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SYMPTOMATOLOGY AND PATHOGENICITY CHARACTERISTICS OF *FUSARIUM OXYSPORUM F. SP. CICERI* IN CHICKPEA SEEDS

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ABSTRACT

Fusarium wilt is a serious disease of chickpea in India and other 32 countries. It is soil borne disease. Observation of symptomatology was record. This pathogen was isolated on culture media and natural host, purified and its pathogenicity was proven in pot culture and on potato-dextrose-agar. Pathogenicity of the fungus carried out on a chickpea variety Radhey, which exhibited wilting after 25 days of inoculation. Out of 75 seeds, 68 germinated in infected condition. Symptom appearances were in 20 plants at seedling stage, 14 plants in adult stage, 34 not show any symptom of wilting. Seeds harvested from wilted plants were lighter and duller than those from healthy plants (Haware and Nene, 1980). Further, on the basis of morphological, cultural characteristics of the pathogen and symptomatology, we have confirmed that fungal pathogen, as Fusarium oxysporum f. sp. ciceri.

Keywords: **Chickpea, Fusarium oxysporum f. sp. Ciceri, Pathogenicity.**

Pulse crops play an important role in Indian agriculture, besides being rich in proteins; they sustain the productivity of the cropping system. Gram (Chana) (*Cicer aritinum* L.) belongs to the sub family *Papilionaceae* of the family *Leguminaceae*. Chickpea is subjected to attack by a number of fungal, viral, bacterial and nematode diseases. 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), causing 10 - 90% annual losses (Singh and Dahiya, 1973; Jalali and Chand, 1992). This pathogen is a facultative saprophyte which can survive in soil up to six years in the absence of susceptible host (Haware et al., 1986). These investigations were undertaken to find out characteristics symptomatology of chickpea wilt complex incidence on field, collection, isolation, purification and pathogenicity of wilt pathogen.

Material and method

Symptomatology:

Roots, collar, bark and internal tissues and foliages of diseased part of chickpea plants showing characteristic symptoms of wilt disease were collected from chickpea field of Plant Pathology section of C. S. A. University of Agriculture and Technology Kanpur at the last week of November, 2002 and March, 2003, for the isolation of *F. oxysporum* f.sp. *ciceri*. Observation of disease symptoms were recorded from start the disease appearance to crop maturity.

Isolation, purification, identification and pathogenicity:

Isolation, purification of pathogen:

Isolation of the pathogen wilt affected gram roots, washed in tap water to remove dust particles. The roots were cut into small piece, sterilized with 0.1 per cent mercuric chloride solution for one minute three times. Later these root pieces were transferred in between two folds of sterilized blotter papers to remove the excess of water. The media

used was potato dextrose 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.

Dried root pieces were finally transferred on two per cent Potato Dextrose Agar (PDA) medium in Petri plates with the help of a sterilized pointed inoculating needle or forceps in the inoculation chamber. These Petri plates were incubated at 28-30^{0}C for the growth of the pathogen. When mycelia growth was visible the growing mycelium in different Petri plates was transferred and purified by repeated transfer of growing hyphae tips to the another sterilized culture tubes and petriplates containing having 2 per cent PDA medium. *F. oxysporum* was purified by single spore method and was identified with the help of relevant literature of Synder and Hausen (1940).

Identification of the pathogen:

The fungus was grown on Potato dextrose agar medium. The measurements of different morphological structures were done under the microscope. Observations on the following morphological characters of the fungus were noted.

(i) Mycelial characteristics: Colour, branching pattern and width of the hyphae etc., will be studied under microscope for identification.

(ii) Sporodochia: Its presence, colour, shape, size and arrangement were recorded under the compound microscope.

(iii) Microconidia: Number, branching pattern, size, shape etc., will be observed under microscope for specific identification.

(iv) Macroconidia: These will also studied under microscope for number, colour, septation, size and arrangement for specific identification.

(v) Chlamydospores: Studies on the position, arrangement, shape, and morphology of the Chlamydospores will be done for better understanding about the pathogen.

Pathogenicity test:

Pathogenicity of the fungus was carried out in pot culture by using chickpea variety Radhey which exhibited wilting after 25 days of inoculation. The initial symptoms produced were light yellow and drooping 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.

Result and Discussion

Symptomatology:

Symptoms of wilt were seen usually in patches of dead seedlings or adult plants seen usually in patches. The disease can affect the crop at any stage. Sudden death, leaves also turn yellow and drop off prematurely, collar region of the wilted plants necrosis and discoloration of the external tissues can be seen. Roots become weak due to disease and when a diseased plant was pulled out, most of the lateral roots remain in the soil. Transverse section of the basal stem or the root reveals masses of fungal hyphae in the vascular bundles and discolorations of vascular cells. 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).

Identification of the pathogen:

Expansion pathogen on potato-dextrose-agar at 25^{0}C is delicate, white, cottony becoming felted and wrinkled; Mycelium of fungus was profusely bunched, creeping, hyaline, cylindrical, septate and measuring 3.20 - 4.6 μm in width.

Colonies of fungus were produces white to light orange sporodochia on potato-dextrose-agar media. Sporodochia was completely covered with arial mycelium, colonies were white initially, radiating in

early stage of growth with wine-red pigmentation of medium in later stages which was clearly visible from the bottom side of the plate. Microconidia were hyaline, single celled, oval to cylindrical, straight to slightly curve and measuring 2.5 - 3.5 X 5 - 11 µm in size while macroconidia were sparse and produced on branched macro-conidiophores. They were fusoid with pointed ends, hyaline septate (3-4 septate) and 3.0-4.5 X 20-55 µm in size. Chlamydospores were usually intercalary, swell form and one produced singly or in pairs on the hyphae and 7.0 -8 X 3.5 - 5.0 µm. They were globose to sub-globose, thick walled and smooth surfaced. The cultural studies results obtained are in agreement with the findings of Chauhan (1962), Prasad and Patel (1964), Godage (1979) and Kewate (1986).

Pathogenicity test:

In order to ascertain the pathogenic ability of the isolates of *Fusarium oxysporum* f.sp. *ciceri*, the pathogenicity test was conducted on chickpea variety Radhey. The results on pathogenicity test are given in Table-1

Table.1: Effect of wilt pathogen on the seed, seedling and plant of chickpea variety Radhey

Treatments	No. of seeds germinated	No. of seedlings	No. of plants wilted		
			Seedling stage	Adult stage	Total wilting
Partially sterilized soil inoculated with *F. oxysporum* f. sp. *ciceri*	75	68	20	14	34
Partially sterilized soil not inoculated with t he fungus (control)	85	85	0	0	0

The results presented in table-1 showed that the fungus had adverse effect on the seed germination and seedling emergence. The seed germination was reduced by 9.33%. Out of 75 germinated seeds, 68 seedlings were emerged in pots containing pathogen mixed partially sterilized soil. The reduction in germination and seeding emergence was due do rotting of seeds and pre-emergence rotting of germinated seeds. The initial symptoms of wilting start appearing after 15 days of sowing in the seedlings as drying of seedling from the tip and 20 plants seedlings got infected within two months where as the wilting recorded at the time of adult stage was 14 plants only.

The fine roots of the wilted plants were mainly affected and bear dark blacks streak beneath of their bark. Such roots were brittle and easily broken when touched. The brown colour mycelium of the pathogen choked the xylem of primary roots, which resulted in to the drying of whole seedling and plant. The fungus was re-isolated from the diseased tissue and was found same as after comparing with the original one and thus proving the Koch's postulate (1882). Pathogenic variability of *Fusarium oxysporium* f. sp. *Ciceri* has been well documented (Haware and Nene, 1982; Gupta et al., 1986; Honnareddy and Dubey, 2006; Rahman et al., 1998). Different levels of virulence in the populations of *F. oxysporium* f.sp. *lentis* have also been reported by Belabid et al. (2004) and they also did not find any correlation between geographical origin and virulence of the isolates.

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