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Cultural and Physiological Studies of *Fusarium oxysporum* f. sp. *ciceri* Causing Wilt of Chickpea and its Management

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Investigations were conducted to find the suitable media, effect of temperature and pH on growth of *Fusarium oxysporum* f. sp. *ciceri*. Chickpea meal medium was found to be best medium for growth and sporulation of *Fusarium oxysporum* f. sp. *ciceri* followed by Potato Dextrose Agar and Corn meal medium. Growth was significantly influenced at different temperatures and pH and maximum radial growth of 81 mm was recorded at a temperature of 28 °C followed by 78 mm at 25 °C and over a pH range of 6.5. Three bioagents viz., *T. viride*, *T. hazanium* and *P. fluorescens* and two fungicides thiram (0.05%) and metiram (0.1%) were used in combination with *T. viride* as seed treatment in pots against *Fusarium oxysporum* f. sp. *ciceri*, the incitant of wilt of chickpea. Significantly minimum (14.48 %) incidence of wilt was observed in combined seed treatment of metiram (0.1%) + *T. viride* and 68.55 per cent disease control which was at statistically at par with Thiram (.05%) + *T. viride* alone which showed 16.45 per cent incidence.

Key words: Chickpea, *Fusarium oxysporum* f sp. *ciceri*, culture media, temperature, pH, management.

Chickpea (*Cicer arietinum* L.) is an important pulse crop which is grown in tropics, sub tropics and temperate regions. It is a rich source of proteins (25.3 – 28.9%). In India it is cultivated in about 8.56 million hectares with a production of 7.35 million tonnes and productivity 858 kg per hectare (Anonymous 2010). The chickpea crop is attacked by 172 pathogens (67 fungi, 22 viruses, 3 bacteria, 80 nematodes and mycoplasma) from all over the world (Nene *et al.* 1996). Wilt of chickpea caused by *Fusarium oxysporum* f. sp. *ciceri* is widely distributed throughout the country and is responsible for heavy reduction in crop yield (Haware *et al*., 1986). The disease is widespread in chickpea growing areas of the world and is reported from at least 33 countries causing 10- 15% annual losses (Nene *et al*. 1996). Considering the importance of the disease, the investigations were under taken on the effect of media, temperature and pH on the mycelial growth and sporulation of *F. oxysporum* f. sp. *ciceri* and its biological management.

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MATERIALS AND METHODS

Collection of disease material

Naturally infected chickpea plants, showing characteristic symptoms of wilt were collected from the field were brought to the

laboratory. These plants were washed and critically examined for the presence of causal organism. The pathogen was isolated with 0.25% sodium hypochlorite from collected diseases leaves on potato dextrose agar (PDA) and purified through hyphal tip method (Rangaswami and Mahadevan, 2004). Pure culture maintained and stored in refrigerator at 5°C for further studies.

Cultural studies of pathogen

Mycelial growth and sporulation of *Fusarium oxysporum* f. sp. *ciceri* in solid medium

In this study ten different natural, synthetic and semi-synthetic solid media viz. Chickpea meal agar medium, Potato dextrose agar medium, Corn meal agar medium, Oat meal agar medium, Chickpea plant extract agar medium, Czapek's agar medium, Asthana and Hawker's agar medium, Brown's agar medium and Richard's agar medium were used. All the media were prepared according to the man-ufacturer instructions. Each Petri dish was poured with 20 ml sterilized medium for solidifica-tion. Equal discs of a 5 mm in diameter of each test grown from the 7-day-old pre-cultured Petri dishes on potato dextrose agar, were taken out with the help of a cork borer and placed at the centre of each set of Petri dishes containing different medium. After inocula-tion, Petri dishes were incubated at 28±2°C. The colony diameter of the pathogen was recorded in millime-ters (mm) in two directions at right angles to each other, and then average colony diameter in millimeters was calcu-lated and recorded. Studies of sporulation on different solid media used, was also undertaken.

Mycelial growth and sporulation of *Fusarium oxysporum* f sp. *ciceri* in liquid broth medium

For conducting this study, ten different natural, synthetic and semi-synthetic broth media viz., chickpea meal medium, potato dextrose medium, corn meal medium, oat meal medium, chickpea plant extract medium, Czapek's medium, Asthana and Hawker's medium, Brown's medium and Richard's medium were used. All the media were prepared using the standard method. 50ml of each liquid media was poured in 150ml conical flasks. The conical flasks were sterilized at 15lb pressure for 15 minutes. Equal discs measuring 5 mm in diameter of test pathogen grown from the 7-day old pre-cultured Petri dishes on Potato dextrose agar, were taken out with the help of a cork borer and placed in each set conical flask contain-ing different medium. After inoculation, flasks were in-cubated at 28±2°C for seven days and were shaken twice every day. Mycelial growth of each of the test isolates was harvested in preweighed moistureless whatmen filter paper No. 42, oven dried at 60°C for 48hr. Subsequently cooled in a desicator and weighed in electric balance. The actual mycelial weight was obtained after weight of fungal growth plus filter paper and dry weight of mycelial mass produced were recorded in milligrams (mg). Studies of sporula-tion on different liquid media used, was also undertaken.

Physiological studies

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

The observations of dry mycelial weight of *F. oxysporum* f. sp. *ciceri* were determined at 5, 15, 20, 25, 28, 30, 35, 40 and 45^{0}C. In this study Potato dextrose agar was used as basal medium and the method of sterilization, incubation, filtration and determination of the dry mycelial weight, were followed as described earlier. The dry mycelia weight were determined after 20 days in incubation at 28± 1^{0}C. Three replications were kept for each treatment.

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

Effect of pH on mycelia growth of *F. oxysporum* f. sp. *ciceri* were studied at 8 pH level viz., 3.5, 4.5, 5.5, 6.5, 7.0, 7.5, 8.5 and 9.0 before autoclaving by Backman pH meter using N/10 sodium hydroxide and N/10 hydrochloric acid. The method of sterilization of the medium, filtration and determination of dry mycelial weight of the fungus were the same as described earlier. The average dry weights of the mycelium were recorded after 20 days in incubation at 28± 1^{0}C. Three replications were kept for each treatment.

Evaluation of bioagents along with fungicides under green house

Pot culture experiment was conducted under green house during Kharif 2006-07. Bioagents viz., *T. viride*, *T. hazanium* and *P. fluorescens* and two fungicides thiram (0.05%) and metiram (0.1%) were used in combination with *T. viride* as seed treatment. Chickpea meal agar was used for mass multiplication of the pathogen. Plastic pots of 30x30 cm diameter containing autoclaved soil were inoculated with Chickpea meal agar @ 50g/kg soil and left for 5 days for establishment of the

inoculum. Chickpea seeds of cv. GT-1 were surface sterilized with sodium hypochlorite, 0.1% solution and sown in sick soil in pots after treating with fungicides and thereafter with bioagents. Spore suspension of 12 days old culture of *T. viride* containing 106 spores ml-1 was prepared and seeds were soaked for five minutes one hour before sowing. Likewise, for bacterial bioagents, cell suspension prepared from 48 hrs old culture was used. For combined seed treatment, the seeds were first treated with fungicides at 24 hrs. before the inoculation of *T. viride* and then sown immediately. The pots were arranged in completely randomized design with three replications for each treatment. Untreated but surface sterilized seeds were sown in sick pots and were maintained as control. Wilt incidence was recorded from initiation of wilting till 85 days of the crop age.

RESULTS AND DISCUSSION

Effect of solid media

The results indicated in table 1 revealed that among the different culture media tested, chickpea meal agar medium and potato dextrose agar medium were the best for the radial growth of *F. oxysporum* f. sp. *ciceri* as this fungus gave maximum growth of 88.5 mm and 87.5 mm, respectively, followed by corn meal agar medium and oat meal agar medium which showed growth of 77.5 and 75mm, respectively. Minimum mycelial growth was recorded on Richards Medium (20.0 mm). Chickpea meal agar medium and potato dextrose agar medium followed by Chickpea plant extract agar produced significantly excellent growth and profuse sporulation.

Effect of liquid media

Nine liquid media were tested for quantification of sporulation, and dry wt. of mycelial mass produced were recorded. The results indicated in table 2 revealed that among the liquid media tested, maximum mycelial growth was obtained in chickpea meal medium and potato dextrose medium which showed growth of 170 mg and 165 mg, respectively followed by corn meal agar medium and oat meal medium which showed growth of 160 mg and 145 mg, respectively. Minimum mycelial growth was recorded on Richards Medium (40 mg).

Different synthetic and non synthetic cultural media have profound influence on cultural and morphological characteristics of fungus (Gupta *et al.,* 2010) The present results confirm the reports of earlier workers doing their physiological studies related to suit-able media for growth and sporulation of *Fusarium* spp. (Kulkarni, 2006; Chittem and Kulkarni, 2008). Cultural studies of *F. oxysporum* f. sp. *dianthi* on solid media

Table 1. Average diameter (mm) of mycelium mat and sporulation of pathogen on different solid media

Culture media	Average fungul growth (in mm)*	Sporulation	Growth characters
Chickpea meal agar	88.5	++++	Mycelium cottony white, compact profuse, colony circular
Potato dextrose agar	87.5	++++	Mycelium cottony white, dense profuse
Corn meal agar	77.5	++++	Mycelium white matty, submerged, loose spread
Oat meal agar	75.0	++++	Mycelium light white, submerged, spears, thin with circular
Chickpea plant extract agar	70.0	++++	Mycelium dark white, dense, profuse, colony circular
Czapek's agar	47.0	+++	Mycelium light pinkish white, fluffy
Asthana and Hawker's agar	36.0	++	Mycelium dirty white, dense, less fluffy
Brown's agar	31.0	++	Mycelium pinkish white
Richard's agar	20.0	+	Mycelium white, compact, submerged, colony circular,
C.D. at 5%	1.33		

*, Average of three replications; ++++, abundant sporulation; +++, good sporulation; ++, moderate sporulation +, poor sporulation

indicated that, the mycelial growth was maximum on potato dextrose agar (Chittem and Kukarni, 2008).

Effect of temperature

Mycelial growth of *F. oxys-porum* f. sp. *ciceri* was studied at nine different temperature viz. 5, 15, 20, 25, 28, 30, 35, 40 and 45^{0}C. As evident from Fig. 1, the fungus grew at the temperature range of 10–35^{0}C but the optimum temperature for its growth was found to be 28^{0}C (0.290 gm). The next best temperature for its growth was recorded 25^{0}C (0.276 gm). However, growth of the fungus was drastically reduced below 15^{0}C and started to decline above 35^{0}C, as these temperatures did not favour for growth of the fungus. Minimum growth was recorded at 5^{0}C. It indicated that pathogen do not survive under cool environment.

Ajid *et al.* (2005) indicated that fungal growth of *Fusarium* Spp. was best between 25^{0}C-30^{0}C. Soil temperature relationship indicated that suitable temperature for development of chickpea wilt is 25-30^{0}C (Chauhan, 1965). Landa *et al.* (2006) found that 25 to 27 ºC temperature is excellent for the growth of the *Fusarium oxysporum* f. sp. *ciceri*. Pandey *et al.* (1996) studied influence of soil temperature on the incidence of Fusarium wilt of chickpea also observed that maximum wilting of gram occurred at 25ºC and lowest at 15ºC. Haware *et al.* (1990) have also reported similar results.

Effect of pH level

Mycelia growth of *F. oxysporum* f. sp. *ciceri* was studied at 8 pH level *viz*., 3.5, 4.5, 5.5, 6.5, 7.0, 7.5, 8.5 and 9.0. The data presented in Fig. 2, that the pH level significantly dif-ferentiates the mycelia growth. The maximum growth was recorded when the pH was at the level of 6.5 (0.200 gm) followed by a pH of 5.5 (0.196 gm). There was gradual reduction in the growth below and above

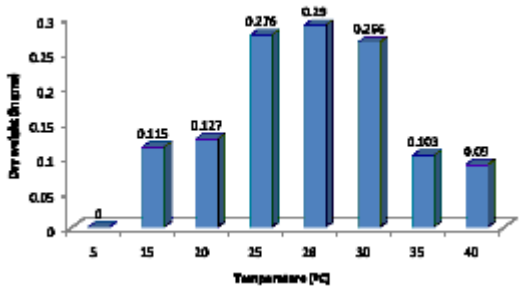

Fig. 1. Average mycelium dry weight of the pathogen at different temperatures

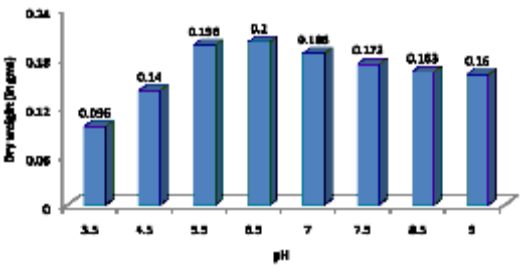

Fig. 2. Average dry weight of the mycelial mat of the pathogen at different pH level after 20 days of incubation

Table 2. Average dry weight of the mycelium mat and sporulation of the pathogen on broth liquid media.

Culture media	Av. (Dry) matter (in mg) *	Sporulation
Chickpea meal	170	++++
Potato dextrose	165	++++
Corn meal	160	++++
Oat meal	145	++++
Chickpea plant extract	140	++++
Czapeak's medium	110	+++
Asthana and Hawker's	80	++
Brown's	60	+
Richard's	40	+
C.D. at 5%	1.80	

*, Average of three replications; ++++, abundant sporulation; +++, good sporulation; ++, moderate sporulation; +, poor sporulation

Table 3. Effect of bioagents alone or combination with fungicides on wilt incidence of chickpea

Treatments	Disease incidence*	% Disease control
T1: *T. viride*	21.65	52.79
T2: *T. harzianum*	21.12	53.93
T3: *P. fluorescens*	35.41	22.77
T4: T1 + T3	19.48	57.51
T5: T2 + T3	23.39	48.99
T6: Thiram(.05%) + T1	16.45	64.12
T7: Metiram (.1%) + T1	14.48	68.41
T8: Control	45.85	-
CV	8.031	-
C.D. at 5%	3.43	

*Figures were angular transformed prior to analysis; **Average of three plants

6.5 pH. Minimum growth occurred at pH 3.5 (0.096gm). It was thus clear that the pathogen preferred slightly acidic medium for its growth. Chauhan (1962) reported decreasing of gram wilt with the increase in soil pH. Sharma *et al.* (2005) studied the effect of pH on the growth and sporulation of *F. oxysporum* f. sp. *lini* and reported that tested *Fusarium* spp. could sporulate and grew well at 5.5 pH.

Evaluation of bioagents with fungicides under glass house

All the treatments of bioagents alone and in combination with fungicides showed significant effect on disease incidence as compared to control. Seed treatment with metiram (0.1%) + *T. viride* was found most effective as it showed minimum of 14.48 per cent wilt incidence and 68.55 per cent disease control. It was statistically at par with Thiram (.05%) + *T. viride* alone which showed 16.45 per cent incidence. Integration of T. viride with P. aeruginosa and thiram (0.05%) was also effective in controlling the disease up to 48.78 percent (Table 3). Seed inoculation with *P. fluorescens* alone was found least effective with 35.41 per cent disease incidence, where maximum wilt incidence of 45.85% was observed in control. Seed treatment with *T. viride* alone and in combination with thiram have been reported to be most effective against wilt of pigeonpea (Pandey and Upadhyay, 1999; Singh and Singh, 2008). Ram and Panday (2011) found that the *T. viride* and in combination with metiram (0.1%) was effective against wilt of pigeonpea.

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REFERENCES

1. Ajid, S., Aroog, S.H., Muhammed, Iqbal and Abdul Rauf, C.H. *International Journal of Agriculture Biology*, 2005; **7**: 275-277.
2. Anonymous. The Hindu Survey of Indian Agriculture. 2010; pp67.
3. Chauhan S. K. A note on soil reaction in relation to Fusarium wilt of gram. *Proc. Nat. Acad. Sci. India***,** 1962; **32**: 385-386.
4. Chauhan, S.K., The interaction of certain soil conditions in relation to the occurrence of *Fusarium* wilt of gram. *Indian J. Agric. Sci*., 1965; **35**: 52.
5. Chittem K. and Kulkarni S. Effect of Media on the Growth of *Fusarium oxysporum* f.sp. *gerberae* and *Fusarium oxysporum* f. sp. *dianthi. Karnataka J. Agric. Sci*., 2008; **21**(2): 303–304.
6. Haware M. P, Jimeneze-Diaz RM, Amin KS, Phillips JC, and Halila H. Integrated management of wilt and root rot of chickpea. In chickpea in nineties proceeding of the second International work shop on chickpea improvement ICRISAT, Patancheru, 1990; pp129-133.
7. Haware M.P., Jimeneze-Diaz, R.M, Amin, KS, Phillips, J.C, and Halila, H. Integrated management of wilt and root rot of chickpea. In chickpea in nineties proceeding of the second International work shop on chickpea improvement ICRISAT, Patancheru, 1990; pp129-133.
8. Haware, M.P., Nene, Y.L. and Natarajan, M. (1986). Survival of Fusarium oxysporum f. sp. ciceri in soil in the absence of chickpea. Paper presented at the disease of crop plants, 8- 10 January 1986. TNAU, Coimbatore, Tamilnadu, India (Abs.).
9. Landa B. B, Navas-Cortes JA, Jimenez – Gasco MDM, Katan J, and Jimenez-Diaz R M. Temperature response of chickpea cultivers to races of *Fusarium Oxysporum* f. sp. *ciceri*. Causal agent *of* Fusarium wilt, *Plant Disease*, 2006; **90**(3): 315-374.
10. Morton DJ and Strobe WH. Antagonistic and stimulatory effect of soil microorganism upon Sclerotium. *Phytopathology*, 1955; **45**: 417-420.
11. Nene, Y.L. and Sheila, V.K. ICRISAT. News letter, proceeding on international workshop on chickpea, improvement, 1996; pp172-180.
12. Nene, Y.L., Sheila, V.K. and Sharma, S.B. A world list of chickpea (Cicer arietinum) and Pigeonpea (Cajanus cajan (L.) Millsp.) pathogens.5th edu. ICRISAT, Patancheru, India, 1996; pp27.
13. Pandey A. K, Arora DK, Pandey RR, and Srivastava, AK. Integrated control of Fusarium wilt of chickpea by solar heating of soil amended with oil seed meal and Fungicides. *Indian Phytopath.*, 1996; **49**: 247-253.
14. Rangaswami, G. and Mahadevan, A. (Eds) Disease of crop plants in India. Prentice-Hall of

India, Private Limited Publisher, New Delhi, India, 2004; pp507

15. Sharma R.L., Singh B.P., Thakur M.P., Thapak S.K. Effect of media, temperature, pH and light on the growth and spor-ulation of *Fusarium oxysporum* f. sp. *lini*. *Ann. Plant Protect. Sci.*, 2005; **13**: 172–174.
16. Pandey, K.K. and Upadhyay, J.P. Comparative study of chemical, biological and integrated approach for management of Fusarium wilt of pigeon pea. *J. Mycol. Pl. Pathol*., 1999; **29**: 214-216.
17. Singh, M. and Singh, P.N. Management of pigeon pea wilt through integrated seed treatment. *J. Food Legumes*, 2008; **21**: 71-72.
18. Ram, H. and Pandey, R.N. Efficacy of bio-control agents and fungicides in the management of wilt of pigeon pea. *Indian Phytopath*., 2011; **64**(3): 269-271.
19. Gupta, V.K., Misra, A.K., Gaur, R. K. Growth characteristics of fusarium spp. causing wilt disease in psidium guajava L. in India. *Journal of Plant Protection Research.* 2010; **50** (4): 452-462.