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Kalpana Yadav
Ph.D., Scholar, Department of Plant Pathology, MPUAT, Udaipur, Rajasthan, India

NL Meena
Assistant Professor, Department of Plant Pathology, MPUAT, Udaipur, Rajasthan, India

Akansha Devra
Ph.D., Scholar, Department of Plant Pathology, MPUAT, Udaipur, Rajasthan, India

Correspondence
Kalpana Yadav
Ph.D., Scholar, Department of Plant Pathology, MPUAT, Udaipur, Rajasthan, India

Occurence of stem and root rot of cucumber in field and Polyhouse conditions in Udaipur region: survey, collection, identification and proveing the pathogenicity of pathogen

Kalpana Yadav, NL Meena and Akansha Devra

Abstract
Root and stem rot of cucumber is believed to be caused by a new formae specialis of *F. oxyspowm*, presently designated *F. oxysporum* f. sp. *radicis - cucumerinum* (FORC) (Vakalounakis, 1996). Fusarium root and stem rot is one of the most damaging diseases of cucumber. This is the most destructive disease of glasshouse cucumber crops in Canada in 1994, in France in 1998, in China in 1999, and in Spain in 2000, causing significant losses in the yield (Punja & Parker, 2000). The present investigation was therefore, undertaken with the Occurrence, identification and pathogenicity of *Fusarium oxysporum* f. sp. *radicis cucumerinum* causing root and stem rot of cucumber in polyhouse and field conditions under Udaipur region.

Keywords: Occurrence, *Fusarium oxysporum* f, sp, radicis cucumerinum, collection

Introduction
Cucumber probably originated in the foothills of the Himalayas and have been cultivated for at least 3,000 years. According to the Food and Agriculture Organization of the United Nations, China produced at least 60% of the global output of cucumber in 2005, followed by Turkey, Russia, Iran and the United States (Anon. 2017) [1-2]. Approximately 75.5 million tonnes of cucumber was produced worldwide in 2017. In India, major cucumber growing states are Karnataka, Andhra Pradesh, Assam, Bihar, Jammu Kashmir, Telangana, Madhya Pradesh, Orissa, Kerala, Jharkhand and almost all states with total production 1.2 million tonnes in 76000 hectare area (Anon. 2017) [1-2]. In Rajasthan, major cucumber growing area is Bharatpur, Alwar, Bhilwara, Jaipur, Tonk, Dholpur, Udaipur, Chittorgarh, and Sawai Madhopur districts. It is cultivated in 2.14 thousand hectare area with production of 15 thousand tons (Anon. 2017) [1-2]. Nutritionally, mineral contents *i.e*. Potassium, Sodium, Magnesium, Sulfur, Silicon, Chlorine and Fluorine is found. Cucumber also has vitamins A, B, C, D, and E. The fruit of cucumber possess various medicinal properties e.g. cooling effect, checks jaundice, anti-cancer properties, and indigestion. Root and stem rot of cucumber is believed to be caused by a new formae specialis of *F. oxyspowm*, presently designated *F. oxysporum* f. sp. *radicis- cucumerinum* (FORC) (Vakalounakis, 1996) [13]. A *Fusariurn* root and stem rot disease on greenhouse cucumber (*Cucumis sativus* L.) has only been reported in Greece, where during the 1989-90 growing season, the disease was limited to a few greenhouses on the island of Crete. Since then, the pathogen has spread to most of the other growing regions of Crete; severe losses occured only 3 years after first being reported (Vakalounakis, 1996) [13]. Some forma speciales of *F. oxysporum*, cause rotting of roots, lower stems and crowns and rotting of seeds and seedlings (damping-off) (Agrios, 2005) [3]. When cucumber is infected with the root and stem rot fungus, the primary root, secondary and tertiary roots and the basal portion of the stem have brown discolorations. Pagoch *et.al*., (2012) [10] collected root and stem parts from cucumber growing areas of Kathua, Jammu, Rajori, Udhampur, Doda and Poonch districts of Jammu region during 2007 and 2008 and revealed that the presence of *Fusarium oxysporum* f. sp. *radicis cucumerinum* and *F. solani* which caused losses 85.72 and 14.29 percent, respectively.

Material and Methods

Survey for Collection of Diseased Material and Isolation of the Pathogen

The survey was carried out from cucumber growing area under Udaipur region *viz*. Salumber, Madar, Dabok, RCA Horticulture farm and RCA Polyhouse during *Kharif* 2016 - 17. Survey was conducted to know the per cent disease incidence of stem and root rot disease in Udaipur district during *Kharif* 2016 -2017 when crop was one month old.

The infected parts of the diseased samples were carefully placed in polythene bags, properly tagged and brought to the laboratory. For isolation of the pathogen, the diseased roots were thoroughly washed first in the running tap water and finally with sterilized water. Then air dried diseased roots were cut in to 0.5 cm long bits. Bits of infected roots were surface sterilized by dipping in 0.1% mercuric chloride solution for 30 second followed by three washings in sterilized distilled water and aseptically plated on Potato Dextrose Agar (PDA) medium and the plates were incubated at 28 ± 2 °C and examined daily for any fungal growth. After five days fungal growth coming from these diseased roots pieces was aseptically picked up on fresh PDA plates. The white pinkish culture so obtained, was further purified by employing hyphal tip method.

Identification of the pathogen

The slides were prepared in lacto phenol solution and mounted by DPX mount. These slides were then observed under compound microscope at 10X and 40X power. The morphological, cultural and formation of Macro conidia and Micro conidia were the principle characters to identify the pure cultures as *Fusarium oxysporum* f. sp. *radicis cucumerium*. These all cultures were maintained on PDA slants at 4ºC for further study.

Preparation of mass culture

For soil inoculation, the fungus was multiplied on sorghum grain for preparation of mass inoculum in laboratory. Sorghum seed were soaked in water for overnight and excess of water was removed. Then about 200-250 gm seeds were placed in each 1000 ml flask. These flasks were sterilized at 1.045 kg/cm^2 pressure for an hour. The contents of the flasks were shaken after sterilization to prevent clumping. Piece (5 mm) of *F. oxysporum* f. sp. *radicis cucumerinum* was aseptically transferred to the cooled flasks. The flasks were incubated at 28±2 ºC for 15 days. To obtain uniform growth, the contents of the flasks were shaken periodically.

Pathogenicity test

The pathogenicity test of *Fusarium oxysporum* f. s*p. radicis cucumerinum* isolate was carried out in cage house in pots using cucumber susceptible variety (cucumber Long desi) by soil inoculation and spore suspension of the fungus having 4.0 × 10^5 spores/ml was used as inoculum. 10 seeds were sown in a pot containing autoclaved soil. Such four replications were kept in each case with suitable un-inoculated control. The pots were labeled, watered as and when required and left undisturbed in net house for germination and development of the symptoms.

Results and Discussion

Disease distribution and collection of disease samples: Survey for distribution of stem and root rot of cucumber were carried out in cucumber grown area of different localities (RCA Polyhouse, RCA Horticulture Farm, Dabok, Salumber and Madar) of Udaipur region during the year of 2016-2017. The disease samples of cucumber plants were collected from severely infected with the stem and root rot pathogen in the farmers field as well as in Polyhouse during *Kharif* season. The disease samples were brought in to the Plant Pathology Laboratory, Rajasthan College of Agriculture, Udaipur for proper isolation, Purification and identification. Five different isolates of the pathogen were isolated and purified. These isolates were identified as *Fusarium oxysporum* f. sp. *radicis cucumerinum* isolates and kept the fungus cultures for further studies. After inoculation in cucumber seedling, observed that Isolate FORC-5 and FORC-3 were highly virulent and produced 80.00 and 70.00 per cent disease incidence in pots condition, respectively. FORC- 1 exhibited moderate virulence with 65 per cent disease incidence followed by FORC-4 with 55 per cent disease incidence. FORC-2 was least virulent pathogen with 17.50 per cent disease incidence recorded.

Table 1: Survey and collection of different isolates of *Fusarium* sp. from different localities of Udaipur District during *Kharif* 2016-17

S. No.	Isolates	Place of collection	Plant Mortality per cent (%)
	FORC 1	Madar, Udaipur	70.00
2.	FORC 2	RCA, Polyhouse, Udaipur	21.50
3.	FORC 3	RCA Horticulture Farm, Udaipur	76.00
4.	FORC 4	Salumber, Udaipur	55.50
5	FORC 5	Dabok, Udaipur	85.00

Symptomatology

The symptoms of stem and root rot of cucumber on different locations showed that rotting of roots, lower stems and crowns and rotting of seeds and seedlings (damping-off) (Agrios, 2005) [3]. General symptomatology of root and stem rot begins with the tap roots of young plants showing a slightly reddish discoloration which later becomes dark red to brown, and can cover the tap root and stem below the soi1. Sudden wilting occurs, especially on lower leaves and in last on the upper leaves. Vatchev, 2007 [12] found similar results as a pale yellow lesion on the stem base was often the first disease symptom most likely to occur 6-8 weeks after sowing. Later in the season, necrosis progressively expanded until the entire crown area and basal part of the stem partly disintegrated into light orange-brown rot. On diseased host tissue, macroconidia and characteristic short phialides were observed. Blue-black microsclerotia developed after 2-3 months and the colonies had a purplish Chlamydospores were also present. These features are characteristic of F. oxysporum (Nelson *et al*. 1983) [8] and consistent with the description of F.o.r.c. by Vakalounakis (1996) [13]. Isolates from B.C. were morphologically indistinguishable from Greek isolates, but differed from the other Fusarium species from cucurbits. Three isolates from B.C., and one from Greece had optimal temperatures for mycelial growth of 24-27 °C.

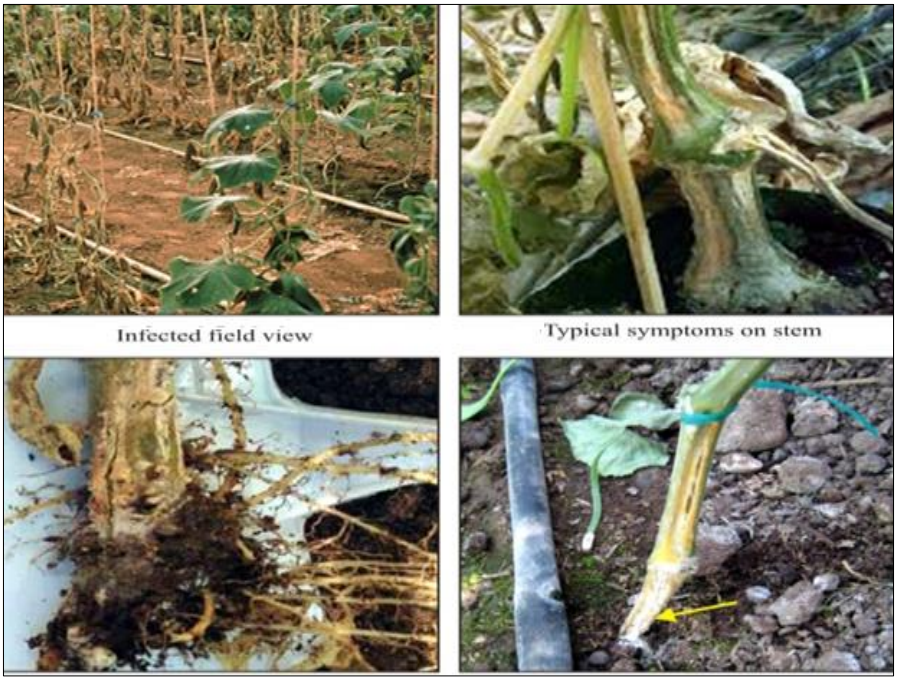

Plate 1: Infected root and stem rot caused by *F. oxysporum* f. sp. *radicis cucumerinum*

Isolation, purification and pathogenicity of the pathogen

Isolation and purification were made from infected cucumber samples collected from different localities of Udaipur district. Pathogenicity test of the culture of *Fusarium oxysporum* f. sp. *radicis cucumerinum* was confirmed on young plants of susceptible variety (Cucumber Long Desi) of cucumber in the pot condition. The same symptoms were initiated after 10-12 days of inoculation as rotting and yellowing, sunken stems and wilting. Disease incidence was highest at Madar followed by salumber and Dabok. It was noticed in patches and later entire field was covered by the disease. Diseased plants poorly yielded, wilted, yellowish and finally complete dry and dead plants were seen in field. Re-isolation of the pathogen was made on PDA plates and identified it on the basis of culture characteristic. At early stages of infection, F.o.r.c. was recovered up to 35 cm distal to the crown, while at advanced stages, it was recovered from healthy-appearing cortical and vascular tissues. Sporulation of F. oxysporum f. sp. radicis-cucumerinum on infected cucumber stems, rockwool blocks, roots, and sawdust growing medium. Thus, internal colonization extends beyond the point of visible disease. The pathogen colonized both cortical and vascular tissues, which distinguished it from fusarium wilt caused by F.o.c. (Blancard *et al*. 1994; Howard *et al*. 1994) [4, 6]. In F.o.r.i., the pathogen could be isolated for distances of up to 25 cm from the crown (Jarvis 1988), and preferentially colonized the cortical tissues in the first 144 h after infection (Charest *et al*. 1984) [5]. In fusarium wilt of tomato, penetration by hyphae into the root was observed as early as 24 h after inoculation (Olivain and Alabouvette 1999) [9].

Plate 2: Symptoms developed in seedlings after proving pathogenicity

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