Journal of Plant Development Sciences
Meerut, India

RESEARCH ARTICLE

SYMPTOMATOLOGY AND PATHOGENECITY OF *FUSARIUM OXYSPORUM* F. SP. *CICERI* IN CHICKPEA VARIETY GNG-1958

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Received-05.08.2023, Revised-16.08.2023, Accepted-28.08.2023

Abstract: *Fusarium* wilt is a serious disease of chickpea in India and world. It is a serious soil borne disease. Pathogenicity of the fungus was carried out on a chickpea variety GNG-1958, which exhibited wilting after 25 days of inoculation. Observations of symptomatology and pathogenecity were recorded. Further, on the basis of morphological and cultural characteristics of the pathogen, it was confirmed that the pathogen was *Fusarium oxysporum* f. sp. *ciceri*. Further the pathogen was identified from ITCC (Indian Type Culture Collection) Lab, IARI, New Delhi.

Keywords: Chickpea, *Fusarium oxysporum* f. sp. *ciceri*, Variety

INTRODUCTION

Pulses occupy an important position in Indian agriculture. They are rich sources of proteins and play a crucial role in atmospheric nitrogen fixation thus sustaining the productivity of the cropping system. Gram, also called chickpea (*Cicer arietinum*) belongs to the family Leguminaceae. Chickpea is subjected to attack by a number of fungal, bacterial, viral and nematodal diseases. The chickpea wilt caused by *Fusarium oxysporum* f. sp. *ciceri* is widely distributed causing high yield losses (Haware *et al*., 1986 and Nene *et al*., 1981), causing upto 10% losses annually (Singh and Dahiya, 1973; Jalali and Chand, 1992). *F. oxysporum* f. sp. *ciceri* is a facultative saprophyte which has the ability to survive in soil for up to six years in the absence of a susceptible host. These investigations were undertaken to find out symptomatology and pathogenecity on chickpea variety GNG-1958 through collection, isolation, purification, inoculation, re-isolation and identification of wilt pathogen.

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MATERIALS AND METHODS

Collection of diseased samples

Infected plants showing typical wilt symptoms were collected from chickpea field of college farm, Ummedganj (Kota), Rajasthan. Samples were brought into the Department of Plant Pathology, College of Agriculture, Ummedganj (Kota) for isolation and further studies.

Cleaning and sterilization

Prior to use, the glasswares were cleaned with chromic acid solution followed by washing with liquid detergent and finally rinsed with tap or distilled water. The dried glass wares were sterilized in hot air oven at 180 ± 2 ºC for 2 hrs. The media and water were sterilized in autoclave at 15 psi for 20 minutes. The inoculation needle, cork borer and other metallic instruments were sterilized by dipping them in alcohol and heating until red over the flame of a spirit lamp. Surface sterilization of diseased plant parts was done by dipping them in 0.1 % $HgCl_2$ for two minute, followed by three washes in sterile distilled water.

Seeds were sterilized in 0.1% of $HgCl_2$ solution for two minute followed by three washings in sterilized

distilled water to remove traces of $HgCl_2$. Soil was sterilized by using 4% formaldehyde and pots were sterilized by 0.1% of $HgCl_2$ solution.

Preparation of PDA medium

For the laboratory experimental studies, standard potato dextrose agar (PDA) medium was used for isolation and culturing of *F. oxysporum* f. sp. *ciceri*. The composition of PDA used is given below:

Ingredients	**: Quantity (g)**
Potatoes, infusion form	: 200.00
Dextrose (Glucose)	: 20.00
Agar	: 15.00
Final pH (at 25°C)	: 5.6 ± 0.2

Two hundred grams of peeled potatoes were cut into pieces. These pieces were boiled in water and the extract was collected by filtering through muslin cloth. Each of 20 g of dextrose and agar-agar were dissolved in potato extract and the final volume was made up to 1000 ml by adding distilled water. A known quantity of such medium was dispensed into number of conical flasks and plugged with non-absorbent cotton and finally wrapped with paper. The flasks containing dispensed medium were sterilized in autoclave at 15 psi for 15 min at 121.6ºC.

Isolation of the fungus

The infected plants were washed thoroughly with running tap water to remove soil particles adhering to roots and other parts. Washed plants were placed in between blotting papers to remove excess moisture. Infected parts of wilted plants were cut into small pieces with the help of sterilized scalpel. These pieces were surface sterilized by 0.1% of $HgCl_2$ solution for one minute followed by 3-4 washings with sterilized distilled water to remove the traces of $HgCl_2$ solution. Such pieces were placed on sterilized PDA plates under sterilized conditions. The inoculated plates were incubated at 28±1°C. After the fungal growth appeared on the PDA plates, the growing tips were sub-cultured. This process was continued till pure culture of the pathogen was obtained. The pathogen was stored in a refrigerator at 4ºC for further studies.

Maintenance of pure cultures

The fungus *F. oxysporum* f. sp. *ciceri* was sub-cultured on PDA slants and allowed to grow at 28±1°C temperature in the incubator for ten days. The culture obtained was stored in a refrigerator at 4±1°C and was sub-cultured once in a month.

RESULTS AND DISCUSSION

Isolation, Pathogenicity, Reisolation and Symptomatology

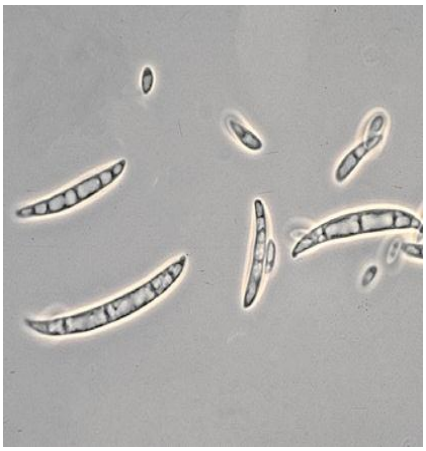
Isolation

Fungus *F. oxysporum* f. sp. *ciceri* (*Foc*) was isolated from the diseased samples of wilt infected chickpea plants collected from diseased field located at College of Agriculture, Agriculture University, Kota. The technique of standard tissue isolation was followed for isolation of fungus. After profused whitish mycelium growth appeared on the potato dextrose agar (PDA) plates, the growing tips were separated and sub-cultured repeatedly. This process was continued till pure culture growth of *F. oxysporum* f. sp. *ciceri* was obtained. The uniform colonies were maintained on PDA slants and culture plates at 4±1°C, which was further used during research investigation.

Identification of the fungus

The pathogen *F. oxysporum* f. sp. *ciceri* formed cottony white growth on the Petri plates containing PDA. The colonies appeared as dull white to pure white mycelial growth and released pinkish pigmentation after 8-9 days of incubation. On the basis of these characters the pathogen was identified as *F. oxysporum* f. sp. *ciceri*. Further the pathogen was identified from ITCC (Indian Type Culture Collection) Lab, IARI, New Delhi. The studies on cultural characteristics were in agreement with the findings of Chauhan (1962), Prasad and Patel (1964) and Kewate (1986).

(A) Pure culture of *Foc*



(B) Microscopic view of *Foc* conidia

Plate 1 Cultural (A) and morphological (B) characteristics of *Fusarium oxysporum* f. sp. *ciceri*

Pathogenecity test of wilt disease pathogen

The pathogenicity of *F. oxysporum* f. sp. *ciceri* was studied on susceptible variety GNG-1958. The pathogenicity test was carried out by two methods *i.e.* seed and soil inoculation methods. The recorded data are presented below:

Seed inoculation

The data in Table 1 indicated that germination was 80.00 per cent in inoculated control and 96.67 per cent in un-inoculated control. It was also observed that inoculated control showed 70.83 per cent mortality of chickpea plants due to wilt disease. On the other hand, un-inoculated chickpea seedlings did not show any symptoms of wilt disease.

Soil inoculation

Data presented in Table 2 indicated 80.00 per cent germination of chickpea plants due to infection by *Foc* in inoculared control as compared to 96.67 per cent of average germination percentage in un-inoculated control. The mortality recorded after 15 days of sowing till maturity was 71.76 per cent. On the other hand, un-inoculated chickpea seedlings did not show any symptoms of wilt disease. Pathogenicity test was confirmed after development of symptoms on infected plants. Pathogenic variability of *Fusarium oxysporum* f. sp. *ciceri* were similar with the findings of Haware and Nene, 1982; Gupta *et al*., 1986; Rahman *et al*., 1998 and Honnareddy and Dubey, 2006.

Table 1. Pathogenicity test of wilt pathogen *(Fusarium oxysporum* f. sp. *ciceri*) by seed inoculation method

Particulars	*Germination (%)	*Mortality (%)
Inoculated control	80.00	70.83
Un-inoculated control	96.67	0.00

*Average of three replications.

Table 2. Pathogenicity test of wilt pathogen *(Fusarium oxysporum* f. sp. *ciceri*) by soil inoculation method

Particulars	*Germination (%)	*Mortality (%)
Inoculated control	80.00	71.76
Un-inoculated control	96.67	0.00

*Average of three replications.

Inoculated control **Un-inoculated control**

Plate 2 Pathogenicity test of *Fusarium oxysporum* f. sp. *ciceri*

Reisolation

Fungus was reisolated on PDA from artificially inoculated wilted plants which produced profused whitish mycelial growth characteristics of *Fusarium oxysporum* f. sp. *ciceri*. The re-isolation of pathogen proved that it was *F. oxysporum* f. sp. *ciceri*.

Symptomatology

Artificially inoculated plants showed typical symptoms like drooping of leaflet, petiole and rachis, ultimately leading to drying of the whole plants. The plants showed gradual loss of green colour (chlorosis). Later the plants turned completely yellow and then became light brown or straw coloured. Within few days, the entire wilted plants got collapsed. When infected stem was splitted open vertically, brown discoloration of internal tissues was observed. It was deduced that *F. oxysporum* f. sp. *ciceri* obtained from naturally wilted plants could also cause infection in chickpea which produced typical wilting symptoms. The wilting was not recorded in pots containing sterilized soil without test fungus. The symptoms of chickpea wilt observed were in corroboration with the findings of Narasimhan (1929), Frisullo *et al*. (1989), Singh and Gangwar (2017), Qureshi *et al*. (2021) and Varala *et al*. (2023).

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