most virulent isolate FOC-2 (Jalna). Effect of different culture media on mycelial growth of *Fusarium oxysporum* f. sp. *ciceri* showed significant differences in growth and sporulation. *In vitro*, culture media studied, Potato dextrose agar (89.66 mm) and Richard agar medium (85.66) produced significantly highest mean mycelial growth and sporulation. The temperature in the range of 25^0C to 30^0C and pH having range of 6.0 to 7.0 produce significantly highest mean mycelial growth and sporulation of the test pathogen.

Key Words : Nutritional, Physiological, Chickpea

How to cite this article : Thaware, D.S., Kohire, O.D., Gholve, V.M., Wagh, S.S. and Chavan, A.A. (2016). Nutritional and physiological studies of *Fusarium oxysporum* f. sp. *ciceri* (Padwick) Snyder and Hansen causing wilt of chickpea. *Internat. J. Plant Sci.*, **11** (2): 213-217, **DOI: 10.15740/HAS/IJPS/11.2/213-217**.

Article chronicle : Received : 28.12.2015; **Revised :** 12.04.2016; **Accepted :** 26.05.2016

Chickpea (*Cicer arietinum* L.) is an important pulse crop, which belongs to Leguminoceae family, ranking third after dry beans (*Phaseolus vulgaris* L.) and dry peas (*Pisum sativum* L.) (Dhar and Gurha, 1998). The Kabuli and Desi chickpea is grown throughout the world with different names *i.e.,* Chickpea (UK), Garbanzo (Latin America), Bengal gram (India), Hommes Hamaz (Arab world), Shimbra (Ethiopia) and Nohud and Loblebi (Turkey). Chickpea is mainly used for human consumption as well as for animal feeds. It is consumed as whole seed, dal fried, boiled, salted or more generally, which is cooked. Fresh green leaves are used as vegetable. The grains also used as vegetable (chhole). Gram flour is mixed with wheat flour to improve the protein content of wheat flour and is used in making missi roti. The flour of dehusked gram called 'besan' is widely used in making pakodas, kadhi, namkeens and several snacks food. Exudation of leaves locally called 'amb' contain oxalic and malic acids, which possess medicinal value for bronchitis, cholera, constipation, diarrhea, digestive disorders, snake-bites, warts and blood purification. In India, chickpea is grown on 10.23 million

MEMBERS OF THE RESEARCH FORUM

Author to be contacted :
D.S. THAWARE, Department of Plant Pathology, College of Agriculture, Vasantrao Naik Marathwada Krishi Vidyapeeth, PARBHANI (M.S.) INDIA
Email: sanju.6771@rediffmail.com

Address of the Co-authors:
O.D. KOHIRE, V.M. GHOLVE, S.S. WAGH AND A.A. CHAVAN, Department of Plant Pathology, College of Agriculture, Vasantrao Naik Marathwada Krishi Vidyapeeth, PARBHANI (M.S.) INDIA

ha area with production 9.88 million tonnes and productivity 967 kg/ha. The production of chickpea in Maharashtra is 1.62 million tonnes with productivity 891 kg/ha which covered nearly 1.82 million ha of area. Maharashtra contributes about 16.42 per cent share in total production of country (Anonymous, 2014).

The major limiting factor in chickpea production is Fusarium wilt which is caused by *F. oxysporum* Schlechtend. Fr. f. sp. *ciceris* (Padwick) Matuo and K. Sato. (Jalali and Chand, 1992 and Haware, 1990 and Nene and Reddy, 1987). It was first reported in Indo-Pak sub-continent (Butler, 1918). McRae (1932) as well as Prasad and Padwick (1939) reported *F. oxysporum* f. sp. *ciceris* pathogenic to chickpea crop which is now accepted worldwide as the causal agent of *ciceri* spp. In general, the disease causes substantial yield losses which may reach even 100 per cent under favourable weather conditions (Jalali and Chand, 1992). The chickpea is cultivated as a rain fed crop in Maharashtra state and yield losses amounted to 10 to 15 per cent (Khillare *et al.* (2009).

MATERIAL AND METHODS

Nutritional and physiological studies of *Fusarium oxysporum* f. sp. *ciceri* :

Effect of culture media :

A total of thirteen culture media (synthetic, readymade, make: Hi media and non-synthetic, prepared) were used to study their effect on growth and sporulation of *Fusarium oxysporum* f. sp. *ciceri*. All the thirteen test media were sterilized in autoclave at 15 lbs/inch^2 pressure for 20 min and autoclaved and cooled media were poured (@ 20 ml/plate) in sterilized glass Petri plates (90 mm dia.) and allowed to solidify at room temperature. On solidification, all the plates (three / media / replication) were inoculated by placing in the centre a 7 mm mycelial disc of actively growing 7 days old pure culture of *Fusarium oxysporum* f. sp. *ciceri* (FOC-2 isolate) and each medium was replicated thrice. Plates were incubated at 28 $\pm$ 2^{0}C. Observations on mycelial growth were recorded at a week and that of sporulation at two weeks of incubation. Conidial production was determined on the basis of microscopic observations.

Effect of temperature regimes :

The effect of seven temperature regimes *viz.*, 10^0, 15^0, 20^0, 25^0, 30^0, 35^0 and 40^{0}C on growth and sporulation of *Fusarium oxysporum* f. sp. *ciceri* was studied using BOD incubators (make: MAC, Delhi). Autoclaved and cooled PDA medium was poured (20 ml / plate) in sterile glass Petri plates (90 mm dia). Three PDA plates / treatment / replication were maintained. All the PDA plates were inoculated by placing in the centre a 7 mm mycelial disc of actively growing 7 days old culture of *Fusarium oxysporum* f. sp. *ciceri* (FOC-2 isolate) and plates were incubated in BOD incubators set at respective temperature regimes. For each temperature regime, three replications were maintained. Observations on colony diameter and sporulation were recorded after at a week and two weeks of incubation, respectively.

Effect of pH levels :

The effect of seven pH levels (hydrogen ion concentration) was studied using PDA as basal medium. For the purpose, 200 ml PDA medium was poured in glass beakers (250 ml) and its pH levels (5.0 to 8.0) were separately adjusted using pH meter (make: MAC, Delhi) by adding 0.1 N HCL and NaOH. Then the PDA medium adjusted with various pH level was sterilized at 15 lbs / inch^2 in an autoclave for 20 min. Autoclaved and cooled PDA medium with various pH levels was poured (20 ml / plate) separately in sterile glass Petri plates (90 mm dia.). Three plates / pH level / replication were maintained. Plates were inoculated by placing in the centre a 7 mm mycelial disc of actively growing seven days old pure culture of *Fusarium oxysporum* f. sp. *ciceri* (FOC-2 isolate) and incubated at 28 $\pm$ 2^{0}C. Observations on colony diameter and sporulation were recorded after at a week and two weeks of incubations, respectively.

RESULTS AND DISCUSSION

The results obtained from the present investigation as well as relevant discussion have been summarized under following heads :

Nutritional and physiological studies of *Fusarium oxysporum* f. sp. *ciceri* :

All the nutritional and physiological requirement studies were undertaken using FOC-2 (Jalna) isolate of *Fusarium oxysporum* f. sp. *ciceri* as representative one.

Effect of culture media :

The results (Table 1) revealed that all of the thirteen culture media tested, encouraged better growth and sporulation of *Fusarium oxysporum* f. sp. *ciceri*. The

mean colony diameter / mycelial growth recorded with the test media ranged from 62.00 mm (Malt agar medium) to 89.66 mm (Potato dextrose agar medium). However, significantly highest mean mycelial growth (89.66 mm) was recorded on Potato dextrose agar medium, followed by Richard's synthetic agar (85.66 mm), Czapek's dox agar (82.00 mm), Asthana and Hawker's (80.66 mm) and Ashby's mannitol agar (75.66 mm) medium. Potato malt agar (70.00 mm), Corn meal agar (69.00 mm); both of these were at par, King's 'B' base (65.33 mm), Sabouraud maltose agar (64.33 mm) and Yeast mannitol agar (64.00 mm) medium, all of thrice were at par. Comparatively minimum mean mycelial growth of the test pathogen were found on Yeast extract agar (63.00 mm), Sabouraud dextrose agar (62.33 mm) and Malt agar (62.00 mm) medium, thrice of which were at par.

Data presented also showed the effect of solid media on sporulation of *F. oxysporum* f. sp. *ciceri*, all the thirteen culture media tested, exhibited a wide range of sporulation from fair (+) to excellent (++++) of the test pathogen. However, Potato dextrose agar, Richard's synthetic agar, Czapek's dox agar and Asthana and Hawker's medium recorded excellent (++++) sporulation. Good (+++) sporulation were recorded with Ashby's mannitol agar and Potato malt agar medium; whereas, there were Corn meal agar, King's 'B' base, Sabouraud maltose agar, Yeast mannitol agar, Yeast extract agar, Sabouraud dextrose agar and Malt agar medium recorded fair (++) sporulation.

Chavan (2004) reported that fungus grew best and sporuled on Potato dextrose agar medium. The least growth of fungus was observed on Malt extract agar medium. Khan *et al.* (2011) showed that Potato dextrose agar (PDA) is the best medium for the growth and sporulation of different Fusarium isolates. Kadam (2012) reported that fungus grew best on Czapek's dox agar medium followed by Potato dextrose agar medium, Asthana and Hawker's medium and Snyder and Han's medium. Awachar (2014) also reported that fungus grew best on Potato dextrose agar followed by Richard's agar, Asthana and Hawker's and Czapek's dox agar medium.

Effect of temperature on radial growth of *Fusarium oxysporum* f. sp. *ciceri* :

Data on effect of temperature for radial growth and sporulation of *Fusarium oxysporum* f. sp. *ciceri* are presented in Table 2. The radial growth of *Fusarium oxysporum* f. sp. *ciceri* was influenced significantly due to different temperatures at 3rd, 5th and 7th days after inoculation. The rate of increase in radial growth was comparatively slow upto 3rd day and during 5th to 7th days after inoculation, it was very fast. Critical examination of data showed that 30°C temperature was most suitable for development of mycelial growth of *Fusarium oxysporum* f. sp. *ciceri*. The mycelial growth at 30^0C was highest at 3rd (53.33 mm), 5th (72.66 mm) and 7th (89.33 mm) days after inoculation, which was significantly superior over rest of the temperature. The radial growth decreased with rise or fall of temperature beyond the most favourable and optimum temperature of 30°C. This was followed by the temperatures of 25^0C (44.00 mm, 62.00 mm and 77.33 mm), 35^0C (41.66 mm,

Table 1 : Effect of different culture media on radial growth (mm) and sporulation of wilt pathogen (*Fusarium oxysporum* f. sp. *ciceri*)

Treatments	Media	Colony diameter of pathogen (mm)	Sporulation
T_1	Sabouraud maltose agar medium	64.33	++
T_2	Malt agar medium	62.00	++
T_3	Corn meal agar medium	69.00	++
T_4	King's 'B' base medium	65.33	++
T_5	Ashby's mannitol agar medium	75.66	+++
T_6	Asthana and Hawker's medium	80.66	++++
T_7	Potato malt agar medium	70.00	+++
T_8	Sabouraud dextrose agar medium	62.33	++
T_9	Czapek's dox agar medium	82.00	++++
T_{10}	Yeast mannitol agar medium	64.00	++
T_{11}	Richard's synthetic agar medium	85.66	++++
T_{12}	Yeast extract agar medium	63.00	++
T_{13}	Potato dextrose agar medium	89.66	++++
	C.D. (P=0.01)	1.82	
	S.E. ±	0.46	

– = No, + = Poor, ++ = Fair, +++ = Good, ++++ = Excellent

55.66 mm and 74.66 mm), 20^{0}C (35.33 mm, 54.00 mm and 67.00 mm) and 15^{0}C (17.66 mm, 25.00 mm and 32.00 mm) at 3rd, 5th and 7th day after inoculation, respectively. Similarly minimum mycelial growth of *Fusarium oxysporum* f. sp. *ciceri* was recorded 40^{0}C (16.00 mm, 23.00 mm and 29.00 mm) followed by 10^{0}C (11.00 mm, 13.00 mm and 19.00 mm) temperature. This means that the optimum temperature range for mycelial growth of test fungus was 25 to 30^{0}C.

Data also showed that, sporulation of test pathogen was excellent (++++) at 30^{0}C, 25^{0}C was good (+++) and fair (++) at 20^{0}C and 35^{0}C. However, there were poor (+) sporulation at 15^{0}C and 40^{0}C. At the temperatures of 10^{0}C, there was none any sporulation of the test pathogen. This means that the optimum temperature range for sporulation of test fungus was 25^{0}C to 30^{0}C.

Gaikwad and Pachpande (1992) and Osman *et al.* (1992) noticed that the optimum temperature for growth and sporulation of test pathogen was 30°C. Wereher (1990) and Chaung and Su (1989) observed maximum growth and sporulation of isolates of F. *oxysporum* between 25-30°C temperature range. Chavan (2004) also reported that the most suitable temperature for development of mycelium growth and excellent sporulation of *F. oxysporum* f. sp. *ciceri* was 25°C to 30°C and significantly lower growth and poor sporulation was recorded at 10°C. Khillare and Ahmed (2012) noticed the growth and sporulation of *F. oxysporum* f. sp. *ciceri* was good at 30°C, which was reduced drastically below 15°C and above 35°C.

Effect of pH on radial growth of *Fusarium oxysporum* f. sp. *ciceri* :

The mycelial growth at pH 6.0 was highest at 3rd (48.00 mm), 5th (70.33 mm) and 7th (88.00 mm) days after inoculation, which was significantly superior over rest of the pH levels. This was followed by the pH level of 6.5 (46.33 mm, 65.00 mm and 84.66 mm), 7.0 (43.66 mm, 63.00 mm and 80.00 mm), 5.5 (43.66 mm, 60.33 mm and 76.33 mm) and 5.0 (41.33 mm, 56.00 mm and 72.33 mm) at 3rd, 5th and 7th day after inoculation, respectively. Similarly minimum mycelial growth of *Fusarium oxysporum* f. sp. *ciceri* was recorded at pH 7.5 (36.66 mm, 54.33 mm and 67.00 mm) followed by 8.0 (31.00 mm, 44.33 mm and 57.00 mm), respectively,

Table 2 : Effect of different temperature on radial growth (mm) and sporulation of wilt pathogen (*Fusarium oxysporum* f. sp. *ciceri*)

Treatments	Temperatures	Colony diameter of pathogen (mm)			Sporulation
		3 DAI	5 DAI	7 DAI	
T_1	10°C	11.00	13.00	19.00	–
T_2	15°C	17.66	25.00	32.00	+
T_3	20°C	35.33	54.00	67.00	++
T_4	25°C	44.00	62.00	77.33	+++
T_5	30°C	53.33	72.66	89.33	++++
T_6	35°C	41.66	55.66	74.66	++
T_7	40°C	16.00	23.00	29.00	+
	C.D. (P=0.05)	2.12	3.00	3.31	
	S.E. ±	0.50	0.71	0.79	

– = No + = Poor ++ = Fair +++ = Good ++++ = Excellent

Table 3 : Effect of different pH ranges on radial growth (mm) and sporulation of wilt pathogen (*Fusarium oxysporum* f. sp. *ciceri*)

Treatments	pH	Colony diameter of pathogen (mm)			Sporulation
		3 DAI	5 DAI	7 DAI	
T_1	5.0	41.33	56.00	72.33	++
T_2	5.5	43.66	60.33	76.33	+++
T_3	6.0	48.00	70.33	88.00	++++
T_4	6.5	46.33	65.00	84.66	++++
T_5	7.0	43.66	63.00	80.00	+++
T_6	7.5	36.66	54.33	67.00	++
T_7	8.0	31.00	44.33	57.00	+
	C.D. (P=0.01)	2.54	2.49	3.18	
	S.E. ±	0.60	0.59	0.76	

– = No + = Poor ++ = Fair +++ = Good ++++ = Excellent

pH levels. This means that the optimum pH levels for mycelial growth of test fungus was 6.0 to 7.0. The radial growth decreased with the increase or decreased of acidity or alkalinity of medium (Table 3).

All the pH level tested, exhibited a wide range of sporulation from poor (+) to excellent (++++). However, excellent (++++) sporulation was recorded at the pH of 6.0 and 6.5. Good (+++) sporulation was recorded at the pH of 5.5 and 7.0; whereas, fair (++) sporulation was recorded at the pH of 5.0 and 7. 5. Poor (+) sporulation was recorded at the pH 8.0.

Shadha *et al. (*1995) reported that pH 6.0 was suitable for optimum growth and sporulation of *F. oxysporum.* Chavan (2004) reported that the maximum radial growth and excellent sporulation was observed at pH 6 and 6.5. Lower radial growth and poor sporulation was recorded at pH 4.0. Khan *et al.* (2011) also showed that optimum pH level for growth of fungus ranged from 6.5 to 7.0. Khilare and Ahmed (2012) found that different pH levels on mycelial growth of *F. oxysporum* f. sp. *ciceri* and suitable growth of fungus was 6.0 and 6.5.

REFERENCES

Anonymous (2014). Directorate of Economics and Statistics, Department of Agriculture and Co-operation. Agricultural Statistics at a glance. pp. 94-96.

Awachar, M.K. (2014). Studies on morphological variability of *Fusarium oxysporum* f. sp. *ciceri* causing wilt of chickpea. M.Sc. (Ag.) Thesis, MPKV, Rahuri, Ahmednagar, M.S. (INDIA).

Butler, E.J. (1918). *Fungi and diseases of plants*. Book published. (M. C. Saxena, K. B. Singh, edi.), CABI Publishing, CAB Int., Wallingford, UK. 233-270.

Chaung, T.Y. and Su, H.J. (1989). Study of *Fusarium oxysporum* f. sp. *cubense.* Memories of college of Agriculture. National Taiwan University, **28**(2):19-26.

Chavan, T.B. (2004). Studies on *Fusarium oxysporum* f. sp. *ciceri* (Padwick) Snyder and Hansen causing wilt of chickpea (*Cicer arietinum* L.). M.Sc. (Ag.) Thesis, Indira Gandhi Agriculture University, Raipur, C.G. (INDIA).

Dhar, V. and Gurha, S.N. (1998). *Integrated management of chickpea diseases*. (Rajeev, K., Upadhyay, K. G., Mukerji, B. P., Chamola and Dubey, O. P. (edi.)), APH Pub. Co., New Delhi. (India). pp: 249.

Gaikwad, S.J. and Pachpande, D.S. (1992). Effect of temperature on wilt of sesamum caused by *Fusarium oxysporum* f. sp. *sesami. J. Man. Agril. Univ.,* **17**(1): 76-78.

Haware, M.P. (1990). Fusarium wilt and other important diseases of chickpea in the Mediterranean area. *Options Mediterr. Ser. Semin.,* **9** : 163-166.

Jalali, B.L. and Chand, H. (1992). *Diseases of cereals and pulses.* (U. S. Singh, A. N. Mukhopadhayay, J. Kumar, and H. S. Chaube, edi.) Prentice Hall, Englewood Cliffs, NY. 1-429-444.

Kadam, N. (2012). Molecular characterization of different isolates of *Fusarium oxysporum* f. sp. *ciceri* causing chickpea wilt from Maharashtra. M.Sc. (Ag.) Thesis, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola, M.S. (INDIA).

Khan, H.S., Saifulla M., Mahesh, S.B. and Pallavi, M.S. (2011). Effect of different media and environmental conditions on the growth of *Fusarium oxysporum* f. sp. *ciceri* causing Fusarium wilt of chickpea.

Khilare, V.C. and Ahmed, R. (2012). Effect of different media, pH and temperature on the growth of *Fusarium oxysporum* f. sp. *ciceri* causing chickpea wilt. *Internat. J. Advan. Bio. Res.*, **2**(1) : 99-102.

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

McRae, W. (1932). Report on Imperial Mycologists Science Agriculture Research Institute, Pusa. pp. 31-78.

Nene, Y.L. and Reddy, M.V. (1987). Chickpea diseases and their control. *Phytopatholgy,* **42**: 499-505.

Osman, M., Sayed, M.A., Mohamed, Y.A.H. and Metwally, M. (1992). Effect of various cultural conditions culture media, temperature and carbon source on *Alternaria alternata* and *Fusarium oxysporum. Microbios,* **71** (286) : 15-26.

Prasad, N. and Padwick, G.W. (1939). The genus *Fusarium* 11. A species of *Fusarium* as a cause of wilt of gram (C. *arietinum* L.). *Indian J. Agric. Sci.,* **9** : 371-380.

Shadha, W.T., Rahma, A.A. and Rageh, S.A. (1995). Damping off of some cucurbitaceous crops in Saudi Arabia with reference to control methods. *J. Phytopath.,* **6** (2): 125-129.

Wereher, M. (1990). Effect of temperature and media composition on growth and sporulation of formae special of *Fusarium oxysporum* Schlecht. *Recozniki Akademi Rolniczej-W. Poznaniu- Ogrodnictwo.,* **18**: 107-125.