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Review on Isolation, Purification and Pathogenicity of *Fusarium oxysporum* f. sp. *radicis-cucumerinum* causing Root and Stem Rot of Cucumber

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ABSTRACT: The aim of study was to include different studies *viz.*, isolation, purification, pathogenicity and identification of pathogen. *Fusarium* root and stem rot caused by *Fusarium oxysporum* f. sp. *radicis-cucumerinum* is one of the most damaging diseases of cucumber. Characteristic symptoms of root and stem rot disease noticed were wilting, shrinking of stem, rotting of crown zone and stem. In severe cases infected plants dried completely with deadly appearance. The tradition of the pathogen turned into isolated from the samples collected from diseased plants of cucumber. The pure culture of pathogen changed into received by means of hyphal tip technique. On the basis of morphological and cultural studies, the pathogen was identified as *F. oxysporum* f. sp. *radicis-cucumerinum* and further its pathogenicity was confirmed. Isolation, Purification and Pathogenicity of *Fusarium oxysporum* f. sp. *radicis-cucumerinum* here under reviewed briefly.

Keywords: Isolation, Purification, Pathogenicity, *Fusarium oxysporum* f. sp. *radicis-cucumerinum*

INTRODUCTION

Cucumber (*Cucumis sativus* L.) belongs to family *Cucurbitaceae* and important vegetable, which is principle source of human edible products and useful fibers. The cucumber has been domesticated in India and it has been cultivated in Western Asia for 3000 years (Kroon *et al.*, 1979). From India, it spread to Greece and Italia, later in China. Cucumber popularly acknowledged in India as '*khira*' and gherkins are drastically grown in tropics, subtropics and milder temperate zones of India. The productivity of the crop is more affected in the polyhouse as well as in field by insects, pest and diseases. Among them, diseases are one of the major constraints affecting quality and quantity of the crop. Many diseases have been reported on cucumbers from different part of the world, but only few of them cause economic losses. Fusarium wilt and foot rot of cucumber caused by *Fusarium oxysporum* f. sp. *Cucumerinum* (Owen) Snyder & Hansen was reported from many parts of the world. Root and stem rot of cucumber is believed to be caused by a new formaespecialis of *F. oxysporum*, presently designated as *F. oxysporum* f. sp. *radicis-cucumerinum* (FORC) (Vakalounakis, 1996). A Fusarium root and stem rot disease on greenhouse cucumber (*Cucumis sativus L.*) has only been reported in Greece, during the 1989-1990. The disease was turned to 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 occur only 3 years after first being reported (Vakalounakis, 1996). Root and stem rot 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). Some forma speciales of *F. oxysporum*, cause rotting of roots, lower stems and crowns and rotting of seeds and seedlings (damping-off) (Agrios, 2005). When cucumber is infected with the root and stem rot fungus, the primary, secondary and tertiary roots and the basal portion of the stem have brown discolorations. On the stem, this discolouration may extend for 40 to 100 cm above the soil line. Fusarium root and stem rot of cucumber has been reported to be favoured at lower soi1 temperatures (17°C) (Vakalounakis, 1996).

Isolation, purification and pathogenicity of *Fusarium oxysporum* f. sp. *radicis-cucumerinum* causing root and stem rot of cucumber;

Typical ailment symptoms of *F. oxysporum* f. sp. *radicis-cucumerinum* include slow wilting and revolution aryyellowing followed by unilateral cortical rot with a longitudinal canker at the hypocotyl which may extend upward for about 20-40 cm and downward to the root system. Primary, secondary and tertiary roots have brown lesions and

isolated unilateral cracks with rots varying in length from 5 to 15 cm, usually with white or pink coloured growth of the pathogen appearing on the upper stem (Vakalounakis, 1996). Owen (1955) found through cross inoculation studies with *F. oxysporum* f. sp. *cucumerinum* and *F. oxysporum* f. sp. *niveum* isolated from cucumber and watermelon in Florida, that *Fusarium* isolates were specifically pathogenic. A change from one forma specialis (*F. oxysporum* f. sp. *niveum*) to another forma specialis (*F. oxysporum* f. sp. *melonis*) has been reported and one isolate of *F. oxysporum* f. sp. *cucumerinum* from the Netherlands is pathogenic to cucumber, muskmelon and watermelon (Geriagh and Blok, 1988). Kawai *et al.,* (1958); Matuo and Yamamoto, (1967) reported that six formaespeciales *i.e .F. oxysporum* f. sp. *niveum*, *F. oxysporum* f. sp. *melonis*, *F. oxysporum* f. sp. *cucumerinum* and *F. oxysporum* f. sp. *radicis-cucumerinum* are globally disbursed and greater essential pathogens from a monetary factor. The other two formaespeciales, *F. oxysporum* f. sp. *lagenaria* and *F. oxysporum* f. sp. *luffae* have only been recognized in a constrained geographic area of Japan. Komada, (1974) also reported *Fusarium* wilts and foot rot of cucumber caused by *Fusarium oxysporum* f. sp. *cucumerinum* from many parts of the world.

Armstrong and Armstrong, (1981) pronounced that within the identification and differentiation of strains of *F. oxysporum* have long been major limitations to studies on the population dynamics. Rupe, (1989) gave the approach of isolation, according to him, the roots were washed in running tap water and divided into lateral roots, taproot epidermal tissue, taproot cortical tissue and tissues from the 5 cm. of stem with vascular discoloration. The epidermis of lower stem were also removed, plant parts were cut into 1 cm. segments and surface disinfested by dipping in 95% ethanol, soaking in 0.5% sodium hypochloride for 5 min, and rinsing in sterile water. The segments were placed on 2% water agar amended with 50 mg/l of streptomycin sulfate. Segments were placed in Petri dishes (five segments per dish) and incubated at room temperature.

Carver *et al.,* (1996) examined the pathogenicity of *Fusarium oxysporum* f. sp. *dianthi* using spore suspensions, introduced into pink plants *via* root dip or cut stem inoculations. The assay on stem sections ended in re-isolation of *Fusarium oxysporum* f. sp. *dianthi* from six of eight plants inoculated by root dip and from seven of eight plants inoculated via a cut stem.

Vakalounakis, (1996) recognized *F. oxysporum* f. sp. *cucumerinum* based on the disease symptoms on cucumber plants and differing pathogenicity on different plant sp. of the *Cucurbitaceae*. This disease caused by the fungus *Fusarium oxysporum* f. sp. *radicis-cucumerinum*, was first observed in Greece and the Netherlands. It was reported in British Columbia (10 % losses), Canada (25% losses) in 1994 and later in Ontario (35 % losses) in 2000.

Martinez *et al*., (2003) studied that cucumber (*Cucumis sativus* cv. Albatros) in several commercial glasshouses in south-eastern Spain (Almeria) exhibited symptoms of wilt, yellowing and necrotic streaks on the stems. Internal, vascular discoloration in affected plants extended from the base of the stem upward. Initial observations were reminiscent of *Fusarium* wilt disease of cucumber, caused by *Fusarium oxysporum* f. sp. *cucumerinum*, as described by Owen, (1955). Pure colonies of fungi were isolated from surface-disinfested (with flame) stem segments, cut at 0.5 m above the hypocotyl, on potato dextrose agar (PDA) medium. Isolates were identified as *Fusarium oxysporum* due to occurrence of typical macroconidia with foot-shaped basal cells, microconidia borne in false heads only on monophialides and chlamydospores. To confirm pathogenicity, eight isolates (1-week-old cultures grown on PDA) were used to root-dip inoculation (1×10^6 conidia ml^{-1}) of one week old seedlings of cucumber *cv* Nevada and Albatros. Seedlings were then transplanted into pots containing vermiculite growth medium and placed in a glass house. Of the tested isolates, all but one was pathogenic, causing wilt symptoms or death in 60–100% of dip-inoculated plants, from which *F. oxysporum* was consistently re-isolated. Symptoms observed in inoculated plants were similar to those observed in cucumber plants in commercial glasshouses. This is the first report of *F. oxysporum* causing wilt of cucumber in Spain.

Garibaldi and Minuto (2004) remoted *Fusarium* spp. consistently and readily from symptomatic vascular tissue on to a *Fusarium*-selective medium from the wilt affected plants. Colonies were identified as *F. oxysporum* after sub-culturing on potato dextrose agar on the basis of morphological observations.

Vatchew, (2007) first time reported *Fusarium oxysporum* f. sp. *cucumerinum*c ausing root and stem rot of cucumber from 21 commercial greenhouses in Bulgaria. He observed disease symptoms expression, host range among plant species of cucurbit family and cultural, morphological variations within isolates of the pathogen.

El-sayed *et al.,* (2008) isolated 23 isolates of *Fusariumo xysporum*, eight isolates of *Fusarium solani*, two isolates of *Verticillium dahliae* and four isolates of *Rhizoctonia solani* from tomato plant showing wilting and root rot symptoms at one of a kind localities in Egypt.

Karaca and Kahveci (2009) examined pathogenicity test for host range by inoculations on *Cucumis sativus*, *Luffa acutangula* L. *aegyptiaca*, *Cucurbita maxima*, *C. moschata*, *Cucurbita ficifolia*, *Cucumis melo* and *Citrullus lanatus* plants in Turkey. Three weeks after inoculation, cucumber plants provided intense severe root and stem rot symptoms and died. *L. aegyptiaca*, *C. melo* and *C. lanatus* plants showed the same symptoms, whereas *L. acutangula* and *C. ficifolia* species were healthy. Two formaespeciales of

F. oxysporum infect cucumber; f. sp. *cucumerinum* causes vascular wilting, whereas f. sp. *radicis-cucumerinum* causes wilting accompanied by root and stem rot. In addition to cucumber, the latter fungus can also infect melon (*C. melo*), watermelon (*C.* lanatus) and sponge gourd (L. *aegyptiaca*).

Sharma *et al.*, (2011) isolated 24 isolates of *F. oxysporum* f. sp. *lycopersici* from tomato vegetation showing ordinary wilt signs from fourteen special areas together with one of a kind agroclimatic condition in India.

Pagoch and Rain (2012) 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 the presence of *Fusarium oxysporum* f. sp. *cucumerinum* and *F. solani* which caused losses 85.72 and 14.29 percent, respectively.

Joshi *et al.*, (2013) isolated sixty isolates from nine different geographical places in Uttar Pradesh of India from soil and plant samples. Among them, thirty nine isolates were identified as *Fusarium oxysporum* on the basis of their morphological and molecular identification. The pathogenicity test was conducted on tomato variety Pant T-3, disease incidence ranged from zero to 78.74%. The results showed that the Isolate no. 40 showed the highest antagonistic activity in inhibiting radial growth of pathogenic isolates.

Benaouali *et al.*, (2014) studied 27 isolates of *F. oxysporum* from different regions of western Algeria (Oran, Mostaganem, Chleff) Allowed on the premise of their macroscopic look to differentiate four morphotypes (downy, cottony, mucous and senescent) with different pigmentation (white, yellow, pink and purple). The pathogenicity test confirmed the presence of *Fusarium oxysporum* f. sp. *radicis-lycopersici* in the western location of Algeria. Of the 27 strains studied 22 have shown signs and symptoms crown and root rot with very high degrees of virulence compared to the references strains and 5 isolates were not pathogen. For a better understanding of the ecology of these isolates a study of the influence of different physiochemical conditions were realized. The results showed a better growth of the isolates on PDA medium with an important pigmentation, mycelial growth of different isolates is quiet faster in the darkness than in light and the optimum pH for growth was 6 and 7 with a rate of humidity ranging from 74 to 80% and an optimal growth temperature of 23°C and 28°C.

Al-Tuwaijri, (2015) tested eight isolates of *Fusarium oxysporum* f. sp. *cucumerinum* for their virulence against the susceptible cucumber cultivar (Beit alpha) in Egypt. Isolate No. 3 was the most virulent isolate (77.33 PDI) followed by isolate No. 4 (74.66 PDI). Less virulent isolate was Isolate no.7 which gave 72.88 percent PDI.

Garibaldi *et al.*, (2016) observed for the first time in Verona, (Italy) in commercial farm where 5 month old cucumber plant in plastic house affected chlorosis, yellowing, and wilting of stem. On the basis of affected stems, whitish-light orange mycelium appeared while vessels were discolored, finally affected tissues collapsed and plants died. The forma sp. *radicis-cucumerinum* was confirmed by Amplification with the specific primer FORCF1 and FORCR2 designed.

Asma *et al.*, (2018) studied to affirm the virulence of all *Fusarium* isolates by performing pathogenicity test on cucumber to fulfill Koch's Postulates. Seventeen isolates of *Fusarium* species were recovered from inflamed cucumber fruits and leaves. All the isolates were recognized and classified based on translation elongation factor (*tef1α*) and beta-tubulin (*β-tub*) sequence analyses. All *Fusarium* isolates were tested for pathogenicity test by soaking the root of 7 days-old cucumber seedling into 2×10^6 spore/ml of conidial suspension for 30 minutes. Sterile distilled water was applied as a control. The most virulent was recorded by *F. oxysporum* isolate D2505C with disease severity of 50%. Three isolates were identified as non-virulent with no visible wilt symptoms, which are *F. longipes* isolate D2504C and *F. incarnatum* isolates N2210C and N2212C. These effects provide beneficial statistics at the diversity and pathogenicity of *Fusarium* species associated with wilt disease of cucumber in Malaysia.

CONCLUSION AND CONFLICT OF INTEREST

Cucumber root and stem rot disease caused by *Fusariumo xysporum* f. sp. *radicis-cucumerinum* leading to rotting of stem, roots. Characteristic symptoms of root and stem rot disease noticed were wilting, shrinking of stem, rotting of crown zone and stem. In severe cases infected plants dried completely with deadly appearance. The information available on the disease as well as pathogen is very limited. Hence, it changed into very important to examine diverse components of the pathogen and also the sickness.

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Conflict of Interest. None.

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