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Fusarium Wilt of Chrysanthemum – Problems and Prospects

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Abstract

Chrysanthemum flower production is adversely affected by many bacterial, fungal and viral diseases. Wilt caused by *Fusarium oxysporum* f. sp. *chrysanthemi* is one of the most serious diseases and causes severe yield losses. The pathogen enters the plant, multiply and blocks the vascular system with or without involvement of toxins and enzymes. Multiplication medium, pH and temperature plays significant role on the growth and sporulation of *Fusarium oxysporum*. Moderate temperatures, high humidity, availability of plant nutrients in soil encourage rapid disease development. High inoculum density causes greater disease incidence, hasty disease advancement and low flower yield. Degree of symptom development is related with the cultivar resistance and susceptibility. Plant extracts, polar and non-polar fractions, their pure compounds, and essential oils have potential antimicrobial activity against *Fusarium oxysporum*. The active phyto-constituents responsible for antifungal properties are low molecular weight phenols, tannins and lignin. *Trichoderma* and other phyto-pathogenic fungi are known to diminish wilt disease caused by *Fusarium oxysporum* f. sp. *chrysanthemi*. Myco-parasitism, antibiosis, nutrient competition and starvation, siderophore production and induction of systemic resistance are the major mechanism employed by *Trichoderma* for controlling pathogens. The present paper is intended to discuss the aspects of epidemiology, pathogenesis and biological control measures of *Fusarium oxysporum* with emphasis on Chrysanthemum wilt.

Key words – botanicals – *Chrysanthemum* – *Fusarium oxysporum* – *Trichoderma*

Introduction

Flower plants are cultivated in India primarily for their application in beautification of garden and avenues. Earlier, flowers were customarily used for sacred purposes, perfumery, and landscaping; but now floriculture has been adapted as a business worldwide. Commercial floriculture favours employment and contributes to the economic growth by increasing both domestic as well as export markets. In India, the area of flower production was 144,000 ha in 2006–07, which increased to 254,000 ha in 2011-12. Analysis of the figures of export and area of flower production clearly specifies that there is a steady and persistent growth in the Indian floriculture market (National Horticulture Board, Govt. of India 2013). The global flower demand is also increasing with a gradual annual increase of about 10–15% in all the importing countries (Table 1 and Table 2). India has a great advantage to become a leader in floriculture trade due to availability of suitable land, cheap labour, educated manpower and ideal geographical location for flower production.

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Table 1 Area and Production of Flowers in India (2006 - 2012)

Year	Area (000 ha)	Production	
		Loose (000 MT)	Cut (Millions)
2006-07	144	880	3716
2007-08	166	868	4365
2008-09	167	987	4794
2009-10	183	1021	6667
2010-11	191	1031	6902
2011-12	254	1652	7506

Source: National Horticulture Board, 2013

Table 2 State wise Area and Production of Flower in India

State/UTs	Area (000 ha)		Flower Production (Loose 000 MT, Cut in Lakh)			
	2009-10	2010-11	2009-10 Loose	2009-10 Cut	2010-11 Loose	2010-11 Cut
And. & Nicobar	0.0	0.0	0.30	-	4.70	-
Andhra Pradesh	21.4	21.8	130.3	6202.0	133.7	6202.0
Arunanchal Pradesh	1.2	1.2	-	2860.0	-	2860.0
Bihar	0.20	0.20	2.30	11.0	2.30	11.0
Chattisgarh	4.1	6.9	13.5	-	27.1	-
Daman & Diu	0.00	0.00	0.0	-	0.0	-
Delhi	5.50	5.50	5.70	1038	5.70	1038.00
Gujarat	12.5	12.5	49.50	5063.0	49.50	5063.00
Haryana	6.2	6.2	60.3	1084.0	60.3	1084.0
Himan. Pradesh	0.70	0.7	0.6	605.0	0.6	605.0
Jammu & Kash.	0.1	0.1	0.2	66.3	0.2	66.3
Jharkhand	1.6	1.6	22.0	1711.0	22.0	1711.0
Karnataka	27.0	27.0	203.9	5860.0	203.9	5860.0
Madhya Pradesh	6.6	7.70	5.0	-	6.0	-
Maharashtra	17.5	17.50	91.1	7914.0	91.1	7914.0
Mizorum	0.0	0.1	0.00	142.0	0.00	162.0
Nagaland	0.00	0.0	0.00	17.0	0.00	17.0
Orissa	7.1	7.4	25.3	5356.0	3.70	5911.0
Pondicherry	0.3	0.30	2.40	-	2.40	-
Punjab	1.7	1.70	82.0	-	82.0	-
Rajasthan	3.3	5.40	4.90	-	9.6	-
Sikkim	0.2	0.20	-	200.0	0.0	230.0
Tamil Nadu	32.0	32.0	247.3	-	247.3	-
Uttar Pradesh	10.4	10.4	17.6	2958.0	17.60	2958.0
Uttaranchal	1.30	1.30	1.0	3414.0	2.30	3416.0
West Bengal	21.9	23.1	55.2	22170.0	59.2	23919.0

Source: National Horticulture Board, 2013

Chrysanthemum is one of the three best merchandisable floriculture crops, globally cultivated for cut as well as loose flowers and also as pot plants. It is the number one flower in terms of its production and demand in Japan and China. In the global market, The Netherlands stands first in *Chrysanthemum* production followed by Germany and the UK.

Chrysanthemum (family Compositae) includes about 200 species producing flowers of different types. About 20,000 diverse varieties of *Chrysanthemum* are grown worldwide, out of which nearly 1000 varieties are cultivated in India. It is commercially cultivated in Maharashtra (Pune, Nasik and Ahmednagar); Karnataka (Bangalore, Kolar, Dharwad, Belgaum and Tumkur); Rajasthan (Udaipur, Jaipur Ajmer, Jaipur and Kota); Gujarat (Anand, Vadodara, Surat, Navsari and Valsad); Haryana (Ambala, Gurgaon and Faridabad); West Bengal (Calcutta and adjoining areas); Delhi; Uttar Pradesh, and Tamil Nadu. *Chrysanthemum* flowers demands are on the rise both in national and in global markets which has consequently resulted in the increase in cultivation area.

Table 3 List of major disease incidents of *Chrysanthemum*

Disease	Causal Organism
Leaf Spot	*Septoria chrysanthemi, Septoria chrysanthemella, Alternaria sp., Cercospora chrysanthemi, Pseudomonas cichorii*
Rust	*Puccinia chrysanthemi*
Wilt	*Fusarium oxysporium* f. sp. *chrysanthemi, Verticillium albo-atrum*
Powdery Mildew	*Erysiphe cichoracarum*
Ray Blight	*Ascochyta chrysanthemi*
Ray Speck	*Stemphylium sp, Alternaria sp*
Gray Mold	*Botrytis cinerea*
Stem rot	*Rhizoctonia solani, Fusarium solani*
Root rot	*Pythium sp., Phytopthora sp., Phoma chrysanthemicola*
Bacterial Blight	*Erwinia chrysanthemi*
Stem necrosis	*Pseudomonas cichorii*
Bipolaris leaf spot	*Bipolaris setaria*
Charcoal stem rot	*Macrophomina phaseolina*
Cylindrosporium leaf	*Cylindrosporium chrysanthemi*
Crown gall	*Agrobacterium tumefaciens*
Fascination	*Corynebacterium fascians*
Mosaic	*CMV, Chrysanthemum virus-B*
Chlorotic mottle	*Chrysanthemum chlorotic mottle viroid*
Chrysanthemum stunt	*Chrysanthemum stunt viroid*

Source: Bhattacharjee & De 2003, Marjan *et al* 2006

Successful cultivation of *Chrysanthemum* plant is hindered by numerous bacterial, fungal and viral diseases (Bhattacharjee and De 2003) (Table 3).

Fusarium wilt of *Chrysanthemum* caused by *Fusarium oxysporium f. sp. chrysanthemi* is one of the most wide spread and destructive disease, causing infection and loss from nursery to flowering stage. The disease is most severe in warm climates (Locke et al. 1985). Fusarium wilt of Chrysanthemum is difficult to control because of the pathogen persistence in the soil and low availability of resistant varieties for its cultivation (Garibaldi et al.2009). The present paper is intended to discuss the aspects of epidemiology, pathogenesis and biological control measures of *Fusarium oxysporum* with emphasis on wilt disease of *Chrysanthemum* plants.

Causal organism and wilt symptoms:

Toop (1963) reported occurrence of *Chrysanthemum* wilt disease in Canada but erroneously identified the pathogen as *Fusarium oxysporum f. sp. callistephi* (Snyd. And Hans.), later was corrected by Armstrong et al. (1970) as *Fusarium oxysporum f. sp. chrysanthemi*. *Chrysanthemum* wilt is caused by *Fusarium oxysporum* f. sp. *chrysanthemi* Littrell, G.M. Armstr. & J.K. Armstr., and is one of the most important diseases and causes massive losses in flower yield. Plants infected with *Fusarium oxysporum f. sp. chrysanthemi* have symptoms of drooping, yellowing and loss of turgidity of leaves; stunted growth and failure in production of normal buds and flowers. Disintegration and discoloration in the roots and lower portions of stem is typically observed due to incursion and proliferation of the pathogen into vascular tissues. As the disease progresses the vascular tissues are blocked, and are not able to translocate water and required nutrients, which ultimately results into plant death. It has been observed that at early stages of the infection, plants shows recovery at night due to low temperature but in severe cases plants do not recover at all. Creamy-white mycelial growth of pathogenic fungi is also observed at the collar region of plants at advance stages of infection (Ghosh and Singh 1982)**.**

The vascular wilt isolates of *Fusarium oxysporum* are known to have an unique ability to penetrate and establish themselves in the vascular system of host (MacHardy and Beckman 1981). Smith (1899) suggested that blocking of the vascular system of infected plants was the main cause

of wilting, but later researchers proposed involvement of toxins and enzymes in causing wilt disease (Haskell 1919, Bisby 1991, Brandes 1919).

The etiology of the pathogen (*Fusarium oxysporum f. sp. chrysanthemi*) was worked out by Murkar *et al.* (1994) and Ghosh and Singh (1982). *Fusarium oxysporum f. sp. chrysanthemi* has white to pink colored septate mycelium bearing micro-conidia (1.5 – 4.2 × 6 – 15µ), macro-conidia (15.9 – 55 × 2.25 – 5.4µ) and chlamydospores (7.0 to 12.0µ). The micro-conidia are abundant, hyaline, aseptate, ovoid or kidney shaped. Macro-conidia are attenuated towards both ends and its size varies with the degree of septation. Chlaymydospores are globose, aseptate, intercallary and individual or in chains of two.

Effect of Environmental Parameters on Disease Development:

Development of disease symptoms in infected plants directly depend on the environmental conditions in which cultivation occurs. Moderate temperature, high humidity, accessibility of plant nutrients in soil are the prime conditions resulting in faster growth of the pathogen and therefore greater disease development. At high temperatures (above 32ºC) the plant displays a preliminary symptom of wilting rather than stunted growth and drooping of leaves (Gardiner *et al.* 1987). *Chrysanthemum* plants inoculated with *Fusarium oxysporum f. sp. chrysanthemi* exhibited wilt symptoms within two weeks followed by complete death when grown at 29 to 32ºC. However, plants showed delayed wilting symptoms when they were kept at lower temperatures. Higher temperatures (27 – 32ºC) favor incidence of infection and disease development (Emberger and Nelson 1981).

Organic matter content availability in the planting medium suppresses growth and development of the pathogen and thereby a reduction in disease development. Chef et al. (1982) amended the planting medium of *Chrysanthemum* plants with mature hardwood bark compost (CHB) and witnessed that amendment of mature CHB inhibits *Chrysanthemum* and flax *Fusarium* wilts, but when green CHB was used disease suppression was less significant.

Susceptibility and Resistance of Cultivars:

Fusarium oxysporum f. sp. chrysanthemi is known to cause moderate to severe wilting symptoms on various cultivars of *Chrysanthemum.* The pathogen has an inimitable capability to alter the host range and it was found that the pathogen may also infect other floriculture plants of family Compositae. *Fusarium oxysporum f. sp. chrysanthemi* has recently been reported to cause infections on four economically important ornamental crops *viz. Chrysanthemum* (*Chrysanthemum morifolium*), Paris daisy (*Argyranthemum frutescens*), African daisy (*Osteospermum sp*.) and Gerbera (*Gerbera jamesonii*). Numerous varieties of *Chrysanthemum* were evaluated for their resistance and susceptibility and only few were found to be resistant against all the isolates of *Fusarium oxysporum f. sp. chrysanthemi* (Garibaldi et al.2009, Minuto et al.2007).

Fisher and Toussoun (1983) evaluated 16 cultivars of *Chrysanthemum morifolium* for susceptibility to wilt caused by *Fusarium oxysporum f. sp. chrysanthemi* and rated symptomatology on a scale of 0-5 and noted variation from resistant to susceptible. They reported that wilt fungus was present in the vascular system of both symptomless and plants which expressed symptoms. *Fusarium oxysporum f. sp. chrysanthemi i*nfected both resistant and susceptible cultivars but progression of disease and visible symptoms were detected on highly susceptible cultivars only. *Chrysanthemum* infected by *Fusarium oxysporum f. sp. chrysanthemi* and *F. oxysporum* f. sp. *tracheiphilum* race 1 showed initial foliage symptoms 7 days after inoculation in very susceptible and in 35 days on highly resistant cultivars. Degree of symptom development is related with the cultivar resistance and susceptibility. Resistance cultivars show less or no symptoms but the susceptible cultivars demonstrate symptoms very fast and at early stage (Engelhard and Woltz 1971).

Survival of *Fusarium oxysporum* in soil:

Survival of *Fusarium* spp. in the soil is generally by chlamydospores, which have the increased capability to endure harsh environmental conditions (Booth 1971, Nash et al.1961). Most of the formae speciales of *F. oxysporum* persist quiescent and immobile in the form of chlamydospores in decaying host tissues (Nelson et al.1981). The chlamydospores have three major stages in their life-cycle, these are formation, dormancy and germination. The formation of chlamydospore is reported to depend on the temperature, pH and CO_2 content of the soil (Banihashemi and Zeeuw 1973, Trujillo and Snyder 1963, Newcombe 1960). In previous studies evidence have been found that the wilt pathogen may persist in soil from 8 to 20 years (Mc Rae and Shaw 1933). Hsieh (1985) reported that the pathogen was present in soil mainly from the surface to a depth of 20 cm and was detectable down to 30 cm.

Inoculum density of *Fusarium oxysporum*:

The initial inoculum density of the pathogen in soil plays a major role in disease progression. In case of *Fusarium* wilt, it has been found that there is increase in the disease severity with an increase in inoculum density, which also reduces the time required for disease development (Ben-Yephet et al.1996, Zote et al.1996). Rekah et al. (2001) reported that the wilt symptoms were apparent when inoculum density in the root zone reached a lethal threshold level of 10^4 per g of soil in tomato. Ben-Yephet et al. (1996) reported that high inoculum density caused greater disease incidence, speedy disease expansion and less flower yield than low inoculum density in carnation. Increased disease incidence with the increasing inoculum was found significant in susceptible and highly susceptible cultivars.

Role of fusaric acid in pathogenesis:

Gaumann (1957) was first to define Fusaric acid as a wilt toxin from *F. oxysporum* f. sp. *lycopersici*, *F. oxysporum* f. sp. *vasifectum* and *Gibberella fujikuroi*. This toxin is produced by many of the formae specialis of *F. oxysporum*. Fusaric acid is highly toxic to the plants and its phyto-toxicity has been demonstrated in several *Fusarium* induced diseases (Drysdale 1982). This toxin has the great ability to inhibit the cytochrome oxidase, mitochondrial respiration and ATP levels on the plasma membrane (Kohler and Bentrup 1983, Gaumann 1958). The toxin is also known to obstruct the synthesis of polyphenol oxidase and peroxidase enzymes (Drysdale 1982, Pegg 1981) that play a significant role in active defense mechanism employed by plants against the pathogens.

Production of fusaric acid by the strains of *Fusarium oxysporum* has been reported by several workers in recent years (Singh & Kumar 2011c, Diniz et al.2009, Hong Sheng et al.2008, Regina et al.2002, Peterson and Rutherford 1991). Regina et al. (2002) reported variability among 12 isolates of *Fusarium oxysporum* with respect to amount of fusaric acid production. Hong-Seng et al. (2008) reported a decrease in height, root length and fresh weight of watermelon plants exposed to various concentrations of fusaric acid produced by *Fusarium oxysporum* f. sp. *niveum*. Fusaric acid produced by *Fusarium oxysporum* f. sp *vasinfectum* is a potent phyto-toxin for the cotton plants (Liu et al.2010). The virulence of the pathogenic isolates of *Fusarium oxysporum* and their competencies to produce fusaric acid can always not be established (Kuo and Scheffer 1964), but in some cases this link has been successfully demonstrated (Davis 1969 and Kern 1972). A positive correlation between virulence and production of fusaric acid in safflower wilt pathogen has been reported (Chakrabarti and Chaudhary 1980). However, various researchers have different opinion about the role of fusaric acid toxin in disease development. Curir et al. (2000) reported only a marginal significance of fusaric acid in the lily basal rot disease caused by *Fusarium*.

Growth characteristics of *Fusarium oxysporum*:

Different isolates express dissimilarity in their growth characteristics when they are grown on single or dissimilar nutritional medium. Gangadhara et al. (2004) reported variation in the growth features of six *Fusarium oxysporum* f .sp. *vanillae* isolates when they were grown on same

or different culture media, pH, temperature and carbon source. PDA and Richards Agar were found to be most suitable as they supported best growth of *Fusarium udum* (Ingole 1995) and *Fusarium oxysporum* f. sp. *vanillae* (Gangadhara et al.2004, Anjaneya 2002). *Fusarium* species react inconsistently towards the pH of the semi-synthetic nutrient media. Strains of *F. coeruleum* could tolerate a pH range ranging between 3.0 to 11.0 (Moore, 1924). Chi et al. (1964) reported pH 5.0 to 5.5 optimum for *F. oxysporum*, while Jhamaria (1972) reported direct correlation in growth of *F. oxysporum* with decrease and increase in pH of the medium. With regard to temperature tolerance, Chi *et al* (1964) and Anjaneya (2002) reported that species of Fusaria could grow between 10^0C to 35^0C with optimum growth between 25^0C to 28^0C. Environmental factors such as media, pH and temperature have substantial influence on the growth and sporulation pattern of *Fusarium oxysporum* (Singh and Kumar 2011c, Gangadhara *et al* 2004).

Biological Methods of controlling *Fusarium* Wilt:

Utilization of botanicals extracts:

Antimicrobial properties of numerous plant extracts, polar and non polar fractions, their pure compounds, and essential oils have been investigated by many researchers against different strains of *Fusarium* (Riaz et al. 2008, Hassannein et al. 2008, Irum 2007, Gomez-Rodriguez et al. 2003, Bansal and Rajesh 2000, Sharma and Trivedi 2002, Singh & Singh 1980, Dubey et al. 1982). Botanicals either in crude form or their fractions, obtained from species of *Eucalyptus, Cinnamonum, Tagetes, Artremisia, Curcuma, Callistemon, Mentha, Ocimum, Valeriana, Azadirachta, Trachyspermum, Caryophyllus, Palmarosa,* and *Cymbopogon* have been reported to efficiently control the growth and sporulation of many phyto-pathogenic fungi (Thind and Suri 1979, Nakhare and Garg 1996, Singatwadia and Katewa 2001, Singh & Kumar 2011b). Growth inhibition of several species and formae species of *Fusarium* have been worked out using extract of *Tagetus erecta* (Riaz et al. 2008), *A. indica* (Hassannein et al. 2008, Bansal and Rajesh 2000, Sharma and Trivedi 2002, Irum 2007, Dwivedi and Shukla 2000), *Mentha sp*. (Ghorbany et al. 2010), *Datura metel* (Irum 2007), *Lantana camera* (Begum et al. 2007, Bansal and Rajesh 2000) and *Calotropis procera* (Bansal and Rajesh 2000, Sharma and Trivedi 2002). Treatment of soil with plant extracts efficaciously reduced the *Fusarium oxysporum f. sp. chrysanthemi* pathogen inoculum. Ten per cent aqueous extract of clove amendment in soil is reported to effectively reduce the population of *Fusarium oxysporum f. sp. chrysanthemi* (Bowers and Locke, 2000).

The active constituents which are considered responsible for the antifungal properties of various phyto-chemicals are generally low molecular weight phenolics (hydroxybenzoic acid, flavanoids, hydroxycinamic acid, acetophenone, stilbenes and lignans) as well as oligo or polymeric forms such as hydrolysable and condensed tannins and lignins (Close and McArthur 2002, Okwu 2004, Okwu and Omodamiro 2005).

Biological Control of *Fusarium oxysporum* by Antagonist

Cook and Baker (1983) defined the biological control as reductions in the amount of inoculum or disease producing activity of a pathogen accomplished by one or more organisms. The biocontrol agents which are presently being used predominantly belong to fungi, bacteria, nematodes, protozoa, and viruses. Fungi such as *Trichoderma, Gliocladium, Aspergillus, Dactylella, Arthrobotrys, Penicillium, Neurospora, Chaetomium,* and *Glomus* have been extensively researched and applied against aerial and soil borne pathogens of economical plants.

Species of *Trichoderma* have been successfully and extensively employed by numerous workers for biological control of *Fusarium* sp. pathogenic to several important crop plants (Muthukumar *et al.* 2005, Manka et al. 1997, Katragadda and Murugesan 1996) Locke et al. (1985) evaluated the efficacy of *Trichoderma viride* and *Aspergillus ochraceus* against *Fusarium oxysporum f. sp. chrysanthemi* and found that there was reduction in the *Chrysanthemum* wilt disease by 40 to 80% as compared to control plants. Biocontrol potential of *Trichoderma harzianum* against *Fusarium oxysporum f. sp. chrysanthemi* has been evaluated under *in vitro* and

in vivo conditions. A significant reduction in the mycelial growth of *Fusarium oxysporum f. sp. chrysanthemi* in lab and infections and disease development in *in vivo* conditions were recorded (Singh & Kumar, 2011ab). Various mechanisms of actions have been suggested to elucidate the suppression of plant pathogens by *Trichoderma* species. These include myco-parasitism, antibiosis, nutrient competition and starvation, siderophore production, induction of systemic resistance, growth promotion etc. (Upadhyay & Mukhopadhyay 1986, Howell 2003).

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