INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print) 2222-5234 (Online)
http://www.innspub.net
Vol. 4, No. 1, p. 143-148, 2014

RESEARCH PAPER OPEN ACCESS

Report primary of *Fusarium Proliferatum* and *Fusarium solani* agents of *Dianthus caryophyllus* wilting in Markazi province in Iran

B. Fattahi[1], H. Rahanandeh[2*], S. Chavoshi[3], H.R. Zamanizadeh[4], H. Bayat[5], M. Moshayedi[6]

[1]*Department of Plant Protection, Research & Science Branch, Islamic Azad University, Iran*

[2]*Member of Department of Agronomy and Plant Breeding, Faculty of Agriculture, Rasht Islamic Azad University P. O. Box 4415866865, Lahijan, Iran*

[3]*Depaetment of Agronomy and Plant Breeding, Faculty of agriculture, Arak Islamic Azad University, Iran*

[4]*Research & Science Branch, Islamic Azad University, Iran*

[5]*National Ornamental Plants Research Station,Mahallat, Iran*

[6]*Young Researchers Club of Rasht, Rasht Islamic Azad University, Iran*

Key words: *F. Proliferatum*, *F. solani,* carnation, crown rot.

doi: http://dx.doi.org/10.12692/ijb/4.1.143-148 Article published on January 01, 2014

Abstract

Wilt disease and root and crown rot important diseases of carnation in carnation greenhouses in Markazi province of Iran. In this study, was reported major cause of the disease, *Fusarium* species. *Fusarium* species associated with different parts of growing carnations isolated in 2010 - 2012 years in Markazi province. 68 isolates of Fusarium species Identification were based on colony morphology and conidial and perithecial characteristics, *F. oxysporum*, *F. proliferatum*, *F. solani*, *F. equiseti*. To our knowledge, this is the first report of a wilt disease caused by *F. proliferatum* and *F. solani* on carnation in Iran.Populations of species was Respectively 25 and 17.64 percent. Experimental results demonstrate that the virulence species *F. proliferatum* is more than *F. solani*.

* **Corresponding Author:** H. Rahanandeh ✉ rahanandeh20@yahoo.com

Introduction

Carnation (*Dianthus caryophyllus* L.) is one of the most important commercially grown flowers of the world (Anon., 2004). The perpetual flowering carnation, *Dianthus caryophyllus*L., is one of ten major cut-flower crops in Iran.

Fusarium wilts, caused by *F. oxysporum* is one of the most widespread and destructive diseases of many major ornamental and horticultural crops.

All over the world in carnation culture, vascular wilt caused by *Fusarium oxysporum* Schelchtend.:Fr. f. sp. *dianthi* (Prill. & Delacr.) W.C. Snyder & H.N. Hans, is the most important fungal disease. *Fusarium oxysporum* f. sp. *dianthi* and *F. avenaceum* (Fr.) Sacc. were consistently isolated from wilted carnation plants in commercial stocks in south-west England (Carver *et al.,* 1996). These are the entities which take heavy toll of plants and cause economic losses to the growers, once established in a greenhouse. Since, greenhouse conditions which favor luxuriant growth of crops, also favor's the development of plant pathogens Garibaldi *et al.* (2004) , isolated *Fusarium* spp. consistently and readily from symptomatic vascular tissue onto 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. The first symptom of wilt of carnation is yellowing of leaves, followed by withering of leaf bases and yellowing of midribs, which progresses from the base of the leaf. The infected leaves gradually become chlorotic and finally wilt. Some times, unilateral development of symptoms producing crook-neck can also be seen (Sohi, 1992).In 2011 the first report of a wilt disease caused by *F. proliferatum* on carnation in China (Zhang *et al*., 2011)... Fusarium important factor causing damage to the carnation in Iran. Fusarium damage in greenhouses up to 60%. *Fusarium oxysporum* species have been reported from Iran.

The purpose of this research is to identify and isolate new strains of Fusarium wilt carnation cause of Iran.

Material and method

Sampling and assessment

A fixed plot survey was taken up during 2010-12, to know the incidence of diseases, occurring on ornamental plants grown under protected cultivation in Markazi Province in Iran districts. The disease incidence was assessed by recording the number of plants showing disease symptoms and the total number of plants present. In each greenhouse, rows were selected randomly and the number of plants showing typical symptoms and the total number of plants were recorded. Per cent disease incidence was calculated by using the formula given by wheeler (1969).

Per cent disease incidence = _No. of plants showing wilting symptoms x 100 Total no. of plants observed

During survey, characteristic symptoms of the diseases were recorded and also samples were collected for isolation of pathogens.

Medium

Purification and identification of the pathogen was cultured, PDA and CLA. Key on the mycological Nelson *et al*., (1983) evaluated growth and generative structures, the fungal isolates were identified.

Isolation of pathogens

The infected plant parts were surface sterilized with 1:1000 mercuric chloride ($HgCl_2$) solution for 30 seconds and washed separately in sterilized distilled water twice to remove the traces of mercury, if any and then transferred to sterilized Petriplates containing potato dextrose agar (PDA). The Petriplates were incubated at room temperature 25^{oC} and observed periodically for the growth of pure colonies. The pure colonies which developed from the bits were transferred to PDA slants and incubated at 25^{oC} for 15 days. Then such slants were used to study the cultural characters in the laboratory (Ben-Yephet *et al*., 1994).

Identification of Fusarium

This method was followed for maintaining pure cultures. Dilute spore suspensions (8-10 macroconidia /ml) of the isolated pathogens (*F. Proliferatum and F. solani*) were prepared in sterile distilled water. One ml of such suspension was spread uniformly on two per cent water agar plates and the excess of which was aseptically drained. Such plates were incubated at 25^{oC} and periodically observed for germination of spores under the microscope. Hyphae coming from each end cell of the single spore was traced and marked with the ink on the reverse side of petriplates. Then tip of hypha was cut and transferred to PDA slants with the help of cork borer under aseptic conditions and incubated at temperature of 25 C for 10 days. Later, mycelial bits of the fungus were placed in the center of Petriplates containing potato dextrose agar medium and incubated at 25 C for 10 days. No saltation or sectoring was observed in the culture and it was concluded that, it was a pure culture of the fungus. The spores of the pathogens were taken from infected plant portions (collar region) and temporary slide mounts were prepared in lactophenol. Then they were observed under high power objective (40x). One hundred spores (macroconidia, microconidia and chlamydospores) of the pathogens were observed under microscope and measured using ocular and stage micrometer (Nelson *et al*., 1972).

Proving pathogenicity by inoculating Dipping the roots in a spore suspension

Roots was placed in a spore suspension to concentration of 10^6 for 25 minutes. The seedlings were grown in pots with a diameter of 14 cm. Then seedlings in pots with a diameter of 14 cm were cultured pasteurized soil. The roots of control seedlings were placed in water. Then seedlings in pots with a diameter of 14 cm were cultured pasteurized soil The pots were kept for 1 month in the greenhouse conditions. Of plants discolored, yellow and faded (leaf chlorosis symptoms) (percent wilting) were recorded (Carver *et al*., 1996).

Proving pathogenicity by inoculating the soil (plants) with a spore suspension

Rooted cuttings (plugs) of carnation was planted in a steam sterilized potting media consisting of soil, sand and farm yard manure in the ratio of 3:1:1. Further, the sick soil was made by inoculating the giant culture of *F.proliferatum* and *F.solani* to the sterile soil respectively. A control treatment was maintained without adding the inoculum. Observations were made regularly for the appearance and development of symptoms. After symptom development, re-isolation was done from the artificially infected plants. The isolate obtained was compared with the original culture for confirmation (Sharma, 2000).

Result

During 2010-2012, this study was conducted in greenhouse carnation in province markazi. Samples were transported to the laboratory for isolation of pathogen.68 cases were related to the genus *Fusarium*, 28 sample genus *Phtophthora* ,25 Number *Pythium*, 22 sample *Alternaria*, 23 sample *Rhizoctonia*. Greenhouses that showed symptoms of infection, the fungus was isolated from five different genera. Abundance fungi are shown in Table 1. Based on the color specifications of colony growth rates of isolates, microscopic characteristics of four species of Fusarium were identified using the keys of Nelson *et al.,* 1983. Two species, *F. Proliferatum* and *F. solani*, first reported of carnation in Iran. Abundance species were studied in 21.79 and 15.38 percent.

Table 1. The number of isolates obtained from the initial sampling.

Isolated fungi	Number of samples
Fusarium spp.	68
Phytophthora sp.	28
Pythium sp.	25
Rhizoctonia sp.	23
Alternaria sp.	22

Fusarium proliferatum var proliferatum

Colony characteristics *Fusarium proliferatum var proliferatum* growth on PDA medium at 25 $^{\circ}C$ after 10 days of 8 cm. Color of colonies on the surface of PDA

medium, cream, purple, turning to purple to dark purple ranged. Relatively abundant aerial hyphae and cotton first medium purple and dark purple to white, then changes color. Give spores. After 2 days of culturing the fungus was produces spores on PDA and CLA Located on the side of phyalides micro aerial conidia production is only, but then the macro produces conidia. May be produced after 2 weeks sporodochium orange color. Conidiophore has conidiogenesis cell monophialide and polyphialide branched and Non-branching. Aerial hyphae produced laterally primary conidiophore. Initially non-branched conidiophore and then will branching But secondary conidiophore are located in dense sporodochium (Fig. 1).Frequency and microconidia formed in chains or false heads and often single cell, but there are two cell. Microconidia shape are elliptical until pear shape but usually elliptical are one side wide and the other side flat and thin. Macroconidia thin, sickle cell is relatively straight, narrow and twisted their apical cells and basal cells are specifically shaped heel. Most of the macro conidia produced 3-5 septate. Conidia size: Microconidia 1-2 cell: 9(5-12.5) × 2.5(2-3.1) µm. Macroconidia 3 septate: 36 (28-43) × 3.5(2.8-4.2), Macroconidia 4 septate: 41(33-45) × 3.6(3-4.3) µm and Macroconidia 5 septate: 45(36-50) × 3.8(3.4-4.4) µm. Chlamydospore: Chlamydospores were not observed(Fig 1).

Table 2. Contamination of plants infected with the fungus isolates of *F. proliferatum*.

Isolate Fungi	rate Infection
F6	3.33
13FF	4.66
F26	4.66
F33	4.66
control	0

Table 3. Contamination of plants infected with the fungus isolates of *F. solani*.

Isolate Fungi	rate Infection
F19	3.33
F24	3
F5	3.33
control	0

Colony characteristics Fusarium solani var solani

Plated on potato dextrose agar and incubated at 25°C. After ten days, individual fungal colonies were subcultured on to PDA plates for identification. Within 10 days, Cream to light violet, aerial mycelium developed. With the aid of an inverted microscope, single conidia were transferred to carnation leaf agar (CLA) medium. after 2-3 days were produced spores After 10 days of incubation, morphological characteristics were found to be identical to those of *F. solani*. On CLA medium, conidia grew in branched conidiophores with false heads bearing monophialides or polyphialides. No conidiospores in chains were observed. Microconidia were ovate to long and oval, 0 to 1 septate, and 9 to 11 × 1.5 to 2.2 µm. Macroconidia are falculate, 3 to 5 septate, and 37 to 44 × 4.3 µm. Many chlamydospores are formed as intermediate and end.

Table 4. Contamination of plants infected with the fungus isolates of *F. proliferatum*.

Isolate Fungi	rate Infection
F6	2.66
F13	3.33
F26	4.66
F33	3
Control	0

Table 5. Contamination of plants infected with the fungus isolates of *F. solani*

Isolate Fungi	rate Infection
F19	3.33
F24	2.66
F5	2.66
control	0

Proving pathogenicity

In this study, none of the control pots wilt symptoms were observed, whereas in all the pots treated with fungal strains, there was some degree of wilting. The comparison of all treatments of disease control were significantly different at the one percent level in all treatments, the disease had occurred.

Root dip inoculation

After a week of planting seedlings, irrigated was done .Sampling was started after the appearance of

symptoms.Factors measured: symptoms of chlorosis, reduced growth, wilting plants infected vascular.The mean percentage of infected plants for each isolate was calculated and the percentage of infected plants (symptoms) were determined for each species(Table 1,2).Pathogens *Fusarium proliferatum* is more than *F. solani.*

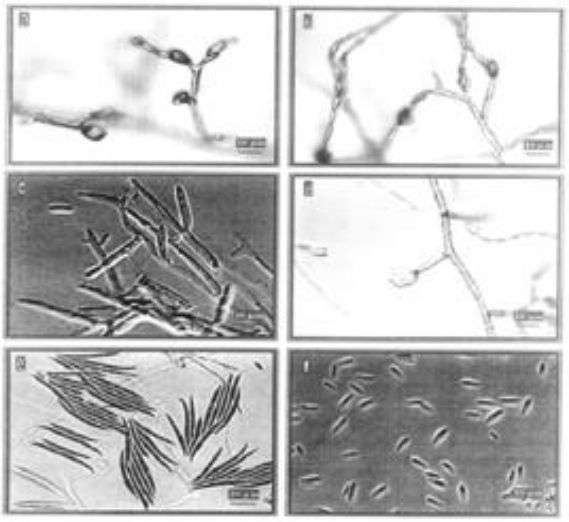

Fig. 1. a,b) Conidiophore with false heads and chains, a,b,c) Conidiophore polyphialide, d) Conidiophore monophialide e) Macroconidia f) Microconidia.

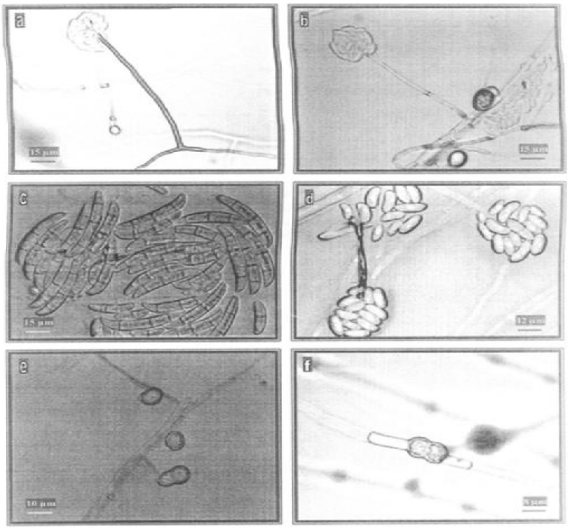

Fig. 2. a,b) conidiophore ،c) macroconidia،d) microconidia ،e,f) chlamydospores.

Soil inoculating spore suspension

After a week of planting seedlings, irrigated was done .Sampling was started after the appearance of symptoms.Factors measured: symptoms of chlorosis, reduced growth, wilting plants infected vascular.The mean percentage of infected plants for each isolate was calculated and the percentage of infected plants (symptoms) were determined for each species(Table 4,5).In this experiment, similar results were obtained with above experiment.

Discussion

In carnation culture, vascular wilt caused by Fusarium is the most important fungal disease (Rattink, 1983). Fusarium crown and root rot in carnation has been reported in USA (Jones *et al.*, 1991). Symptoms were observed on carnation included severe vascular browning, root and crown rot. In this study, the species *Fusarium proliferatum* and *Fusarium solani* was isolated for the first time on Carnation in Iran. *Fusarium* was consistently isolated from all wilt affected carnation plants. Garibaldi *et al.,* (2004) and Garibaldi and Minuto (2007), Carver *et al.*, (1996) reported isolation of *Fusarium oxysporum* f. sp. *dianthi* and *F. avenaceum* from wilt affected greenhouse grown carnation plants in southwest England. Generally the disease incidences were higher in the high temperature period than in the low one, and the disease outbreaks were found only during the high temperature period in markazi previous. Nelson *et al.* (1975) reported the disease occurred especially during the summer when it is not possible to manage greenhouse environmental conditions which favor the disease development. Inoculated carnation plugs developed wilt symptoms within 30 days after inoculation, symptoms started as yellowing of leaves, later turned straw coloured, finally wilting and death of the plants was occurred. This is in acceptance with Carver *et al.,* (1996) who examined and proved the pathogenicity of *Fusarium oxysporum* f. sp. *dianthi* using spore suspensions, introduced into carnation plants via root dip or cut stem inoculations. *F. proliferatum* microconidia measured Average 9 x 2.5 µm, macroconidia were Average 40.67 x 3.63µm in diameter and chlamydospores not observation. The microconidia, macroconidia and chlamydospores of *F. solani* microconidia measured Average 10 x 1.85 µm,macroconidia Average 40.1 x 4.3 µmand 4.5 x 9.5

µm in diameter. These observations are in accordance with Booth (1971) and Nelson *et al*., (1983).

Reference

Anonymous. 2004. Horticultural Statistics of Karnataka State At a Glance, 2002-03. Statistical wing, Directorate of Horticulture, Dept of Horticulture, Govt. of Karnataka, Lalbhag, Bangalore **32,** 107 p.

Ben-Yephet Y, Reuven M, Genizi A. 1994. Effects of inoculum depth and density on Fusarium wilt in carnations. phytopathology **84**, 1393-1398. http://dx.doi.org/10.1094/Phyto-84-1393.

Booth C. 1971. The genus *Fusarium*. Commonwealth Mycological Institute, Kew Surrey, UK, 142-143 p.

Carver CE, Pitt D, Rhodes DJ. 1996. Aetiology and biological control of Fusarium wilt of pinks (*Dianthus caryophyllus*) using *Trichoderma aureoviride*. Plant Pathology **45,** 618-630. http://dx.doi.org/10.1046/j.1365-3059.1996.d01-162.x

Garibaldi A, Minuto A, Bertetti D, Gullino ML. 2004. Fusarium Wilt of Gerbera in Soil and Soilless Crops in Italy. Plant Disease **88,** 311. http://dx.doi.org/10.1094/PDIS.2004.88.3.311C

Garibaldi A, Minuto A. 2007. Fusarium wilt of gerbera in Spain in soilless crops. Plant Disease **91,** 638. http://dx.doi.org/10.1094/PDIS-91-5-0638C

Johnson LF, Curl EA, Bond JH, Fribourg HA. 1959.Methods for studying soil microflora. Plant Disease Relationships. Burgers Publishing Co. Minneapolis., USA.

Nelson PE, Pennypacker BW, Toussoun TA, Horst RK. 1975. *Fusarium* stub dieback of carnation. Phytopathology, **65,** 575-581. http://dx.doi.org/10.1094/Phyto-65-575.

Nelson PE, Toussoun TA, Marasas WFO. 1983. *Fusarium* species, an Illustrated manual for identification.The Pennsylvania State University Press,Univ. Park and London. 193.

Rattink H. 1983. Spread and control of Fusarium wilt in carnations on artificial substrates **141,** 103-108. http://www.actahort.org/books/141/141_14.htm

Sharma P. 2000. An Integrated approach for the management of Carnation wilt caused by Fusarium oxysporum f. sp. dianthi (Pril. And Del.) Snyd. And Hans 2001. New Botanist **27,** 143-150.

Sohi HS. 1992 Diseases of ornamental plants in India. Publication and information division, Indian Council of Agricultural Research, Krishi Anusandhan Bhavan, New Delhi, India. 46.

Wheeler BEJ. 1969. An introduction to plant disease. John Wiley and Sons Ltd., London,UK, pp. 301.

Zhang J, Xingxing W, Yunqing B, Yixin W, Guanhui l, Yueqiu H, Zichao M. 2011. First Report of *Fusarium proliferatum* Infecting Carnation (*Dianthus caryophyllus* L.) in China. Journal of Phytopathology **161,** 850-854. http://dx.doi.org/10.1111/jph.12128