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Review Article

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Fusarium Wilt of Cucumber- A Review

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Abstract

Cucumber (*Cucumis sativus* L.) wilt is an important disease caused by *Fusarium oxysporum*. The disease is prevalent in most of the countries of the world with yield losses ranging from 40–70%. Characteristic symptoms of the disease include pre and post-emergence damping-off, stunting, chlorosis, necrosis and finally wilting of the older leaves with brown vascular discoloration. Two formae speciales of *F. oxysporum* have been described in cucumber. The fungus grows profusely on the culture media and produces macroconidia which are tapered with a slight hook, microconidia oval in shape and terminal or intercalary chlamydospores. The disease prevails under the conditions having abundant soil moisture and soil temperature ranging from 24–27°C and no growth of the fungus was observed above 37°C. The pathogen is host-specific to cucumber with muskmelon and watermelon being slightly sensitive. Due to environment and health concerns associated with large scale use of chemicals, the application of resistant germplasm, bio-control agents, and bio formulations is more reliable. This review describes the use of resistant germplasm, bio-control agents, bio-formulations and fungicides to control Fusarium wilt of cucumber.

Keywords: Bio-control agents, bio-formulations, cucumber wilt, germplasm, fungicides

1. Occurrence

Cucumber (*Cucumis sativus*) is an important cucurbitaceous crop which is among the oldest cultivated vegetable crops dating back to about 5,000 years (Wehner and Guner, 2004). This crop is originated in India probably in the foothills of Himalayas. Cucumber contains a rich amount of minerals and vitamins along with about 1% dietary fiber. India is also among the global producers of cucumber and the states having a major role in its cultivation are Haryana, Karnataka, Madhya Pradesh, and Tamil Nadu. In Himachal Pradesh, it is mainly grown as an off-season crop under greenhouse conditions. Fusarium wilt (*Fusarium oxysporum* (Schlechtend.:Fr.) Synd. and Hans) is among the most destructive diseases of cucumber. It was firstly reported from Florida in 1955 as the cause of losses in commercial field-grown cucumber crops (Owen, 1955) while in greenhouse cucumbers, the disease was reported for the first time from North Carolina in 1979 (Jenkins and Wehner, 1983). On cucumber, two formae speciales of *F. oxysporum* have been described, *F. oxysporum* f. sp. *cucumerinum* Owen, causing Fusarium wilt and *F. oxysporum* f. sp. *radicis-cucumerinum* Vakalounakis, causing root and stem rot (Vakalounakis et al., 2004).

Cucumber wilt disease is prevalent in most cucumber growing regions around the world such as South Africa, Japan, China, Thailand, England, France, Germany, Pakistan and Australia (Armstrong and Armstrong 1978; Huang, 1990; Huang et al., 1994; Sultana and Ghaffar, 2013; Fareed et al., 2016). In India, the incidence of Fusarium wilt was reported for the first time from Jammu and Kashmir. Isolates of the pathogen were recovered from diseased samples collected from Kathua, Jammu, Rajouri, Udhampur districts of Jammu division, and based upon the characterization, the pathogen was designated as *Fusarium oxysporum* f. sp. *cucumerinum* (Pagoch and Raina, 2012). Later, the disease was reported from Lucknow (Srivastava, 2017).

2. Losses and Symptomatology

The pathogen has been found responsible for causing up to 40% losses in Florida and wilt increased with successive field crops, increasing to a 70% infection rate in the third consecutive season (Owen, 1956). Cucumber cultivation as a monoculture for three consecutive seasons can lead to wilt incidence as high as up to 70% (Shen et al., 2008) along with yield reduction up to 50% or even complete failure of the cucumber crop can occur (Chen et al., 2010). The disease often occurred in replanting fields leading to great losses (Owen, 1955; Jenkins and Wehner, 1983). The characteristic disease symptoms include seedling damping-off, plant stunting, yellowing, and wilting of older leaves with brown vascular discoloration. The wilt fungus attacked the cucumber plants at all stages of development. Three to five days after emergence, young infected seedlings became dull green and damped-off. Symptoms of the disease include pre-emergence rot and damping-off during propagation. The infection of older plants usually resulted in the wilting of the entire plant.

Wilting occurred over a period of 3–5 days with the infested plant often partially reviving at night and wilting in the middle of the day during the early stages of the disease. Finally, the entire plant collapsed rapidly (Owen, 1955). Vascular discoloration of roots and stem extending up to 8–10 nodes is of common occurrence (Takeuchi et al., 1978; Vakalounakis, 1993). The wilting symptoms are accompanied by chlorosis and finally necrosis of interveinal areas of the leaves. Early infection of plants prevented fruit set, while later infection resulted in small, abnormal fruits. Cracks often appear on diseased vines. The central part of the taproot shows a deep red color (Kim et al., 1993; Majdah and Tuwaijri, 2015).

3. Causal Organism and Identification

Fusarium oxysporum (Schlechtend.:Fr.) is a genetically diverse and ubiquitous fungus. No sexual state has been found for *F. oxysporum* but related species are thought to be anamorphs of the ascomycete *Gibberella species* (Snyder and Hansen, 1940). *F. oxysporum* includes morphologically indistinguishable plant pathogenic strains and non-pathogenic strains, for which no host has yet been defined. Pathogenic strains of *F. oxysporum* can cause vascular wilt or root rot on a broad range of plants, many of which are economically important crops. The taxonomy of Fusarium has undergone a major revision. Based on virulence specificity to a set of differential cultivars within the same plant species, some of the formae speciales are further divided into races (Armstrong and Armstrong, 1978; Armstrong and Armstrong, 1981).

F. oxysporum f. sp. *cucumerinum* (cucumber) the most economically important ones causing Fusarium wilt of cucumber (Hegde et al., 1955; Bhaskaran et al., 1971). *Fusarium oxysporum* f. sp. *cucumerinum* has been reported as the causal organism of Fusarium wilt of cucumber from different parts of the world (Owen, 1955; Booth, 1971; Armstrong and Armstrong, 1978; Jenkins and Wehner, 1983; Huang, 1990). Morphological characteristics of *Fusarium oxysporum* f. sp. *cucumerinum* have been described by various workers. The fungus grew rapidly, producing white aerial mycelium in young culture while, in the old culture, the substratum, and sometimes the aerial mycelium assumed various shades of purple (Owen, 1955). The pathogen has pale pink fluffy hyphal growth on PDA as well as dark magenta-violet pigment on PDA media slates (Scarlett, 2013).

The fungus produces macroconidia, microconidia, and Chlamydospores on PDA media. Macroconidia (3-4.5×40-50 µm) are tapered and curved to almost straight with a slight hook, thin-walled with usually three septa and are produced from monophialides and microconidia which are non-septate (2-4×59-12 µm) are oval or elliptical, usually non-septate and form in false heads from short monophialides (Burgess et al., 1994). Chlamydospores (7-13 µm), both smooth and rough, thick-walled, are produced intercalary or terminal in the hyphae and form abundantly in most isolates, usually in pairs or singular. The appearance of *F. oxysporum* isolates as cottony, thin, flat, and fluffy, thread-like spreading at the periphery. The growth of the colonies was delicate and wooly to cottony. The color of the colonies varied from creamy white to white while some isolates produced pinkish violet pigments in colonies after 15 to 20 days of incubation at 25°C on PDA medium.

4. Pathogenicity

Pathogenicity which is defined as the absolute ability of an infectious agent to cause disease in a host is a pre-requisite to test whether an infectious agent is pathogenic or not. Different methods of inoculation had been tested for proving the pathogenicity of Fusarium on cucumber plants. A cucumber variety Marketer was tested for its pathogenicity by using soil as well as root inoculation method. Soil inoculation method showed that first damping off of the seedlings occurred within 8 days after planting. After 14 days, there was a high incidence of damping-off in all cucumber varieties. Root inoculation method showed that the cucumber plants began to wilt in around 10 days and within 13 days all the plants had wilted (Owen, 1955). Disease severity ranged from 78–100% with all isolates.

The root dip method of inoculation has resulted in higher disease incidence as compared to the sick soil inoculation method due to forcible inoculation brought about by root damage Rani et al. (2008). *Fusarium oxysporum* f. sp. *cucumerinum* was found to be virulent on cucumber cv. Super Long Green under artificial conditions and produced true wilt symptoms resembling those incited by the fungus under natural conditions (Pagoch and Raina, 2012). Scarlett (2013) inoculated stems of cucumber plants by placing one drop of conidial suspension (1×10^{6} conidia ml^{-1}) onto either a wounded stem site or an unwounded stem site using a sterile pasture pipette. Wounding involved breaking and removing the first true leaf from the petiole base of 4-week old plants. Unwounded plants were inoculated by placing one drop of conidial suspension on the base of the first true leaf, where the petiole joins the stem.

The roots of cucumber plants were inoculated by dipping the root system of four-week-old seedlings into the conidial suspension, swirling for ten seconds, and then replanting back into the coir peat fiber (Martyn and McLaughlin, 1983; Latin and Snell, 1986; Beckman, 1987; Freeman and Katan, 1988). After 14–20 days, wilt symptoms including necrosis of the lower stem and wilting of lower leaves was observed in the cucumber plants (disease severity score 2 and 3). After 6 weeks, the adult plants showed severe wilting and necrosis of the stem base. Internal examination of the root tissue showed symptoms characteristic of Fusarium wilt of cucumber, including browning of the stem base and roots and presence of orange hyphal pigmentations. All control plants remained asymptomatic with no detection of the pathogen through isolation (Scarlett, 2013).

5. Epidemiological Studies

Soil moisture and temperature play an important role in the initiation and development of infectious plant diseases. These are among the most important environmental factors strongly affecting wilt diseases since these influences each of the three biotic components involved in the development of wilt diseases, i.e. the pathogen, the host plant, and soil microorganisms. A positive correlation between high soil moisture and high wilt incidence has been reported by various workers. Mustafee and Chatopadhyay (1971) reported that a 40% saturation level seemed to be effective for multiplication of pathogen in the soil while, at 80% level, there was a steady decline of *Fusarium solani*.

Effect of different moisture levels viz. 25, 50, 75 and 100% on the development of Fusarium root rot in pea was also observed and found out that soil moisture at 75% of field capacity had significantly lower disease incidence than those grown in soil at 100%, 50% or 25% of field capacity. Sekhon and Singh (2007) studied the effect of different soil moisture regimes viz. 10, 20, and 40% on Fusarium wilt development in muskmelon. Mortality of muskmelon plants due to wilt was maximum at 20% water holding capacity which decreased with further increase in moisture level. It was observed that 75% of water holding capacity was favorable for the growth and multiplication of *Fusarium* spp. Wilt induced by *Fusarium* spp. is markedly affected by soil temperature. The effect of soil temperature on stem colonization and wilt occurrence varies in different pathosystems (Marios and Mitchell, 1981; Bosland et al., 1988). Huang et al. (1988) studied the effect of different temperature regimes on spore germination and hyphal growth of *Fusarium* sp. They found optimum growth of the pathogen at 24–27^{o}C and no growth occurred above 37^{o}C. Sekhon and Singh (2007) reported that wilt development in muskmelon was greater at 25^{o}C as compared to 20, 30, and 35^{o}C temperatures. Similar results were reported by Scarlett (2013) who found 25^{o}C temperature best for the development of Fusarium wilt of cucumber.

Chen et al. (2013) reported that *F. oxysporum* was able to grow at wide temperature range, and the highest growth rate was observed at 23–24°C while, Sekhon and Singh (2007) in their studies observed that a temperature range of 21–26^{o}C to be most effective against Fusarium wilt of watermelon. Attri et al. (2016) studied the effect of different temperature regimes on the development of Fusarium wilt of bell pepper and observed that incidence of disease was maximum (100%) at a soil temperature of 25°C followed by 30°C (75.0%) and 20°C (37.5%).

6. Host Range

Isolates of *F. oxysporum* are generally host-specific however; seedling assays have revealed that the host range distinction between formae speciales of *F. oxysporum* is not as obvious as originally postulated. Several studies have shown cross infectivity of some formae speciales to other hosts, especially in the family Cucurbitaceae (McMillan, 1986; Gerlagh and blok, 1988; Kim et al., 1993). *F. oxysporum* f. sp. *niveum* isolates from watermelon were found to infect *Cucurbita pepo* cultivars (Martyn and McLaughlin, 1983). *F. oxysporum* f. sp. *cucumerinum* isolates have shown to be pathogenic and cause severe vascular wilt symptoms on rockmelons (McKeen, 1951; McMillan, 1986) and a watermelon (Gerlagh and Blok, 1988). Nomura (1992) demonstrated pathogenicity of *F. oxysporum* f. sp. *lagenariae* to pumpkins (*Cucurbita* spp.). The pathogen is host-specific to cucumber, with muskmelon (*Cucumis melo* L.) and a watermelon (*Citrullus vulgaris*) being slightly sensitive (Vakalounakis, 1996).

The inoculated cucumber and melon seedlings one day after their emergence as well as 25 days after emergence was found infected with all *F. oxysporum* f. sp. *cucumerinum* isolates and were pathogenic to cucumber (Cafri et al., 2005), while approximately two-thirds of the isolates were pathogenic to both cucumber and melon. However, in more recent work (Vakalounakis, 1996; Vakalounakis and Fragkiadakis, 1999), isolates of the proposed '*F. oxysporum* f. sp. *cucurbitacearum* race cu' were shown to belong to a new formae speciales named *F.oxysporum* f. sp. *radicis-cucumerinum*. *F. oxysporum* f. sp. *radicis-cucumerinum* has since been found to infect and cause serious disease on various cucurbits, including melon, watermelon, bottle gourd, and sponge gourd (Vakalounakis et al., 2004).

F. oxysporum isolates showed significantly higher virulence on oriental melon and watermelon as the isolates of *Fusarium oxysporum* f. sp. *melonis* and *Fusarium oxysporum* f. sp. *niveum*; however, these isolates were differentiated in their virulence on cucumber. This suggests the most abundant formae speciales of *F. oxysporum* distributing in oriental melon greenhouses may be *Fusarium oxysporum* f. sp. *melonis* or *Fusarium oxysporum* f. sp. *niveum* type with high virulence on the oriental melon (Seo and Kim, 2017).

7. Disease Management

For the management of wilt diseases, different crop management practices viz., crop rotation, solarization, use of resistant germplasm, sanitation, seed and seedling dip treatments in fungicides, soil fumigation and soil amendments with fungal and bacterial biocontrol agents have been proposed (Chavan et al., 1977; Dong and Chen, 1993; Ahmad et al., 1996; Punja and Parker, 2000; Bora et al., 2004; Harman, 2006; Vakalounakis et al., 2004; Hu et al., 2010; Nisa et al., 2011).

7.1. Resistant germplasm

Wilt pathogen being soil-borne in nature and thus survives in soil for many years and is not controlled by simple methods such as crop rotation. Due to environmental and health concerns, non-chemical practices are encouraged over the large use of pesticides and chemicals. However, not much work has been done on screening of germplasm against Fusarium wilt of cucumber. Resistance against Fusarium wilt pathogen in cucumber has been reported by Dong and Chen (1993) and Punja and Parker (2000).

Tuwaijri (2015) screened 11 cucumber genotypes viz., Medina, Zeina, Sweet crunch, Mena, Thamin, Biet alpha, seina 2, Shorouk, El-hout, Samara and Melita against Fusarium wilt pathogen and found that all the genotypes were susceptible to *F. oxysporum* f. sp. *cucumerinum* and differed significantly in their susceptibility. El-hout was found to be most susceptible followed by Shorouk and Biet alpha. Zeina, Sweet Crunch, and Seina were less susceptible. Fareed et al. (2016) screened 12 varieties of cucumber against Fusarium wilt. Out of these 12 varieties, 9 varieties viz. Green cucumber, Hashim, Rocky, Cu-05, Happy, Durga, Guard HC-1, Cu-30, and Qasim were found to be a moderately susceptible while; Shaheen and HCU-163 gave a moderately resistant response. However, Local variety was found highly susceptible to the disease.

7.2. Bio-control agents

Bio-control is an important component of integrated disease management (IDM), which is non-polluting, bio-degradable, selective in the mode of action, unlikely to harm human beings, and other beneficial microorganisms. Several factors precluded mycoparasitic interactions, competition for space and nutrients and antibiosis of fungal biocontrol agents like *Trichoderma harzianum* isolates to *Fusarium* sp. have been explained in the literature (Lifshitz et al., 1986). Such interactions were observed infrequently and occurred at 24 h or more after mycelial contact in dual culture. Jee and Kim (1987) observed several mycoparasitic mechanisms such as coiling, penetration, overgrowing, and lysis on a dual culture of water agar between antagonistic fungi *Trichoderma harzianum*, *T. viride* and *F. oxysporum* f. sp. *cucumerinum.* Moon et al. (1988) reported that the mode of mycoparasitism of *T. harzianum* appeared to be coiling around and its attachment on the host or penetration into the host hyphae or breaking septa of both the hyphae and the conidia. Volatile metabolites produced by *Trichoderma harzianum* and *T. viride* are also responsible for their excellent antagonistic activity against *F. oxysporum* under *in vitro* conditions.

Karimi et al. (2012) evaluated rhizosphere isolated *B. subtilis* (B28) and reported 51.1% inhibition against *F. oxysporum* f. sp. *cicerisin vitro* and concluded that the use of rhizobacteria against Fusarium wilt of chickpea not only suppressed the disease incidence and decreased the number of seedlings with wilting symptoms, but also increased growth parameters of the plant.

7.3. Bio formulations

Cow urine and cow dung based bio-formulations have been gaining acceptance in recent times due to their ability in managing the disease to some extent as well as because of increment in yield of the crop. Basak and Lee (2001) proved that cow urine and cow dung had some effectiveness in the suppression of conidial germination and mycelial growth of *Fusarium oxysporum* f. sp. *cucumerinum* causing Fusarium wilt of cucumber plants. While Basak et al. (2002) recorded the efficacy of cow urine against *Fusarium solani* f.sp. *cucurbitae* causing root rot disease of cucumber.

The effect of various concentrations of fresh and stored cow urine (3 months) viz., 5, 10, 20, and 40% were tested against the mycelial growth of test fungi by poison food technique. Both fresh and stored cow urine displayed concentration-dependent inhibition of test fungi. Inhibitory efficacy was recorded higher for stored cow urine than fresh cow urine. The inhibition of test fungi was >50% at 20% and higher concentrations of cow urine. It can be concluded that cow urine has got potential to inhibit pathogenic fungi causing rhizome rot of ginger *in vitro*.

7.4. Soil and seed treatment with bio-control agents and bio formulations

The application of biocontrol agents to suppress soil-borne pathogens has been widely used (Bora et al., 2004; Harman, 2006; Hu et al., 2010). Soil application of antagonistic microbes with a suitable substrate has been reported to be more effective than the direct application (El-Hassan and Gowen, 2006; Trillas et al., 2006).

Soil application of *Gliocladium virens* and *Trichoderma harzianum* as soil inoculation in pots infested with *Fusarium oxysporum* f sp. *cucumerinum* was more effective in reducing the incidence of cucumber wilt than direct application conidial suspension (Cho et al., 1989) Larkin and Fravel (1998) tested isolates of *Gliocladium virens, Trichoderma hamatum*, *Pseudomonas fluorescens* and *Bacillus* and found that these reduced the Fusarium wilt compared to control. Seed treatments with *Trichoderma viride* and *Trichoderma harzianum* resulted have 30% disease control of root rot of chickpea as compared to the check (Gurha, 2001). The bacterial treatment on seeds significantly increased the growth and fruit yield of tomato plants and also provided a high level of protection against the disease caused by *Fusarium* pathogen (Prasad et al., 2002).

The combination of seed treatment, seedling dip, and soil drenching of the liquid formulation of *P. fluorescens* reported to have the minimum disease of Fusarium wilt on tomato under glasshouse (17.33%) and field (4.81%) conditions (Manikandan et al., 2010). Devi and Singh (2012) found that inhibition of *Fusarium* spp. was maximum with *Trichoderma* harzianum followed by *Trichoderma viride*, *Pseudomonas fluorescens* and *Bacillus subtilis*. Application of strain B068150 of *Bacillus* sp. in the germination seeds resulted in a lower Fusarium wilt disease incidence under pot conditions using the soil application method.

Sultana and Ghaffar (2013) concluded that microbial antagonist's viz., *Trichoderma harzianum*, *T. viride*, *Gliocladium virens*, *Bacillus subtilis* and *Stachybotrys atra* significantly reduced seedling mortality and root rot infection of *Fusarium oxysporum* in bottle gourd and cucumber under *in vivo* conditions. Among the antagonists, *Trichoderma harzianum* was better in inhibiting the mycelial growth of *Fusarium oxysporum*, followed by *Trichoderma viride* whereas *Pseudomonas fluorescens* and *Bacillus subtilis* were least effective Singh et al. (2017) while, Srivastava (2017) reported about 93.62% control of Fusarium wilt of cucumber with *Trichoderma* spp. coated seeds in infected soil over untreated seeds in Fusarium infested soil taken as a check.

7.5. Fungicides

The use of fungicides is the most effective method of plant disease management. New fungicides are being introduced now and then and evaluated for plant disease control. Use of the same fungicides time and again leads to resistance development. It is, therefore, important to evaluate new fungicides for the management of soil-borne diseases.

The effectiveness of systemic fungicides has been reported against *Fusarium oxysporum* by many workers (Chavan et al., 1977; Hussain et al., 1981; Ahmad et al., 1996; Arshad et al., 1996). Benlate has been reported to be the most effective chemical for checking the mycelial growth of *F. oxysporum* at low concentrations while similar findings were reported by Poddar and his co-workers in 2004. Zhao et al. (2006) tested nine different fungicides against *Fusarium oxysporum* f. sp. *melonis* and found that carbendazim and fludioxonil most effective against pathogen. Complete inhibition of *F. oxysporum* growth was recorded by Musmade et al. (2009) with carbendazim (0.1%).

Nisa et al. (2011) explained that systemic fungicides at different concentrations significantly inhibit the mycelial growth of *F. oxysporum*. Taskeen et al. (2011) reported that systemic fungicides at different concentrations significantly inhibit the mycelial growth of *F. oxysporum*. They concluded that hexaconozole at the highest concentration (1000 ppm) caused the highest reduction of mycelial growth followed by carbendazim. Fungicides evaluation under field conditions has been done by various workers to study the efficacy of various contact and systemic fungicides against the disease. It has been reported by various workers that among the various fungicides tested benzimidazole group of fungicides was found to be effective.. Four concentrations of fungicides (0.1, 1, 10 and 100 μg ml^{-1}) were tested for controlling Fusarium wilt on tomato plants. They observed that soil drenching with prochloraz, bromuconazole, benomyl, and carbendazim were effective in reducing Fusarium wilt in tomato.

Sultana and Ghaffar (2013) screened seven fungicides against Fusarium wilt of cucumber under field conditions. Benlate and carbendazim were found most effective and only 4 and 8% seedling mortality and root infection were observed in these fungicides, respectively. Vitavax, Aliette, Topsin M and Ridomil were also found effective and significantly reduced seedling mortality to 8–16% and root infection to 10–18% whereas, mancozeb was found least effective. Behrani et al. (2015) reported that carbendazim followed by Antracol appeared as the most effective fungicide showing a remarkable increase in seedling emergence of treated plants inoculated with *F. oxysporum* as compared to the untreated plants.

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