

Full Length Research Paper

Survey, surveillance and cultural characteristics of chickpea wilt caused by *Fusarium oxysporium* f. sp. *ciceri*

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Accepted 24 January, 2011

Survey and surveillance of chickpea wilt in the Latur district revealed average wilt complex to the tune of 12.26%. Tashilwise survey report indicated maximum wilt incidence in Tashil Ausa (15.4%) followed by Jalkot (14.8%) and Renapur (14.0%). Further study indicated that *Fusarium oxysporium* f. sp. *ciceri* was associated with majority of the cases. The pathogen was isolated, purified and its pathogenicity was proven in pot culture. Further, on the basis of morphological, cultural characteristics of the pathogen and symptomatology, the fungal pathogen was identified as *Fusarium oxysporium* f. sp. *ciceri*.

Key words: Survey, chickpea wilt, *Fusarium oxysporium* f. sp. *ciceri*.

INTRODUCTION

In Maharashtra, the total area under chickpea was 756 thousand ha with the production of about 451 thousand tonnes and the average productivity of 596 kg/ha which is very low compared to national average (Anonymous, 2002). 50 different pathogens have so far been reported on chickpea (Nene et al., 1989) including diseases caused by fungal, bacterial, nematodes mycoplasma and viral pathogen. The chickpea wilt caused by *Fusarium oxysporum* f. sp. *ciceri* was reported to be widely distributed in near about 32 countries of the world and at national scenario; six fungal diseases have been reported to be important and causing considerable damage to the crop (Haware et al., 1986a; Nene et al., 1996). Considering the variable types of wilt reactions of the released variety in the farmer's field and sick plot at different locations and yield losses caused, this investigations were undertaken to find out the major causal organism involved in chickpea wilt complex in Marathwada region of the Maharashtra state. Survey and surveillance of chickpea wilt complex incidence on farmer's field, collection, isolation, purification and pathogenicity of wilt pathogen was done.

MATERIALS AND METHODS

Survey and surveillance

A roving survey of chickpea fields was conducted in the eight Tashils districts viz., Latur, Ausa, Nilanga, Udgir, Ahmedpur, Chakur, Jalkot and Renapur of Latur district during the month of December to record the occurrence and distribution of chickpea wilt. On an average, 10 farmers' fields of chickpea in each Tashil were visited and the percent wilt incidence was recorded. Chickpea plants showing typical wilt symptoms were collected in separate paper bags and brought to the laboratory for further investigations.

Isolation

Chickpea plants naturally infected and wilted with typical symptoms of wilt were collected from farmers' field and brought to the laboratory. All samples collected from different locations were subjected to isolation on potato dextrose agar (PDA) in the laboratory.

Pathogenicity

Pathogenicity of the organism was confirmed by sick soil inoculation technique in earthen pots under green house condition by using susceptible cultivar JG-62. The culture of *Fusarium oxysporum* f. sp. *ciceri* was multiplied on sand maize flour medium (1:1). 15 g of maize flour was mixed in 85 g of river bed sand and was filled in the

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Table 1. Survey and surveillance to record chickpea wilt on farmer's field.

S/N	Name of Tahsil	Number of location traversed	Location	Average % of wilt in each Tahsil
1.	Latur	12	Bhatangli, Gharni, Takali, Bhatkheda, Malwati, Shirala, Sirsi, Gangapur, Pakharsangvi, Bamni, Kolpa, Pedgaon.	10.00
2.	Ausa	10	Bhada, Aalamla, Tungi, Devtala, Killari, Bhusni, Nagarsoga, Haregaon, Dapegaon, Jawalgawadi,	16.50
3.	Nilanga	10	Tagarkheda, Nithur, Sonkhed, Ambulga, Hangarga, Malegaon, Hisamnagar, Hadga, Kedarpur, Sonkhed	9.15
4.	Udgir	9	Dongarshelki, Haknakwadi, Kaaradkhel, Astamode, Hali Handarguli, Nagalgaon, Togri, Telegaon, Wadhona.	12.25
5.	Ahmedpur	7	Shirur tajband, Hiware bazaar, Kingaon, Nandura, Ujna Vilegaon, Godhala.	15.5
6.	Renapur	15	Bitergaon, Pangaon, Karepur, Kharola, Sangavi, Kamkheda, Ghanshargaon, Poheragaon, Bhokamba, Darji bargaon, Andalgaoon, Mohgaon, Sumthana, Faradpur, Harwadi.	13.2
7.	Chakur	7	Achola, Ashtha, Nandgaon, Belgaon, Hadoli, Zari, Nalegaon.	12.0
8.	Jalkot	10	Hokharna, Umberda Kektsindgi, Seheldara, Dhamangaon, Dongaon, Mangrul, Wanjarwada, Jirga, Nalgir.	9.55
		80	Mean incidence	12.26

conical flasks of 250 ml capacity (50 g/flask) and sterilized in autoclave at 15 Lbs for 30 min. Then, the flasks were inoculated aseptically with pure culture of *F. oxysporum* f. sp. *ciceri* and incubated at room temperature for 15 days. After 15 days of incubation, the inoculum was taken out from the flask and mixed thoroughly with sterilized sand plus soil mixture (1:1) at 100 g inoculums per kg soil. This potting mixture (sand + soil + inoculum) was filled in the earthen pots sterilized with 5% solution of copper sulphate, watered lightly and incubated for four days. Then, the seeds of highly susceptible variety, JG-62 were sown (at 10 seeds/pot) in the earthen pot. The pots with uninoculated soil served as control. All these pots were then watered lightly and kept in a glass-house for further recording of observations on per cent seed germination, seedling mortality, etc. The observations on wilt incidence were recorded after 15 days of sowing up to wilting. Reisolation of the fungus was made from roots of artificially inoculated and diseased plants showing the typical symptoms of wilting. The fungus growth obtained was transferred on potato dextrose agar slants for comparison with the original culture of *F. oxysporum* f. sp. *ciceri*. The symptoms of wilting were observed and recorded right from the initiation of the disease till the complete wilting of plants both in pot culture as well as under field condition. The culture of the pathogen obtained was identified on the basis of morphological and cultural characteristics.

Cultural studies

Growth characters and sporulation ability of the isolated *Fusarium oxysporum* f. sp. *ciceri* were studied by growing it on different agar culture media. The media used were potato dextrose agar, host leaf extract agar, Sobouraud's agar, Richard's agar, oatmeal agar

and corn meal agar. These agar media were prepared by following standard laboratory procedure given by Twite (1969), sterilized by autoclaving, poured into the sterile Petri plates, (ten plates of each medium) and allowed to cool down and solidify. Then, the plates were inoculated by placing a fungal disc (5 mm diameter) at the centre of the medium in plates and incubated at room temperature for a week.

RESULTS

Survey

The data is presented in Tables 1 and 2. From the data presented in Table 1, it is revealed that, heavy disease incidence was noticed in Ausa tashil (16.5%) followed by Ahmedpur (15.5%) and moderate in Renapur (13.2%) and Latur (10%) Tashils. The lowest disease incidences were in Nilanga (9.15%) and Jalkot tashil (9.55%) and average disease incidence was recorded in Latur district which was to the tune of 12.26%. Similar findings were reported previously by Zote et al. (1991). Data from Table 2 on percent wilt incidence for the different chickpea cultivars grown on farmers field indicated that, the maximum incidence of wilt (average 13.0%) was found in the local chickpea cultivar followed by Annegiri-1 (7.6%) and Vijay (7.5%) and the lowest incidence (3.25%) was recorded in BDN-9-3. Tashilwise wilt incidence revealed

Table 2. Per cent wilt incidence in chickpea cultivars on farmers' field.

S/N	Name of Tahsil	Number of location traversed	Percent wilt			
			BDN-9-3	Annegiri-1	Vijay	Local
1.	Latur	12	2.4	8.5	8.2	12.4
2.	Ausa	10	1.9	6.8	7.4	10.2
3.	Nilanga	10	3.2	7.0	7.2	11.6
4.	Udgir	9	2.8	6.7	9.1	13.0
5.	Ahmedpur	7	3.0	7.2	6.6	12.8
6.	Renapur	15	4.1	8.2	7.0	14.0
7.	Chakur	7	4.9	8.7	7.8	15.4
8.	Jalkot	10	3.7	7.7	6.8	14.8
	Average		3.25	7.6	7.5	13.02

Table 3. Study of organisms associated with chickpea wilt.

S/N	Name of Tahsil	Isolation study	Type of pathogen (%)		
			FOC	<i>R. bataticola</i>	<i>S. rolfisii</i>
1.	Latur	10	7	2	1
2.	Ausa	10	8	1	1
3.	Nilanga	10	7	2	1
4.	Udgir	10	9	1	0
5.	Ahmedpur	10	10	0	0
6.	Renapur	10	8	1	1
7.	Chakur	10	8	1	1
8.	Jalkot	10	8	2	0
	Total	80	65 (81.25%)	10 (12.5%)	05 (6.25%)

that in all cultivars including the local maximum incidence was recorded in Tashils Ausa (15.4%) followed by Jalkot (14.8%) and Renapur (14.0%). The results in Table 3 revealed that out of the 80 samples isolated and studied, nearly about 65 samples (81.25%) proved the association of *F. oxysporum* f. sp. *ciceri* followed by 10 samples (12.50%) of *R. bataticola* and 5 samples (6.25%) of *S. rolfisii* with chickpea wilt complex in Latur district. These results clearly indicated that the major pathogen associated with chickpea wilt complex was *F. oxysporum* f. sp. *ciceri* during early as well as later stages of the crop and to some extent *R. bataticola* and *S. rolfisii* especially in the early stage of the crop. Hence, *F. oxysporum* f. sp. *ciceri* was confirmed as the major pathogen involved in the chickpea wilt complex and therefore, further studies were continued and concentrated on *F. oxysporum* f. sp. *ciceri*. The fungus *F. oxysporum* f. sp. *ciceri* was isolated from the wilted plant collected during the survey of Latur district. After five days of incubation, the whitish profuse mycelial growth of the fungus *Fusarium* was obtained on potato dextrose agar medium. The pure culture was obtained by hyphal tip method, was sub-cultured

frequently and maintained on PDA slants for further studies.

Pathogenicity

Pathogenicity of the fungus was carried out in pot culture by using chickpea variety JG-62 which exhibited wilting after 25 days of inoculation. The initial symptoms produced were light yellow and dropping of leaves and final wilting of host. Symptoms of wilting produced by the artificially inoculated and diseased plants were identical and confirmed with those symptoms observed on naturally infected and wilted chickpea plants in the field.

Identification of fungal pathogen

The pathogenic culture isolated from the diseased plants was identified on the basis of the morphological characters as *F. oxysporum* f. sp. *ciceri* and was confirmed from NIKU, BIO-Research Lab and PUNE-411002.

Table 4. Growth characteristic and sporulation of *Fusarium oxysporum* f. sp. *ciceri* on different media

S/N	Medium	Mean colony diameter (mm)*	Sporulation	Growth character
1.	Potato dextrose agar	83	++++	Mycelium dirty white, compact submerged with concentric rings, colony circular
2.	Host leaf extract agar	90	++++	Mycelium dirty white, loose, submerged and aerial hyphae, colony and circular
3.	Richards agar	86	++++	Mycelium cottony white, compact, submerged and colony circular.
4.	Sobouraud's agar	76	++++	Mycelium pinkish, compact, submerged with circular colony
5.	Oat meal agar	71	++	Mycelium white, submerged, spreaded, sparse, thin with colony circular.
6.	Corn meal agar	73	+++	Mycelium dirty white, submerged, loose spreaded and colony circular.
	S.E.	0.85		
	C.D. at 5%	2.67		

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

Cultural studies

The results in respect of the growth characters of *F. oxysporum* f. sp. *ciceri* on various culture media are presented in Table 4. From the results, it was revealed that the maximum growth was obtained on medium host leaf extract of susceptible cultivar JG-62 (90 mm) followed by Richard's agar (86 mm) and potato dextrose agar (83 mm). All the three media proved to be significantly superior in favoring the growth of *F. oxysporum* f. sp. *ciceri* than the rest of the media tested. The least growth was observed on oat meal agar (71 mm). Hence, for the multiplication and maintenance of *F. oxysporum* f. sp. *ciceri*, PDA medium was used. Cultural characteristics of *F. oxysporum* f. sp. *ciceri* were studied on different agar media (Table 4). Host leaf extract agar (90 mm) followed by Richard's agar (86 mm) and Potato dextrose agar (83 mm) produced significantly good growth and profuse sporulation.

DISCUSSION

The symptoms produced were exactly identical to those described earlier by Westerlund (1974), Frisullo et al. (1989) and Haware et al. (1986b). Reisolation studies revealed the presence of the same fungus identical to the original one obtained from naturally wilted plants. The morphological and cultural characteristics of the *F. oxysporum* f. sp. *ciceri* obtained after reisolation were

similar to those reported earlier by several workers (Chauhan, 1962; Gupta et al., 1986 and Kewate, 1986). Pure culture of *F. oxysporum* f. sp. *ciceri* was again when reinoculated on chickpea cultivar JG-62 in pot culture and the symptoms that appeared were dropping of petioles and rachis along with leaflets. Such seedlings further got collapsed retaining almost their green colour, but when uprooted showed uneven shrinking of stem above and below the collar region. Diseased plant roots when split opened showed discoloration of internal tissues. The symptoms of chickpea wilt observed were similar to those recorded earlier by Narasimhan (1929), Westerlund et al. (1974), Haware et al. (1986b) and Frisullo et al. (1989). The cultural studies results obtained are in agreement with the findings of Chauhan (1962), Prasad and Patel (1964), Godage (1979) and Kewate (1986) who reported both host leaf extract agar and Richard's agar medium as best for the ramification and sporulation of *F. oxysporum* f. sp. *ciceri*.

REFERENCES

- Bendre NJ, Barhate BG (1998). A souvenir on Disease Management in Chickpea. M.P.K.V., Rahuri during 10th Dec. 1998.
- Chauhan SK (1962). Physiologic variations in *Fusarium arthoceras* App. and Wr. var. *ciceri* Padwick, causing wilt of gram (*Cicer arietinum* L.) Proc.Nat. Acad. Sci., 32: 78-84.
- Frisullo SF, Cioccarese M, Amenduni, Zamani HR (1989). Wilt of chickpea (*Cicer arietinum* L.) caused by *Fusarium oxysporum* f. sp. *ciceri* in Southern Italy. Difesa delle Piante, 12(1-2): 181-185.
- Godage TU (1979). Studies on the wilt of gram (*Cicer arietinum* L.) caused by *Fusarium oxysporum* f. sp. *ciceri* (Padwick) Snyder and

- HanSen. M.Sc. (Agri.) Thesis submitted to M.P.K.V., Rahuri,
- Gupta OM, Khare MN, Kolasthane SR (1986). Variability among six isolates of *Fusarium oxysporum* f. sp. *ciceri* causing wilt of chickpea. Indian Phytopath., 39 (1) : 279-281.
- Kewate SB (1986). Studies on wilt of gram (*Cicer arietinum* L.) caused by *Fusarium oxysporum* f. sp. *ciceri*. M.Sc. (Agri.) Thesis. submitted to M.P.K.V., Rahuri,
- Khune NN, Patil MJ (1992). Transmission studies of *Fusarium* wilt by isolation of *Fusarium oxysporum* f. sp. *ciceri* from various plant parts of chickpea. New Agriculturist, Dept. of Pl. Patho.. Post Graduate Institute, P.K.V., Akola, India, 3(2): 227-228.
- Narasimhan R (1929). A preliminary note on the *Fusarium* parasitic on Bengal gram (*Cicer arietinum* L.). Madras Agri. Dept. Year Book, pp. 5-11.
- Nene YL, Shelia VK, Sharma SB (1989). A world list of chickpea and pigeonpea diseases. Legume Pathology Progress Report – 7, ICRISAT Publication.
- Nene YL, Shelia VK, Sharma SB (1996). A world list of chickpea and pigeonpea pathogens 5th Edn. Patancheru, Andhra Pradesh, India. ICRISAT, p. 27.
- Prasad N, Patel PN (1964). Studies on *Fusarium* wilt of Cumin. Plant Dis. Repr., 47: 528-531.
- Twite J (1969). Plant Pathological Methods. Fungi and Bacteria. Burgess Publishing Co., pp. 12-80.
- Westerlund FV, Jr Campbell RN, Kimble KA (1974). Fungal root rots and wilt of chickpea in California. Phytopathology, 64(4): 432-436.
- Zote KK, Mote TS, Kulkarni SV, Shaikh MH (1991). Survey of Association of fungi with chickpea wilt disease. Paper presented in 4th Annual Meeting WZ Department of Botany, Marathwada University, Aurangabad.