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Wilt in Cucumber-An Emerging Disease in Mid Hills of Himachal Pradesh

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ABSTRACT

Fusarium wilt of cucumber caused by *Fusarium oxysporum* was found to occur in major cucumber growing areas of mid hills of Himachal Pradesh. During surveys conducted in 2018-2019, maximum disease incidence was recorded at Dedghrat (43%) followed by Jadari (31%). Symptoms of disease appeared as chlorosis followed by necrosis from lower to middle leaves leading to complete wilting of the plant. Associated fungus, from the diseased samples was isolated in pure form and designated as *Fusarium oxysporum* on the basis of cultural and morphological characters. Pathogenicity test was carried out with three different methods of inoculation, where soil inoculation with mass culture method was found most effective with an incubation period of about 10 days. Five cucurbits (bottle gourd, bitter gourd, zucchini, sponge gourd and pumpkin) were evaluated against *Fusarium oxysporum* to study its host range and it was found that all the cucurbits were susceptible to the disease except bitter gourd.

Key Words: Cucumber Fusarium wilt, Host range, Occurrence, Pathogenicity.

INTRODUCTION

Fusarium wilt (*Fusarium oxysporum*) is one of the most destructive diseases especially under protected conditions reducing the potential yields to the tune of 70 per cent. The occurrence of the disease was first time reported from Florida in 1955. However, in India, the Fusarium wilt was first reported from Jammu and Kashmir (2012), then Lucknow (2017) followed by Punjab and Solan (Himachal Pradesh) in 2018. Change in climatic conditions and repeated cultivation of cucumber under protected conditions, has led to increase in the incidence of the wilt disease in mid hills of Himachal Pradesh. Though the disease is occurring regularly in the state but, very little scientific work has been carried out so far on different aspects of the disease in this area. Keeping in view a huge amount of losses caused by the disease, present studies were undertaken to study occurrence, pathogenicity and host range of the pathogen.

MATERIALS AND METHODS

Occurrence

To record the prevalence of Fusarium wilt, periodic surveys of cucumber growing areas of Solan district of Himachal Pradesh were undertaken from June to September during 2018 crop season. During the course of surveys, the diseased plants were collected in paper bags and brought to the laboratory for immediate isolation of the associated pathogen and also kept in refrigerator for further use.

In order to assess the magnitude of the Fusarium wilt, the incidence of the disease was recorded during periodic surveys conducted in different cucumber growing areas of Solan district and per cent disease incidence was calculated. Above and below ground symptoms of Fusarium wilt of cucumber were studied on the diseased samples collected during the surveys. The fungus was isolated in pure form using standard procedure.

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The isolated pathogen was purified by single spore isolation method. Morphological characters of the isolated fungus were studied both on potato dextrose agar medium and under microscope. The characters observed were compared with the standard authentic descriptions and taxonomic keys given by Booth (1971) in "The Genus Fusarium".

$$\text{Disease incidence (\%)} = \frac{\text{Total number of plants infected}}{\text{Total number of plants observed}} \times 100$$

Pathogenicity test

Pathogenicity test of the causal organism was carried out on three weeks old seedlings of cucumber hybrid "KH-1" by artificial inoculation under laboratory conditions using three methods viz. soil inoculation by mass culture, by spore suspension and root dip method. To raise the seedlings of KH-1, seeds of the hybrid were sown in pots/polybags filled with sterilized mixture of soil: sand: FYM in the ration of 2:1:1. After three weeks, these seedlings were transferred to pots for pathogenicity test.

Preparation of mass culture of *Fusarium oxysporum*

The mass culture of *Fusarium oxysporum* was prepared on corn: sand meal medium (Dohroo, 1988) which was inoculated with mycelial bits of *Fusarium oxysporum* from the margins of an actively growing culture and incubated at 25±2°C in BOD incubator for 15 days. The bags were shaken regularly after 3 days so that fungus grows uniformly.

Preparation of conidial suspension

For inoculation, conidial suspension was prepared from one week old culture of *Fusarium oxysporum*. Sterilized water was added in each culture plate in order to harvest the spores of fungus. The culture was gently rubbed with spatula to scrap off the spores. The spore suspension was then collected in a beaker and passed through a muslin cloth. The filtrate was resuspended in distilled water and spore concentration was adjusted to $1x10^7$ conidia/ml with the help of haemocytometer.

Soil inoculation by spore suspension

About 50ml of the spore suspension was drenched in each pot (4 inch dia.) filled with sterilized soil. After inoculation, pots were kept as such for about seven days for buildup of inoculum. Thereafter, about 2-3 seedlings of cucumber were transplanted in each pot and pots were kept in the glasshouse for symptom development. Pots without spore suspension served as control. Observations in terms of incubation period (days) were recorded.

Soil inoculation by mass culture

Mass culture of *Fusarium oxysporum* was mixed @ 10g/pot in sterilized soil contained in pots upto 5 cm depth. After inoculation, soil was sprayed with sterilized distilled water and kept covered with polythene for about seven days for buildup of inoculum. Pots without inoculum served as control. Two to three seedlings were thereafter transplanted in each pot and pots were kept in the glasshouse for symptom development. Pots without mass culture served as control. Observations in terms of incubation period (days) were recorded.

Root dip method

Roots of the healthy seedlings were washed with water and trimmed gently. Seedlings were inoculated by dipping their roots in suspension for about 5 minutes and thereafter, 2-3 seedlings were transplanted in pots containing sterilized soil. Seedlings treated with sterilized water served as control. Thereafter, pots were kept in the glasshouse for symptom development. Pots without root dip treatment served as control. Observations in terms of incubation period (days) were recorded.

Host range studies

Seedlings of cucurbits like bottle gourd (*Lagenaria siceraria*), pumpkin (*Cucurbita moschata*), bitter gourd (*Momordica charantia*), zucchini (*Cucurbita* pepo) and sponge gourd (*Luffa*

Fig 1: Symptoms of Fusarium wilt of cucumber

cylindrical) were raised in pots filled with sterilized soil. Seedlings at about 3-4 true leaf stage (21 days old) were transplanted in poly bags inoculated with mass culture of *Fusarium oxysporum*. The polybags were placed in glass house, observed for symptom development and incubation period (days) was recorded.

RESULTS AND DISCUSSION

Occurrence

Out of the fifteen locations surveyed in Solan district, three locations viz., Adda, Mansar and Damkari were found to be free from the disease. However, at other locations, disease incidence varied from 5 to 43 per cent with mean disease incidence of 13.53 per cent. The highest disease incidence was recorded at Dedgharat (43%) followed by Jadari (31%) and Nauni (25%) whereas, minimum disease incidence was recorded at Darwa (5%) and Palhech (5%). Initially symptoms appeared as drooping of lower and middle leaves progressing upwards to the top of the plant. Wilting symptoms were accompanied by chlorosis and finally necrosis (Fig 1A) leading to the death of the plant. Plants with or without vascular discolouration of roots and stems were observed (Fig 1B). During surveys, it was observed that disease incidence was more under polyhouse conditions as compared to the open field conditions. This might be due to the prevalence of high temperature and moisture conditions under polyhouse conditions which are favourable for the survival of the pathogen. Higher disease incidence at certain places could be due to the buildup of soil borne inoculum due to monoculture of susceptible cultivars. The occurrence of the disease has been reported from different cucumber growing regions of the world (Owen ,1955; Armstrong and Armstrong, 1978; Jenkins and Wehner, 1980; Ogura and Ma, 1992; Sultana and Ghaffar 2013; Fareed *et al,* 2016).

Cultural and morphological characters of fungus

The fungus produced white, flat, cottony spreading type of colonies without any pigmentation (Fig 2A). Hyphae of the fungus were branched, hyaline and septate with average diameter of about 3.37µm (Fig 2B). Microconidia were oval to oblong, single celled measuring 6.2-17.4 X 1.5-3.8µ (Fig 2C), macroconidia were straight or slightly curved, attenuated towards each end and pedicillate, mostly 3 septate having average size of 28.9X4.32 µ (17.0-48.0 X 2.5-4.8µ). Chlamydospores were abundant in old cultures terminal or intercalary, globose to sub-globose, thick walled and smooth with average size of 9.6X8.2 µ. On the basis of morphological characters compared with standard authentic description (Owen, 1955; Booth, 1971), the fungus was identified as *Fusarium oxysporum*. Morphological and cultural characters of the pathogen observed were in agreement with other workers (Owen, 1955; Pagoch and Raina, 2012; Sultana *et al*, 2014; Seo *et al,* 2017).

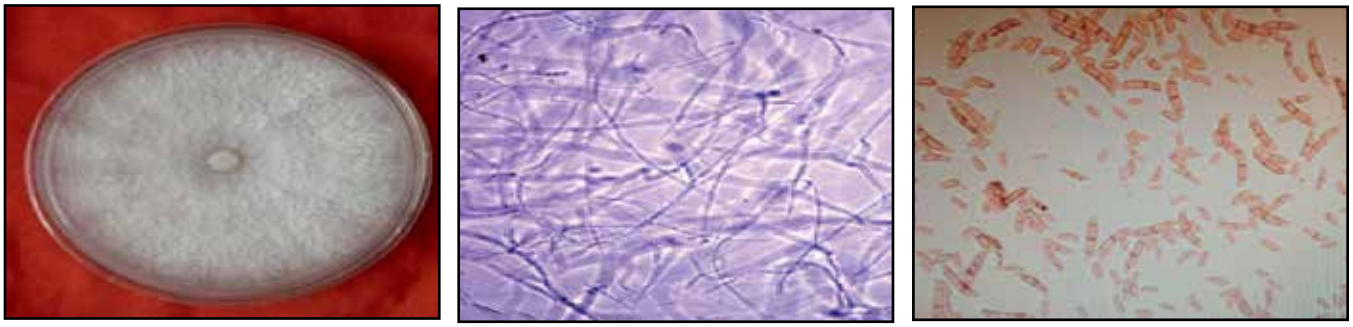

Fig 2: Cultural and morphological characters of *Fusarium oxysporum*

Pathogenicity

Among different methods of inoculation used for proving pathogenicity of *Fusarium oxysporum* under glass house conditions, soil inoculation by mass culture method (Fig 3A) was found best with least incubation period of 10 days followed by root inoculation by root dip method (Fig 3B) having incubation period of 13 days and soil inoculation by spore suspension method (Fig 3C) with incubation period of 14 days. However, no disease was recorded in un-inoculated control. The pathogen was re-isolated from the infected crown and stem portion on PDA medium, thereby proving Koch's postulates. Results are in agreement with Parasiya (2008) and Saengnak *et al* (2013) who reported soil inoculation method best for proving pathogenicity of *Fusarium oxysporum* f. sp. *capsici*. Contrary to this, Pagoch and Raina (2012) and Nirmaladevi and Srinivas (2012) observed standard root dip method of inoculation better for proving pathogenicity of *Fusarium oxysporum* f. sp. *cucumerinum* and *Fusarium oxysporum* f. sp. *lycopersici*.

Host range studies

It is evident that except bitter gourd, all the cucurbits were found susceptible to the disease (Fig 4). Sponge gourd was the most susceptible with 11 days of incubation period, followed by bottle gourd (12 days). Pumpkin and zucchini had 13 days of incubation period. Although isolates of *Fusarium oxysporum* are generally host specific, however cross pathogenicity among formae speciales of cucurbits is not uncommon (McKeen, 1951; Owen, 1955; McMillan, 1986; Gerlagh and Blok ,1988; Nomura, 1992; Cafri *et al*, 2005).

CONCLUSION

Incidence of Fusarium wilt in cucumber growing areas of mid hills of Himachal Pradesh varied from 5 to 43 per cent with mean disease incidence of 13.53 per cent. Among different methods of inoculation used for proving pathogenicity of *Fusarium oxysporum*, soil inoculation by mass culture method was found best with least incubation period of 10 days. Except bitter gourd, all the cucurbits were found susceptible to the disease.

Fig 3: Pathogenicity test of *Fusarium oxysporum* under pot conditions

Fig 4: Evaluation of host range of *Fusarium oxysporum* under pot conditions

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