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Survey on Carnation Wilt Under Protected Cultivation in the Nilgiris and Diversity Analysis of *Fusarium oxysporum* f. sp. *dianthi* Using RAPD Markers

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Carnations grown under protected cultivation were surveyed for Fusarium wilt caused by *Fusarium oxysporum f.sp. dianthi* in major carnation growing areas in Ooty, Coonoor and Kotagiri areas of the Nilgiris district in Tamil Nadu, India during 2011-12. The results indicated that the wilt incidence ranged from 19.66 to 44.46 per cent. During the survey, eight different isolates were brought into pure culture and confirmed for pathogenicity. Diversity analysis of *F.o.f.sp. dianthi* through RAPD analysis revealed three different groups. The pathogen was confirmed as *F.o.f.sp. dianthi* using specific primers through polymerised chain reaction (PCR) and through sequence analysis. The isolates FOD1, FOD3 and FOD4 were submitted to NCBI and assigned with accession numbers JX036529, JX036531 and JX036532.

Key words: Carnation, *Fusarium oxtysporum* f.sp. *dianthi*, Pathogenicity, PCR, RAPD

Floriculture is a fast emerging venture throughout the world. Indian flower export markets are estimated to transact about 11 billion US dollars at present and expected to raise up to 20 billion US dollars by 2020 (Gian Aggarwal, 2011). Carnation (*Dianthus caryophyllus* L.), a "Divine Flower", native of Mediterranean region belonging to the family Caryophyllaceae, is an introduced cut flower crop to India and adapted well in the regions with cool climatic conditions commonly prevailing in places like in The Nilgiris, Kodaikanal, Bangalore, Pune and Shimla. Continuous cultivation of carnation and exploitation of soil under protected cultivation, indiscrimin.ate use of fungicides for the management of soil borne diseases and availability of different cultivars of carnation make carnation varieties highly susceptible for infection by Fusarium wilt pathogen *Fusarium oxysporum* f.sp. *dianthi* Schlechtend:Fr.(Prill&Delacr.) W.C. Snyd. & H.N.Hans. Intensive cultivation of carnation under monoculture deteriorates soil health and induces the Fusarium wilt, causing considerable yield loss leading to deterioration in quality and quantity of marketable blooms.

Materials and Methods

Carnation varieties were surveyed for the incidence of Fusarium wilt in The Nilgiris district of Tamil Nadu, India. Commercial varieties of carnation with different colours like White (White Dona, Hunza, Baltico and White Liberty), Red (Big Red, Tuareg, Turbo, Gaudina and Domin.go), Yellow (Elisir, Kiro, Harvey and Liberty), Light pink (Big Mama, Pink Dovar and Charment), Dark pink (Dona, Dumas and Bizet) and bicoloured (Happy Golem, Folgore, Diana, Falicon, Solar, Rendez-vouz, Yellow Viana, Alibaba, Felica, Scorpio and Beads) were surveyed in Ooty, Coonoor and Kotagiri of The Nilgiris district in Tamil Nadu, India. The varieties were supplied by different companies (Hilverda Kooij from Holland, Barbarat and Blanc from Spain and Selecta from Italy. In India, the carnations were distributed at Pune and Bangalore) and grown in Ooty, Coonoor and Kotagiri. It was surveyed in vegetative and bud formation stage during I, II and III flushes. The typical symptoms of the disease include yellowing of leaves followed by withering of leaf bases, epinasty of the older leaves. The infected plants gradually become stunted, wilted and finally die (Agrios, 1988). Browning of the vascular tissue and the per cent wilt incidence in different varieties were recorded. The per cent disease incidence was calculated as follows,

$$\text{Percent disease incidence} = \frac{\text{Number of plants showing wilting symptoms}}{\text{Total number of plants observed}} \times 100$$

Isolation of vascular wilt pathogen

The vascular wilt pathogen *F. oxysporum* f.sp. *dianthi* was isolated from infected carnation roots of susceptible varieties on potato dextrose agar (Aneja, 1993). The fungus was purified using the single hyphal tip technique and used for further study. Individual conidia were observed under microscope and monoconidial cultures were developed for all the isolates. They were stored as conidial suspensions in glycerol at −80°C.

Identification of Fusarium oxysporum f.sp. dianthi

The pure cultures on PDA were used for observation of phenotypic characters such as colony features, growth rate and pigmentation. For microscopic characteristics, pure cultures of 5mm mycelia disc were transferred on PDA and incubated at 28±2°C. After 10 days of growth, the morphological characteristics were observed and evaluated

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according to Burgess *et al.* (1994) using a light microscope (Labomed – IVU 5100) and photographed using a Labomed camera model LX400 with an image analyser – pixel pro programme. The morphological characteristics of the fungi were observed from slide cultures and *in situ* observation on PDA. Keys of Burgess *et al.* (1994) and Leslie and Summerell (2006) were used for the identification of *Fusarium* isolates upto species level.

Pathogenicity

Potting mixture comprised of laterite soil, sand and compost. They were mixed at 3:1:1 and steam sterilized at 1.42 kg cm^{-2} pressure for 1hr on alternate days. The sterilized potting mixture was filled in the pots @ 5Kg / pot. The vascular wilt pathogen *F. oxysporum* multiplied in potato dextrose broth, consisting of 106 conidia/ml was inoculated @ 1% to the soil weight. Later, the rooted cuttings of carnation were planted. Similarly, uninoculated control was also maintained. The pots were watered regularly upto saturation on alternate days. Observations were made regularly for the appearance and symptom development. Different isolates, FOD1, FOD2, FOD3, FOD4, FOD5, FOD6, FOD7 and FOD8 were inoculated to confirm the ability to cause the wilt symptom in carnation. Similarly, non- pathogenic isolate of *F. o*. f.sp. *lycopersici* was also inoculated in carnation. After symptom development, re-isolation was done and compared with the original culture for confirmation of the pathogen identity.

DNA extraction and amplification of Fusarium

Genomic DNA was extracted from the suspension culture of Fusarium by the Cetyl Trimethyl Ammonium Bromide (CTAB) method as described by Knapp and Chandlee (1996). For confirmin.g the isolates as F. *oxysporum* species 16s rDNA intervening sequence specific FU F (5'CAACTCCCAAACCCCTGTGA3'); FU R (5'GCGACGATTACCAGTAACGA3') primers were used to get an amplicon of 389 bp size (Singh and Kumar, 2001). Amplification was conducted with a total reaction volume of 25µl in Eppendorf Master Cycler, Germany. Polymerase Chain Reaction (PCR) conditions were hold of 2 min. at 95°C, 30 cycles of 1 min. at 94°C, 30 sec at 54°C and 1min. at 72°C and a final extension of 10 min. at 72°C. PCR products were resolved on two per cent agarose at 50 V, stained with ethidium bromide (0.5µg/ml), photographed and analyzed using gel documentation system.

Diversity analysis of F. oxysporum f.sp. dianthi using RAPD

Nucleotide sequencing of PCR products was done at Chromous Biotech Pvt. Ltd, Bangalore, India. The rDNA homology searches were performed using the BLAST program through the internet server at the National Center for Biotechnology Information (National Institutes of Health, Bethesda, USA). DNA sequences of eight *F. oxysporum* species were subjected to multiple alignment and phylogenetic analysis using Clustal X to determin.e proximity of relationship by similarity matrix. Sequences and accession numbers for compared isolates were retrieved from the GenBank database as outgroup. Sequence pair distances among related and outgroup were scored with the Clustal X program and phylogenetic tree constructed with Treecon version 1.15. Newly obtained sequences were submitted in the GenBank database, New York, USA.

Random Amplified Polymorphic DNA (RAPD) analysis of F. oxysporum f.sp.dianthi

DNA was extracted according to Knapp and Chandlee (1996) method. Template DNA concentration is very important in RAPD studies, so the DNA was quantified by dotting onto ethidium bromide (400 µl/ 100 ml of agarose) supplemented agarose (0.8% w/v). Different isolates of *F. oxysporum* were used for RAPD analysis following the method recommended by Bhat and Jarret (1995) with required modification. The cocktail of 20µl for the amplification was prepared as follow in 0.2 ml PCR tubes: DNA 25 ng/µl - 4.00 µl, Primer (Operon Technologies Inc., USA) - 4.00 µl, dNTP mix - 8.00 µl, sterile distilled H_20 - 4.00 µl. Then the 0.2 ml PCR tubes were loaded on to a Eppendorf Master Cycler, German. PCR conditions are hold of 10 min at 94°C, 45 cycles of 1min at 94°C, 1min at 35°C and 2 min at 72°C and a final extension of 10 min at 72°C. The PCR products were resolved on two per cent agarose at 50 V, stained with ethidium bromide (0.5µg/ml), photographed and analyzed using gel documentation system. The amplification products were visualized with a UV transilluminator and photographed in the gel documentation system (Alpha Innotech Corporation, San Leandro, California). All RAPD-PCR reactions were repeated atleast three times and only the RAPD bands, which appeared consistently were evaluated for polymorphism.

List of random primers used for RAPD analysis

Primer	Sequence
OPA1	3'- CAGGCCCTTC - 5'
OPA2	3'- TGCCGAGCTG - 5'
OPA3	3'- AGTCAGCCAC - 5'
OPA4	3'- AATCGGGCTG - 5'
OPA7	3'- GAAACGGGTG - 5'
OPA11	3'- CAATCGCCGT - 5'
OPA13	3'- CAGCACCCAC - 5'

Analysis of RAPD-PCR results

The banding patterns were scored for RAPD and microsatellite primers in each *F. oxysporum* isolates starting from the small size fragment to large sized one. Presence and absence of each band in each isolate was coded as 1 and 0 respectively. The scores were used to create a data matrix to analyze genetic relationship using the NTSYS-PC program version 2.02 (Exeter Software, New York, USA) described by Rohlf (1993). A dendrogram was constructed based on Jaccard's similarity coefficient (Jaccard, 1908) using the marker data from *F. oxysporum* isolates with UPGMA.

Results and Discussion

Survey for the incidence of Fusarium wilt in carnation

The results of the survey revealed that the average wilt incidence in white coloured carnation at Nilgiris district was maximum 44.46 per cent in Baltico variety, whereas the minimumwilt incidence of 25.13 per cent was observed in Hunza variety. Survey for the Fusarium wilt of red coloured varieties showed that maximum incidence of 44.20 per cent in Gaudina variety and the minimum incidence of 18 per cent was observed in Big Red variety. The survey of yellow coloured carnations revealed that the mean average wilt incidence of 31.93 per cent was observed in The Nilgiris district in Harvey variety. The minimum incidence of 28.80 per cent was observed in Elisir variety. The maximum wilt incidence of 30.33 per cent was observed in light pink coloured carnation variety in Big Mama and the minimum of 25.20 per cent disease incidence in the variety, Charment. Among the dark pink coloured carnation varieties the maximum wilt incidence of 29.73 per cent was recorded in the variety Dona, while the minimum incidence of 20.06 per cent was observed in the variety, Dumas. Whereas Golem, the purple coloured carnation variety showed 23.00 per cent wilt. Farida, the light purple coloured variety exhibited 37.33 per cent of wilt as against 29.20 per cent in the green coloured variety Darjeeling (Table 1). The highest mean average wilt incidence of 36.20 per cent was observed in bicoloured carnation variety, Felica and the minimum incidence of 11.33 per cent was observed in the variety Folgore (Table 2).

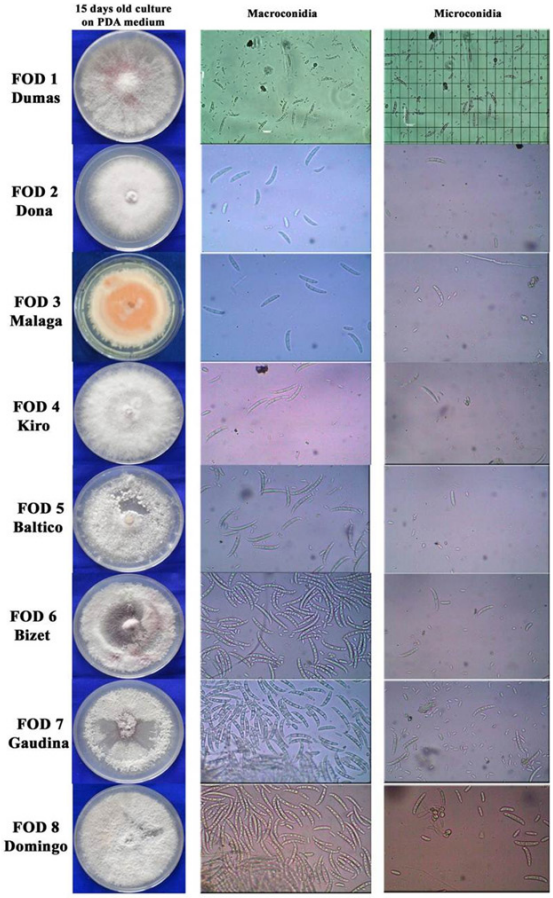
Fig 1. Vascular wilt symptoms of carnation caused by *Fusarium oxysporum f. sp. dianthi*

Table 1. Survey for the occurrence of Fusarium wilt of carnation varieties under protected cultivation in the Nilgiris

Variety	Colour	Percentage of wilt incidence			
		Ooty	Coonoor	Kotagiri	Mean
White Dona (RR)	White	40.00	32.00	28.80	33.60
Hunza (RRR)	White	25.00	28.20	22.20	25.13
Baltico (R)	White	48.20	47.80	32.40	44.46
White Liberty (R)	White	47.40	28.80	30.40	35.53
Big Red (RRRR)	Red	18.00	20.20	20.80	19.66
Tuareg (RRR)	Red	32.00	24.00	20.00	25.33
Turbo (RR)	Red	42.00	38.00	39.40	39.80
Gaudina (RRR)	Red	49.80	32.40	50.40	44.20
Domingo (RRRR)	Red	38.00	47.00	28.20	37.73
Elisir (RR)	Yellow	32.00	30.00	24.40	28.80
Kiro (RRR)	Yellow	34.20	18.00	36.20	29.46
Harvey (R)	Yellow	38.00	29.60	20.40	29.33
Liberty (R)	Yellow	36.00	31.20	28.60	31.93
Big Mama (RRR)	Light pink	28.00	28.80	34.20	30.33
Pink Dovar (RR)	Light pink	36.40	20.00	28.40	28.26
Charment (RR)	Light pink	28.40	20.40	26.80	25.20
Dona (RR)	Dark pink	34.40	32.00	22.80	29.73
Dumas (RRRR)	Dark pink	32.20	12.00	16.00	20.06
Bizet (RR)	Dark pink	38.00	20.00	14.00	24.00
Golem (RRRR)	Purple	34.80	18.00	16.20	23.00
Farida (R)	Light Purple	48.00	38.00	26.00	37.33
Darjeeling (RRRR)	Green	32.00	27.40	28.20	29.20
Mean		36.04	28.35	27.04	30.55

Ben-Yephet *et al*. (1992) reported that, the wilt incidence in carnation varied depending upon the cultivar, mono-cropping and stage of infection. The *Fusarial* wilt incidence in different cultivars of carnation varied from 40 to 79% (Katoch, 1999). Similarly, in the present study, the incidence of carnation wilt varied depending on carnation varieties.

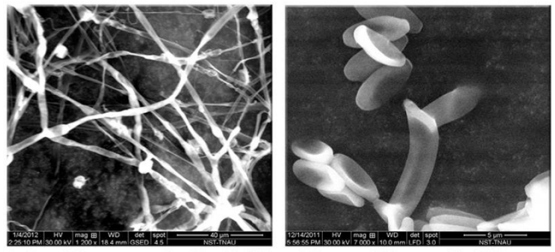
Fig 2. Isolates of *Fusarium oxysporum f.sp. dianthi* (FOD)



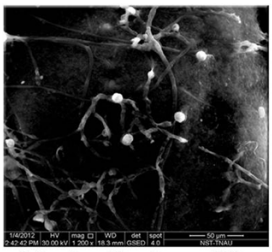
Symptomatology

Wilt symptoms were found to be associated in rooted stem cuttings and in older plants. The symptoms associated with wilt in the stem cutting stage (30 days old) include, yellowing of lower most leaves, and it subsequently spread to entire plant. Affected leaves drooped down followed by wilting of entire plant. The longitudinal section of the infected roots and stems exhibit discolouration of the vascular tissues.

Fig 3. Environmental Scanning Electron Microscope (ESEM) images of *Fusarium oxysporum f.s.p. dianthi*



Hyphae of *F. oxysporum* f.sp.dianthi 1200x Macro and Micro conidia 7000x



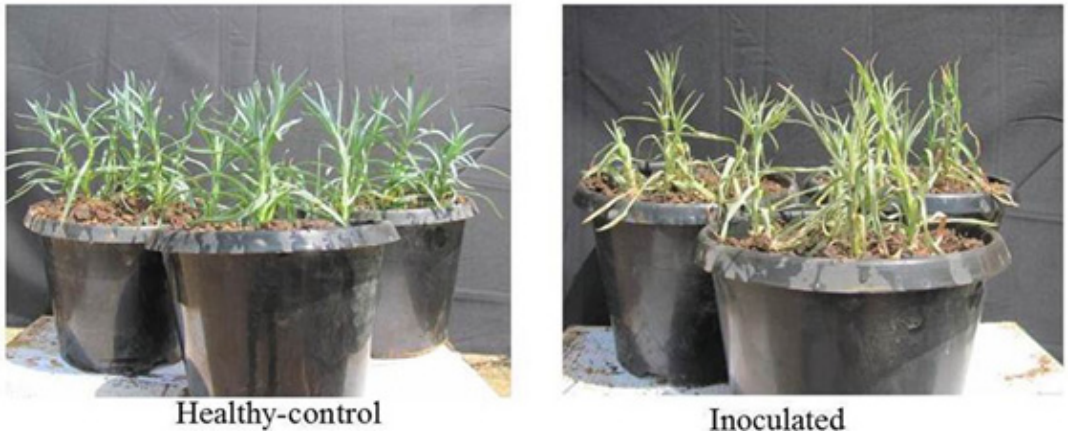
Intercalary chlamydospores 1200x

However, if the infection occured during later stages of the crop growth (70 days after planting), yellowing of the leaves are noticed on few shoots. Later the affected leaves drooped down and dried. Subsequently, the infection gradually spread to other shoots. It lead to wilting of the entire plant within 3 to 4 weeks after infection. Examination of the infected plants showed the presence of vascular discolouration in roots and stems (Fig 1).

Phenotypic characterization of pathogen associated with carnation wilt

Pathogen associated with wilt was isolated from carnation varieties *viz*., Dumas (Light Pink), Dona (Pink), Malaga (orange), Kiro (yellow), Baltico (White), Bizet (Pink), Gaudina (Red), and Domingo (Red). Eight different isolates of *Fusarium* spp., were

Fig. 4 Pathogenicity for carnation wilt caused by Fusarium oxysporum f.sp. dianthi



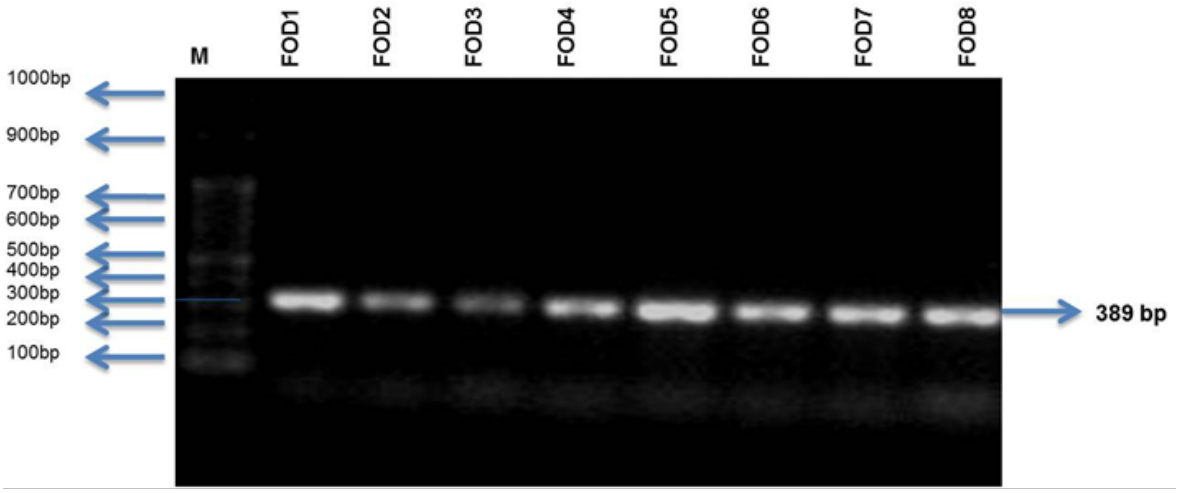
Healthy-control Inoculated

isolated. The mycelium of the fungal culture on PDA medium was initially white and later turned light pink to orange in different isolates. Macroconidia was sparse, and fusoid, 2-3 septate and measured 17.0-24.0 x 3.5-4.0 µm. Microconidia were abundant, hyaline, continuous, ovoid and measured 4.5-8.0 x 2.0-3.5 µm. Chlamydospores were hyaline, spherical and measured 4.5 – 7.5 µm in diameter. Based on these phenotypic characters, the pathogen was confirmed as *Fusarium oxysporum* f.sp. *dianthi* (Table 3). The colour of the mycelium on PDA medium was white, orange purple and pink with abundant microconidia. In a similar case, the culture of the fungus on potato dextrose agar had been reported to possess pinkish to purple colored mycelium (Booth, 1971). Likewise, 8 races of FOD were reported to be associated with the Mediterranean carnation ecotypes in Italy, France, and Spain (Garibaldi, 1983). The race 2 is the most prevalent and a virulent isolate seen all over the world (Baayen *et al*., 1997).

Table 2. Survey for the occurrence of Fusarium wilt of bi-coloured carnation varieties under protected cultivation in Nilgiris

Variety	Percentage of wilt incidence			
	Ooty	Coonoor	Kotagiri	Mean
Happy Golem (RRRR)	18.00	12.00	10.00	13.33
Folgore (RRRR)	14.00	12.00	8.00	11.33
Diana (RRRR)	18.00	12.20	14.60	14.93
Falicon (RRR)	14.00	12.80	18.20	15.00
Solar (RR)	32.00	30.40	30.20	30.86
Rendez-vouz (RRR)	18.00	16.20	17.20	17.13
Yellow viana (RRR)	18.80	16.80	11.20	15.60
Alibaba (R)	38.00	30.00	26.20	31.40
Felica (RR)	32.00	38.40	38.20	36.20
Scorpio (RRR)	24.00	22.40	15.40	20.60
Beads (RRRR)	28.60	26.80	24.00	26.46
Mean	23.22	20.90	19.38	21.16

Fig 5. PCR amplification of ITS region of *Fusarium oxysporum*



FOD 1 to FOD 8 - Isolates of Fusarium oxysporum f.sp. dianthi

Booth (1971) described that *F. oxysporum* f.sp. *dianthi* was characterized by the presence of microconidia, macroconidia and chlamydospores. The microconidia were abundant, hyaline, aseptate or single septate and ovoid. Macroconidia were sparse, fusoid and variable, 3 septate, rarely 4 to 5 septate and measured 18.5-28.0 x 3.0-4.5 µm. Chlamydospores were hyaline vacuolated, spherical and measured 3.5 – 8.0 µm in diameter. Observation of FOD isolates under compound and scanning electron microscope confirmed with all the descriptions of FOD as reported by Booth (1971).

Environmental Scanning Electron Microscope (ESEM) images of F. oxysporum f.sp. dianthi isolate FOD1

The actively growing hyphae of *F. oxysporum* f.sp. *dianthi* along with macro, micro conidia and chlamydospores were observed under 7000x magnification. It revealed the presence of chains of ovoid microconidia. The fusoid macroconidia were seen in sparse. Besides, spherical intercalary chlamydospores were also observed under 1200x magnification.

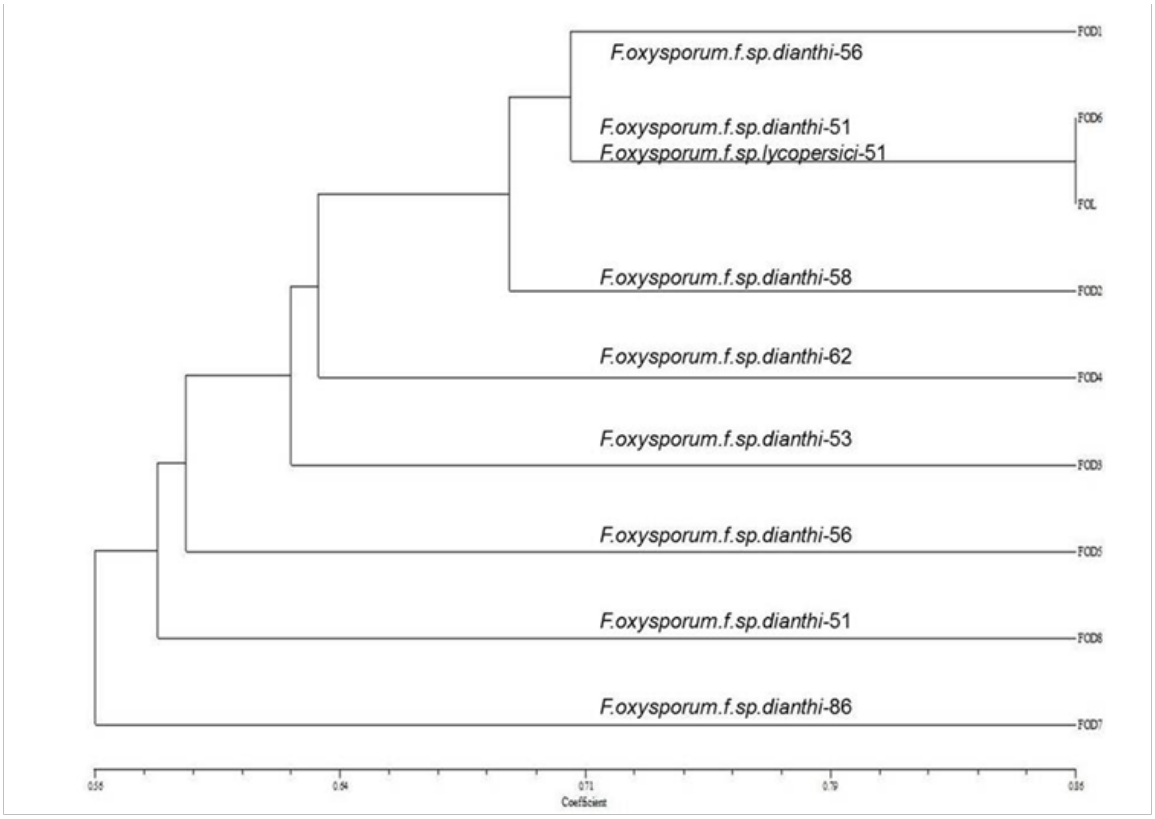
Pathogenicity

Inoculation of eight isolates of F. o. f.sp. dianthi

conidia (FOD1 to FOD8) into the healthy carnation rooted cuttings of Gaudina (Red) (30 days old) expressed the typical symptoms of vascular wilt after 30 days of inoculation and the Koch postulate was confirmed. Among the eight isolates of FOD tested in root cuttings of Gaudina, cent per cent wilt incidence was noticed with FOD1, FOD2, FOD6 and FOD8. Ninety per cent wilt incidence was noticed with FOD5 and FOD7. FOD3 and FOD4 isolates exhibited 80 per cent wilt incidence. However, FOL isolated from wilt infected tomato plants did not exhibit wilt incidence in carnation. Frequency of re-isolation from the wilt infected plants revealed that, 100 per cent re-isolation was observed in the Gaudina (Red) inoculated with FOD1, FOD2, FOD6 and FOD8. Similarly, pathogen was recovered to ant extent of 90 per cent in the plants inoculated with the isolates FOD5 and FOD7. However, only 70 per cent recovery of the pathogen was obtained in the plants inoculated with the isolates FOD3 and FOD4 (Fig 4).

Werner and Irzykowska (2007) reported considerable variation on wilt occurrence within

Fig. 6 Phylogenetic relationship of different FOD isolates of carnation



different groups of *F. oxysporum*. Similarly, among the eight isolates of FOD tested in rooted cuttings of Gaudina, considerable variations in the pathogenicity were also observed between the FOD isolates. Almost cent per cent wilt was noticed with the isolates FOD1, FOD2, FOD6 and FOD8, whereas FOD5 and FOD7 showed 90 per cent incidence; FOD3 and FOD4 isolates exhibited only 80 per cent wilt incidence.

Amplification and sequencing of Fusarium wilt pathogen genus-specific loci

PCR was carried out to specifically identify the *F. oxysporum* using gene-specific primers. The Internal Transcribed Spacer (ITS) primers amplified a DNA fragment of 389 bp corresponding to the region of 16S-23S rRNA intervening sequence for *F. oxysporum*. Eight isolates were examined for the amplification of 16S-23S rRNA region and all the isolates amplified the DNA product with the size of 389 bp. The partial sequences of F.o.f.sp. *dianthi* FOD1, FOD3 and FOD4 were submitted to the NCBI (accession number *viz*., JX036529, JX036531 and JX036532, respectively) (Fig 5).

Variability analysis of FOD

Eight isolates of F. o. f.sp. dianthi pathogenic isolates originating from Dianthus caryophyllus and a non-pathogenic F. oxysporum strain isolated from tomato were screened for genetic variation. Twelve polymorphic DNA PCR products were obtained with OPA-1, OPA-2 and OPA-4 primers. Fourteen polymorphic DNA PCR products were obtained with OPA-3 primer. 11, 9 and 10 polymorphic DNA PCR products were obtained with OPA-6, OPA-11 and OPA13 primers respectively. The RAPD PCR products size varied from 0.1 to 1 Kb. Comparative analysis of the PCR product profile for each primer was done on the basis of presence (1) and absence (0) of the same length, which are considered to be a single genetic locus. The diversity among the isolates using the RAPD markers was grouped by the UPGMA method. The genetic distance between the

isolates is represented in the form of a dendrogram and similarity matrix**.** Results of the cluster analysis using the RAPD polymorphic DNA products revealed high level of DNA polymorphism, which resulted in the possible occurrence of several distinct groups in relation to *F. o.* f.sp. *dianthi* isolates. Three main groups of isolates were resolved at 3.2% similarity level. Isolates in group I include FOD7, FOD8 and FOD5. Isolates in group2 include FOD3 and FOD4. Isolates in group3 include FOD1, FOD2 and FOD6 (Fig 6). Hence, all the analyzed isolates exhibited very high level of DNA polymorphism making a subdivision of main groups possible.

Table 3. Phenotypic characterization of the isolates of *Fusarium oxysporum* infecting carnation

Name of the isolates	Variety	Phenotypic characters of the isolates
FOD1	Dumas (Light pink)	The mycelium of the fungal culture on PDA medium was initially white and later turned light pink. Microconidia were abundant, hyaline, continuous, ovoid and measured 4.5-8.0 x 2.0-3.5 µm. Macroconidia were sparse and fusoid, 2-3 septate and measured 17.0-24.0 x 3.5-4.0 µm. Chlamydospores were hyaline, and spherical, measured 4.5 – 7.5 µm in diameter.
FOD2	Dona (Pink)	The mycelium of the fungal culture on PDA medium was initially white and later turned light pink. Microconidia were abundant, hyaline, continuous and ovoid measured 4.0-8.0 x 2.0-3.0µm. Macroconidia were sparse fusoid and measured 16.0-25.0 x 3.8-4.0 µm. Chlamydospores were hyaline, and spherical, measured 4.4 – 7.2 µm in diameter.
FOD3	Malaga (Orange)	The mycelium of the fungal culture on PDA medium was initially white and later turned to orange. Microconidia were abundant, hyaline, continuous and ovoid measured 4.7-8.5 x 2.0-3.5 µm. Macroconidia were sparse and fusoid 2-3 septate and measured 18.0-27.0 x 3.8-4.2 µm. Chlamydospores were hyaline, spherical, and measured 4.2 – 7.5 µm in diameter.
FOD4	Kiro (Yellow)	The mycelium of the fungal culture on PDA medium was white. Microconidia were abundant, hyaline, continuous, ovoid and measured 4.2-8.5 x 2.0-3.3µm. Macroconidia were sparse and fusoid, 2-3 septate and measured 19.0-25.0 x 3.8-4.2 µm. Chlamydospores were hyaline, spherical, and measured 4.3 – 7.4 µm in diameter.
FOD5	Baltico (White)	The mycelium of the fungal culture on PDA medium was white. Microconidia were sparse, hyaline, continuous ovoid and measured 4.5-8.0 x 2.0-3.5µm. Macroconidia were abundant fusoid, 2-3 septate and measured 17.0-24.0 x 3.5-4.0 µm. Chlamydospores were hyaline, spherical and measured 4.5 – 7.5 µm in diameter.
FOD6	Bizet (Pink)	The mycelium of the fungal culture on PDA medium was initially white and later turned to violet. Microconidia were abundant, hyaline, continuous, ovoid measured 4.5-8.0 x 2.0-3.5 µm. Macroconidia were sparse fusoid, 2-3 septate and measured 17.0-24.0 x 3.5-4.0 µm. Chlamydospores were hyaline, spherical, measured 4.5 – 7.5 µm in diameter
FOD7	Gaudina (Red)	The mycelium of the fungal culture on PDA medium was white and later turned to violet. Microconidia were abundant, hyaline, continuous and ovoid measured 4.5-8.0 x 2.0-3.5µm. Macroconidia was sparse, and fusoid, 2-3 septate and measured 19.0-29.0 x 3.8-4.0 µm. Chlamydospores were hyaline, and spherical, measured 4.3 – 7.0µm in diameter
FOD8	Domingo (Red)	The mycelium of the fungal culture on PDA medium was initially white and later turned to violet. Microconidia were abundant, hyaline, continuous ovoid and measured 4.6-8.5x 2.2-3.4 µm. Macroconidia was sparse, and fusoid, 2-3 septate and measured 21.0-27.0 x 3.0-4.0 µm. Chlamydospores were hyaline, spherical, and measured 4.8 – 7.3 µm in diameter

The random amplification procedure has shown efficiency in the evaluation of clusters within the FOD population as described previously (Alves-Santos et al. (2007); Jimenez-Gasco et al. (2001) and (Kerenyi et al., 1997). In the current study, variations observed in the FOD population suggested that changes in the commercial carnation cultivars used by growers would have directed the diversity of the pathogen population in the soil, favouring the more adapted groups of isolates.

References

Agrios GN, 1988. Plant Pathology, (3rd.) ed. Academic Press, Inc. New York. 803p.

Aneja, K.R. 1993. *Experiments in Microbiology, Plant Pathology and Tissue Culture.* Wishwa Prakasham, New Delhi. pp.11-195.

Baayen, R.P., Van Dreven, F., Krijger, M.C. and Waalwijk, C. 1997. Genetic diversity in *Fusarium oxysporum* f. sp. *dianthi* and *Fusarium redolens* f. sp. *dianthi*. *Eur. J. Plant Pathol.*,**103:** 395-408.

Ben-Yephet, Y., Reuven, M., Lampel, M., Nitzani, Y. and Mor, Y. 1992. *Fusarium oxysporum* f. sp. *dianthi* races in carnation. (Abstr.) *Phytoparasitica,* **20:** 225.

Booth, C. 1971. *The genus Fusarium*. Common wealth Mycological Institute, Kew Surrey, UK., pp.142-143.

Burgess, L.W., Summerell, B.A., Bullock, S., Gott, K.P. and Backhouse, D. 1994. Laboratory Manual for Fusarium Research. University of Sydney, 3rd Ed. 133 p.

Garibaldi, A. 1983. Resistenza di cultivar di garofanoneiconfronti di ottopatotipi di *Fusarium oxysporum*f. sp. *Dianthi* (Prill. *et* Del.) Snyd.*Et* Hans. (in Italian) *Riv. Ortoflorofrutti. Ital*., **67:** 261-270.

Gian Aggarwal 2011. Indian Greenhouse Industry. *Floriculture Today*, **16:** 30-31.

Jimenez-Gasco, M.M., Perez-Artes, E. and Jimenez-Diaz, R.M. 2001. Identification of pathogenic races 0, 1 B/C, 5 and 6 of *Fusarium oxysporum* f. sp. *ciceris* with random amplified polymorphic DNA (RAPD). European *J. Plant Pathol.,* **107:** 237–248.

Katoch, R. 1999. Studies on Fusarium wilt of Carnation. M.Sc. Thesis. *Univ. Hort. For., Nauni, Solan,* India. pp. 53.

Kerenyi, Z., Taborhegyi, E., Pomazi, A. and Hornok, L. 1997. Variability amongst strains of *Fusarium poae* assessed by vegetative compatibility and RAPD polymorphism. *Plant Pathol.,* **46:** 882–889.

Knapp, J. and Chandlee, J.M. 1996. Rapid, small-scale dual isolation of RNA and DNA from a single sample of orchid tissue. *Biotechniques*, **21:** 54 - 55.

Rohlf, F.J. 1993. NTSYS-pc: Numerical taxonomy and multivariate analysis system, v. 2.0. Exeter Software. Setauket, New York.

Werner, M. and Irzykowska, L. 2007. The pathogenicity and DNA polymorphism of *Fusarium oxysporum* originating from *Dianthus caryophyllus*, *Gypsophila* spp. and soil. *Phytopathol. Pol.,* **46:** 25 - 36.

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