Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2017; 6(5): 1055-1059
Received: 01-07-2017
Accepted: 02-08-2017

S Ashwathi
Department of Plant Pathology, Centre for Plant Protection Studies, Tamilnadu Agricultural University, Coimbatore, Tamil Nadu, India

C Ushamalini
Department of Plant Pathology, Centre for Plant Protection Studies, Tamilnadu Agricultural University, Coimbatore, Tamil Nadu, India

S Parthasarathy
Department of Plant Pathology, Centre for Plant Protection Studies, Tamilnadu Agricultural University, Coimbatore, Tamil Nadu, India

S Nakkeeran
Department of Plant Pathology, Centre for Plant Protection Studies, Tamilnadu Agricultural University, Coimbatore, Tamil Nadu, India

Correspondence
C Ushamalini
Department of Plant Pathology, Centre for Plant Protection Studies, Tamilnadu Agricultural University, Coimbatore, Tamil Nadu, India

Morphological and molecular characterization of *Fusarium* spp. associated with Vascular Wilt of Coriander in India

S Ashwathi, C Ushamalini, S Parthasarathy and S Nakkeeran

Abstract
Coriandrum sativum in Tamil Nadu were surveyed for the association of soil borne fungal pathogens and it was recovered from infected plants. Cultural and microscopic characterization of *Fusarium oxysporum* causing vascular wilt was observed. *Fusarium* spp. produced white, white with pinkish and white with brown tinch like mycelial growth on artificial medium. Micro conidia are scattered freely in the mycelial mat, they are small, oval shaped, hyaline and single or bicelled. The size of micro conidia ranges from 8.70 µm to 14.05 µm in length and 3.30 µm to 5.00 µm in width. Macroconidia were sickle shaped hyaline and multicelled with two to three septate. The size of macro conidia ranges from 16.90 µm to 31.60 µm in length and 3.89 µm to 5.53 µm in width. The number of macroconidia was more as compared to macro conidia in artificial culture. Also, abundant chlamydospores were observed terminally and intercalary. Further, PCR amplification of ITS gene region in the ten isolates of *F. oxysporum* was performed using universal ITS primers. *Fusarium* the genus was amplified as a fragment of 560 bp corresponding to the region of the 18S-28S rRNA intervening sequence for *Fusarium* spp. Then the selected virulent isolates of *Fusarium* spp. was sequenced and submitted in NCBI database with the accession numbers of KX265248 (Foc1), KX265249 (Foc2), KX265250 (Foc3), KX265251 (Foc4), KX265251 (Foc5). The soil-borne fungal pathogen of coriander was identified as *F. oxysporum* based on its cultural, morphological and molecular characteristics.

Keywords: Coriander; *Fusarium oxysporum*; Molecular; Morphology; Vascular Wilt

Introduction
India is the largest producer, consumer and exporter of seed spices in the world and widely known as the land of spices. According to the Spices Board of India, 52 spices are grown in the country. Share of spices in total agricultural exports of India is around 6 per cent. India's share in world spices trade is 45-50 per cent by volume and 25-30 per cent by value. After cumin, coriander is the second most important seed spice with respect to exports and getting foreign exchange earnings [20]. Coriander (*Coriandrum sativum* L.) is one of the important seed spices belonging to the family Apiaceae. It is the most widely used condiment throughout the world. It is mainly grown for its aromatic and fragrant seed which is botanically a cremocarpic fruit. The fresh green stems, leaves and fruits of coriander have a pleasant aromatic odour. Coriander bark oil has high germicidal activity and can be used as fungicide [11]. Pests and diseases are the major constraints in the production of coriander. Coriander cultivation is affected by several diseases like wilt caused by *Fusarium oxysporum* [19, 25], stem gall caused by *Protomyces macrospores* [8], grain mould diseases caused by *Helminthosporium* spp., *Fusarium* spp., *Curvularia* spp. and *Alternaria* spp. [23], powdery mildew (*Erisyphe polygoni*), rust and leaf spots. Among these the wilt of Coriander is a serious problem and affected plants grew poorly and were stunted. Root infection results in dropping of terminal shoots, followed by withering and drying of leaves. Partial infection shows yellow to pink foliage as disease progressed, plants eventually died [21]. The wilt disease causes up to 60 per cent yield loss in coriander [17]. The seed yield losses caused by *Fusarium* wilt ranges from 5 to 60 per cent in Rajasthan and 15 to 25 per cent in Gujarat [22]. Whereas, early reported an average seed yield loss of 20 per cent in Rajasthan due to *Fusarium* wilt with disease incidence of 70 to 80 per cent [18]. Not much attention has been paid on certain aspects of disease especially on ecology and variability in pathogen and effective management. During infection of host roots, the fungus crosses the cortex, enters the xylem tissues and then spreads rapidly upward direction through the vascular system, becoming systemic in the host tissues, and may directly infect the seed [24]. Mostly *Fusarium* fungus enters the vascular system of the infected plant via the roots [13]. It produces enzymes that degrade the cell walls so that gels are formed that block the transport system of plant. In India, *Fusarium oxysporum* is distributed in cosmopolitan in

nature and it is an anamorphic species that includes numerous plant pathogenic strains causing wilt diseases of a broad range of agricultural and horticultural host plant species [4, 2]. *Fusarium* can cause various types of diseases, including vascular wilts, head and seed blights, stem rots, root and crown rots and canker diseases, with some species capable of simultaneously causing multiple or overlapping disease syndromes depending on the host and the environment. *Fusarium oxysporum* Schlecht. causes vascular wilt diseases in a wide variety of economically important crops. Plant pathogenic forms of *F. oxysporum* are divided into *formae speciales* based on the hosts they attack [3]. Further subdivisions of *formae speciales* into races are often made based on their virulence to a set of differential host cultivars. Vascular wilt has been a major limiting factor in the production of many agricultural and horticultural crops, including banana (*Musa* spp.) (*F. oxysporum* f.sp. *cubense*), cabbage (*Brassica* spp.) (*F. oxysporum* f.sp. *conglutinans*), cotton (*Gossypium* spp.) (*F. oxysporum* f.sp. *vasinfectum*), flax (*Linum* spp.) (*F. oxysporum* f.sp *lini*), muskmelon (*Cucumis* spp.) (*F. oxysporum* f.sp. *melonis*), onion (*Allium* spp.) (*F. oxysporum* f.sp. *cepae*), pea (*Pisum* spp.) (*F. oxysporum* f.sp. *pisi*), tomato (*Lycopersicon* spp.) (*F. oxysporum* f.sp. *lycopersici*), watermelon (*Citrullus* spp.) (*F. oxysporum* f.sp. *niveum*), china aster (*Calistephus* spp.) (*F. oxysporum* f.sp. *callistephi*), carnation (*Dianthus spp.)* (*F. oxysporum* f.sp. *dianthi*), chrysanthemum (*Chrysanthemum* spp.) (*F. oxysporum* f.sp. *chrysanthemi*), gladioli (*Gladiolus* spp.) (*F. oxysporum* f.sp. *gladioli*) and tulip (*Tulipa* spp.) (*F. oxysporum* f.sp. *tulipae*) [3, 15]. The *formae speciales* of *Fusarium oxysporum* are normally hemibiotrophs, initially surviving on live hosts and eventually killing the infected cells [14]. Despite the significance of the coriander growing sphere in India, this area is partially threatened by soil-borne *Fusarium* spp. Thus, the importance of wilt on coriander in India dictates the need for investigation on the identification and characterization of *Fusarium* isolates to provide useful information about *Fusarium* isolates and suggest strategies to prevent and control pathogen.

Materials and Methods

Fusarium isolates and culture conditions

Damping-off infected samples were collected from different coriander growing districts of Tamil Nadu *viz*., Coimbatore, Mettupalayam, Tirupur and Dharmapuri. These infected samples were used for the isolation of pathogens. Infected collar portion and root bits were cut into small pieces and surface sterilized with 0.1% mercuric chloride for 30 seconds and subsequently three washings were given with sterile distilled water. Then, they were placed in sterilized Petri dishes containing Potato dextrose agar (PDA) medium by half plate technique and incubated at the laboratory conditions at 25 ± 2 ºC. The hyphal tips of fungi growing from these pieces were transferred aseptically to PDA slants for maintenance. The isolates of *Fusarium* spp. were grown on Potato dextrose agar medium to study their growth and variability in colony characters. From the three day-old culture plates, five mm disc of the fungus was cut by a sterilized cork borer and placed at the centre of each sterile Petridish (90-mm-dia) containing 15 ml of sterilized and solidified PDA. The plates were incubated at room temperature (28 ± 2 ºC) for 3 days. The mycelial growth, colony characters, and spore characters were recorded three days after inoculation (DAI).

Pathogenicity study

The fungus isolated was multiplied in sand maize medium. Sand and ground maize seeds were mixed at the ratio of 19:1 respectively. The mixture was moisture with water and sterilized at 121 ºC at 15 psi for 2 h. The fungus was inoculated into sand maize medium and incubated for 15 days at room temperature (28 º ± 2 ºC) for multiplication. Potting soil (Red soil: sand: cow dung manure @ 1:1:1 w/w/w) was sterilized in autoclave at 121 ºC at 15 psi for 2 h for consecutive days. The different fungi multiplied on sand maize medium were incorporated separately into the sterilized soil at the rate of 5% (w/w). Coriander seeds were sown at the rate of 10 seeds /pot and maintained under glass house conditions. Three replications were maintained for each isolate and monitored regularly.

Genomic DNA extraction

Genomic DNA was extracted from 23 pathogenic *Fusarium* isolates. The mycelium of each isolate was collected by harvesting the mat grown on the surface of inoculated PD broth. After grinding 100 mg of fungal mycelia from each isolate in liquid nitrogen, was incubated in 5 ml, 2 % CTAB extraction buffer [10 mM trisbase (pH 8.0), 20 mM EDTA (pH 8.0), 1.4 M NaCl, CTAB (2 %), mercaptoethanol (0.1%) and PVP (0.2%)] at 65 ºC for 1 h. The suspension was added with equal volume of phenol-chloroform-isoamylalcohol (25:24:1) mixture. It was vortexed to mix two phases, followed by a centrifuge at 12,000 rpm for 5 min. The supernatant was transferred to clean tube and mixed with equal volume of ice cold isopropanol. It was incubated at 25 ºC for DNA precipitation. The precipitate was collected by centrifugation and the pellet was wash with 0.1 M ammonium acetate in 70% ethanol. Again incubation was given for 15 min. The pellet was resuspended in TE buffer (10mM Tris, 1mM EDTA, pH 8.0) and the DNA concentration and purity was determined using a NanoDrop ND1000 spectrophotometer (NanoDrop Technologies Inc). Aliquots of samples were also analyzed on a 1% agarose gel to check DNA quality.

PCR amplification and sequencing

The universal primers Pa 1 (Forward) and ITS 2 (Reverse) were used to amplify the ITS regions of *Fusarium* spp. (Abd El-Salam *et al*., 2003) [1].

ITS 1 - 5'-TCCGTAGGTGAACCTGCGC-3'

ITS 4 - 5'-TCCTCCGCTTATTGATATGC-3'

PCR reaction mixture consisted of 10µl *viz*., 5 µl of PCR master mix, 1µl of forward primer and 1 µl reverse primer, template DNA 2 µl and 1 µl of sterile water. The amplification was carried out in Thermocycler. PCR reaction was performed with initial denaturation step at 94 ºC for 5 min, 35 cycles of amplification denaturation 94 ºC for 1 min, annealing ration step at 55 ºC for 30 sec annealing and 72 ºC for 30 sec extension and final extension was carried out at 72 ºC for 10 min in PCR Palm Cycler (Corbett Research, Australia). The amplified PCR products were run on 1.5% agarose gel in Tris-acetate buffer. The gel was stained with ethidium bromide, visualized on an UV-transilluminator and photographed in the gel documentation unit (Alpha Innotech Corp, USA). The sizes of the PCR products were determined by comparison with standard 100 bp or 1 kb molecular marker.

Statistical analysis

The data obtained were statistically analyzed[10] and the treatment means were compared by Duncan' package used for analysis was IRRISTAT version 92 developed by the International Rice Research Institute, Biometrics Unit, The Philippines.

Table 1: Morphological and cultural characters of *Fusarium oxysporum* isolates

S. No.	Districts	Place of Collection	Colour of Culture in Petri plate	Micro conidia		Macro conidia		Chylamydospore	
				Length (µm)	Width (µm)	Length (µm)	Width (µm)	Length (µm)	Width (µm)
1.	Coimbatore	Mettupalayam	White	11.30 bcd (19.62)	4.60ab (12.37)	29.40ab (32.81)	5.30ab (13.29)	4.60b (12.37)	3.80cd (11.23)
2.		Idikarai	White with brown tinch	10.40cde (18.79)	3.76cde (11.17)	21.30e (27.46)	4.70a-d (12.51)	5.00b (12.91)	4.40bc (12.09)
3.		Gomangalam	White	14.05a (22.00)	5.00a (12.91)	31.60a (34.18)	5.53a (13.59)	5.30b (13.29)	6.50a (14.75)
4.		Kanjampatty	White with pinkish tinch	9.80cde (18.22)	3.50de (10.77)	23.06cde (28.68)	3.23g (10.34)	5.00b (12.91)	4.30bc (11.95)
5.		TNAU Orchard	White with yellow tinch	8.56e (16.99)	4.12bcd (11.70)	25.63b-e (30.39)	3.89d-g (11.36)	5.00b (12.91)	4.50bc (12.23)
6.	Tirupur	Pethapampatty	White	9.30de (17.74)	4.20bcd (11.81)	26.40bcd (30.89)	4.50b-e (12.23)	4.60b (12.37)	3.50d (10.77)
7.		Palladam	White with brown tinch	8.70e (17.14)	3.30ef (10.45)	23.20cde (28.77)	4.80abc (12.64)	6.60a (14.87)	5.90a (14.04)
8.	Dharmapuri	Thenkaraikottai	White	11.40bc (19.71)	2.80f (9.62)	23.73cde (29.13)	4.20c-f (11.81)	4.40b (12.09)	5.00b (12.91)
9.		Menasi	White	11.06bcd (19.40)	3.80cde (11.23)	27.90abc (31.86)	4.60b-e (12.37)	4.5b (12.23)	4.80b (12.64)
10.		Ramiyanahalli	White	11.20bcd (19.53)	3.40ef (10.61)	16.90f (24.25)	4.80abc (12.64)	6.20a (14.40)	6.00a (14.16)

*Values are mean of three replications

Values in parentheses are arcsine transformed values

Means followed by a common letter are not significantly different at the 5% level by DMRT.

Result and Discussion

Occurrence of coriander wilt has been reported from few parts of India *viz*., Gwalior and Guna districts of Madhya Pradesh, Kota division of Rajasthan and Coimbatore district of Tamil Nadu [21]. In this study, ten isolates of *Fusarium* spp. were isolated from infected coriander samples using Potato Dextrose Agar (PDA) medium by half plate technique. The pure culture was maintained in PDA slants in a refrigerator at 4 ºC for further studies (Plate 1). Pathogenicity test was conducted for *Fusarium* spp. as per Koch's postulates. Isolated *Fusarium* spp. from infected plants were multiplied in sand maize medium and used as inoculums source for pathogenicity assay. The pathogen inoculated plants showed reddish brown lesions at collar portion, girdling and toppling down of the seedlings on 15th day (Plate 2). The pathogens were re-isolated from artificially infected coriander plants. The re-isolated pathogens were similar to the original culture and pathogenicity was proved. In 1967, scientists in India first described the symptoms of wilt disease caused by *F. oxysporum* f. sp. *corianderii* in coriander [5]. A species of *Fusarium* has been found to cause localized lesions on coriander at soil level or at various heights above the soil[26]. Multiple symptoms observed at various stages of plant growth and were recognized as pre-emergence, post emergence and adult stage symptoms [16]. Early, noticed wilt disease of coriander in all the stages of plant growth and drooping of the leaves were the characteristic symptoms. In advanced stages of infection, the leaves were found to dry up and the plants could be easily pulled out[21]. Brown discolouration of roots was also seen conspicuously. Severity and incidence of diseases caused by each *Fusarium* species vary according to the geographical location, climatic factors and cultural practices [7]. The colony colour of *Fusarium* isolates varied from white, white with pinkish and white with brown tinch. The margin of the colony was smooth to irregular. The mycelial topography was flat to fluffy. The colony diameter varied from 7.70 cm to 8.56 cm. *Fusarium* produced two types of conidia *viz*., micro and macro conidia. Micro conidia were small, oval shaped, hyaline and single or bicelled. The size of micro conidia ranges from 8.70 µm to 14.05 µm in length and 3.30 µm to 5.00 µm in width. Macroconidia were sickle shaped hyaline and multicelled with two to three septate. The size of macro conidia ranges from 16.90 µmto 31.60 µm in length and 3.89 µm to 5.53 µm in width. The number of macroconidia was more as compared to macro conidia. Abundant chlamydospores were observed terminally and intercalary. Based on the morphological characters it is identified as *Fusarium oxysporum* (Plate 3; Table 1). The present results confirm the reports of earlier workers doing their morphological studies related to suitable media for growth and sporulation of *Fusarium* spp. [12, 6]. The cultural and morphological characters of *Fusarium* spp was described in detail [18]. Microconidia produced by the fungus are scattered freely in the mycelial mat, they are hyaline, unicellular, ovoid, ellipsoid or reniform. Macroconidia are formed in sporodochia, they are borne on simple conidiophores. Conidia are stout, fusiform, falcate, gradually tapering at both ends, pedicellate with rostrate apex and delicate walls and septation. Chlamydospores are terminal or intecalary, spherical or globose, smooth, one celled, occasionally in chains, dark in colour and double walled. Based on the structure in or on which conidiogenous hyphae are borne, *Fusarium* spp. are classified under the Hyphomycetidae subclass of the Deuteromycetes. PCR amplification of ITS region in the ten isolates of *Fusarium* spp. was performed using the universal primers. *Fusarium* the genus was amplified as a fragment of 560 bp corresponding to the region of the 18S-28S rRNA intervening sequence for *Fusarium* spp. (Plate 4). Then the selected virulent isolates of *Fusarium* spp. was sequenced and submitted in NCBI database. It showed 100 % homology with *F. oxysporum* with the accession numbers of KX265248 (Foc1), KX265249 (Foc2), KX265250 (Foc3), KX265251 (Foc4), KX265251 (Foc5). Hence, the pathogen was identified as *Fusarium.* Sequencing the ITS gene region is effective for identifying some species of *Fusarium* [27]. Phylogenetic relationships exist among fungal isolates of *Fusarium oxysporum* f.sp. *cumini* was early reported in India [9], collected from different

regions of Rajasthan correlates with variation exist at pathogenicity. Two taxon-selective primers, ITS-Fu-f and ITS-Fu-r were designed by comparing the aligned sequences of internal transcribed spacer regions (ITS) of a range of *Fusarium* species [1]. The primers showed good specificity for the genus *Fusarium*, and approximately 389-bp product was amplified. Also reported that the ITS primers 1 and 4 have been used to amplify the genus-specific PCR assay for the rapid identification in different isolates of *Fusarium*. The soil-borne fungal pathogen of coriander was identified as *F. oxysporum* based on its cultural, morphological and molecular characteristics. Further study on epidemiology and development of suitable disease management practices of wilt of coriander is crucial in sense. Therefore, precautionary action is needed to prevent any future outbreaks of this disease.

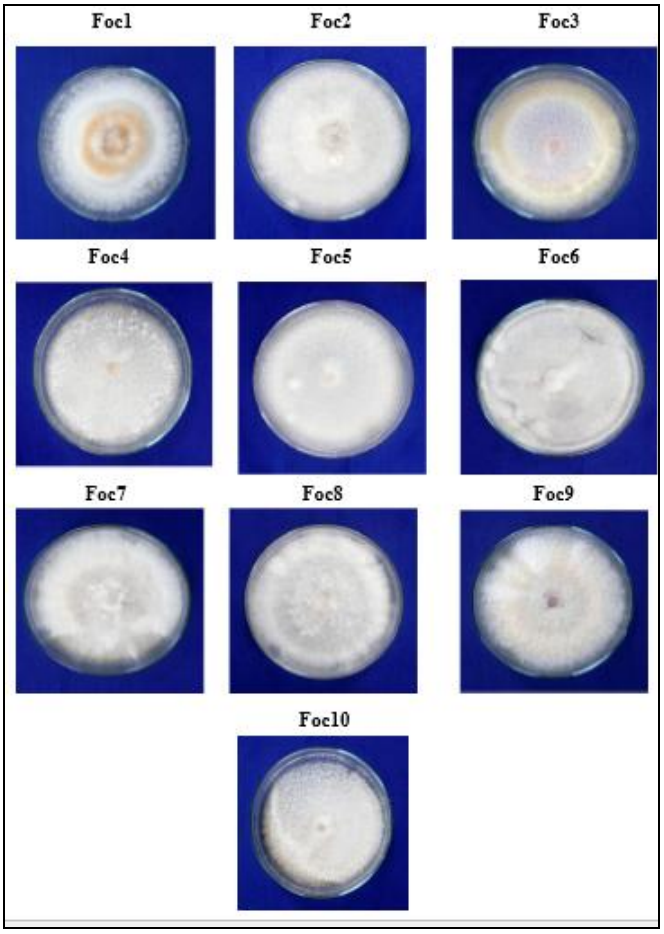

Plate 1: Isolates of *Fusarium oxysporum*

Plate 2: Pathogenicity test for *Fusarium oxysporum*

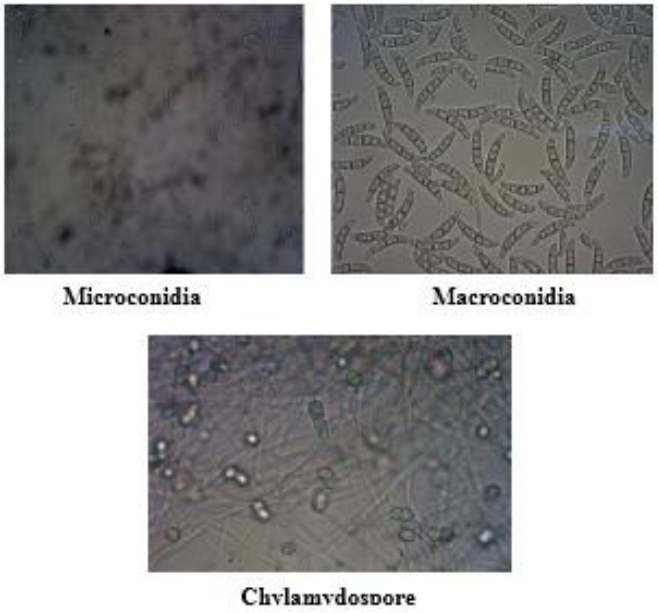

Plate 3: Morphological characters of *Fusarium oxysporum*

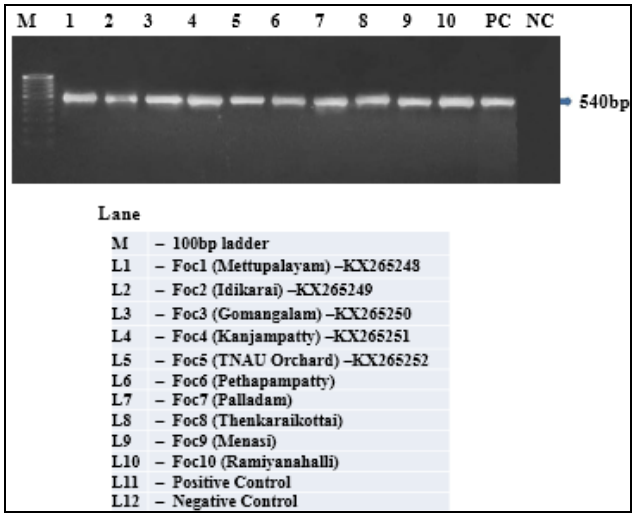

Plate 4: Molecular characterization of *Fusarium oxysporum*

Table 2: Pathogenicity of *Fusarium* sp. for wilt incidence

Isolates	Source	Infected plants (%)*
Foc1	Mettupalayam	43.51ef (41.26)
Foc2	Idikarai	54.34de (47.49)
Foc3	Gomangalam	85.01a (67.82)
Foc4	Kanjampatty	68.20bc (55.75)
Foc5	TNAU orchard	35.71f (36.68)
Foc6	Pethapampatty	80.04a (63.76)
Foc7	Palladam	62.23cd (52.11)
Foc8	Thenkaraikottai	75.21ab (60.31)
Foc9	Menasi	48.90e (44.36)
Foc10	Ramiyanahalli	62.52cd (52.29)

*Values are mean of three replications.
Values in parentheses are arcsine transformed values.
Means followed by a common letter are not significantly different at the 5% level by DMRT.

References

1. Abd El-Salam KA, Schneieder F, Khalil MS, Aly AA, Verreet JA. Genetic variation at the intra- and inter specific level in *Fusarium* spp. associate with Egyptian cottons. Journal of Plant Disease and Protection. 2003; 110(1):46-53.
2. Appel DJ, Gordon TR. Relationships among pathogenic and non-pathogenic isolates of *Fusarium oxysporum*

based on the partial sequence of the intergenic spacer region of the ribosomal DNA. Molecular Plant-Microbe Interactions. 1996; 9:125-138.
3. Armstrong GM, Armstrong JK. Formae speciales and races of *Fusarium oxysporum* causing wilt diseases. In *Fusarium:* Diseases, Biology and Taxonomy (P.E. Nelson, T.A. Toussoun & R.J. Cook, eds), The Pennysylvania State University Press, University Park, 1981, 391-399.
4. Booth C. The genus *Fusarium*. Kew, Surrey, UK. Common wealth Mycological Institute, 1971, 237.
5. Chattopadhyay SB, Satyabratamaiti. Diseases of betelvine and spices. Indian Council of Agricultural Research, New Delhi, 1967, 160.
6. Chittem K, Kulkarni S. Effect of Media on the Growth of *Fusarium oxysporum* f. sp. *gerberae* and *Fusarium oxysporum* f. sp. *dianthi.* Karnataka Journal of Agricultural Sciences. 2008; 21(2):303-304.
7. Daami-Remadi M. Etude des fusarioses de la pomme de terre. Thèse, Institut Supérieur 424 Agronomique de Chott- Mariem, Tunisie, 2006, 238.
8. Das AM. Studies on stem gall disease of coriander incited by *Protomyces macrosporus*. Abstracts of papers presented at the Second International Symposium on Plant Pathology (IARI) New Delhi, 1971, 80-81.
9. Deshwal RK, Kumari N. Regional variation in genetic structure and pathogenecity of *Fusarium oxysporum* f.sp. *cumini* Isolated from *Cuminum cyminum* L. Asian Journal of Biological Sciences. 2012; 5:30-38.
10. Gomez KA, Gomez AA. Statistical Procedure for Agricultural Research. John Wiley and Sons, New York, 1984.
11. Krishna DA. Spice: Some known and unknown facts. Science and Culture. 1999; 65:220-228.
12. Kulkarni SP. Studies on *Fusarium oxysporum* Schlecht Fr f. sp. *gladioli* (Massey) Snyd. & Hans. causing wilt of *Gladiolus.* M.Sc. (Agric.) thesis, University of Agricultural Sciences, Dharwad, India, 2006, 245.
13. Leslie JF, Summerell BA. The *Fusarium* Laboratory manual. (Blackwell Publishing: Iowa, USA), 2006.
14. Ma LJ, Geiser D, Proctor R, Rooney A, O Donnell K, Trail F *et al*. Fusarium pathogenomics. Annual Review of Microbiology. 2013; 67:399-416.
15. MacHardy WE, Beckman CH. Vascular wilt Fusaria: Infections and Pathogenesis. In *Fusarium:* Diseases, Biology and Taxonomy (P.E. Nelson, T.A. Toussoun & R.J. Cook, eds), The Pennysylvania State University Press, University Park, 1981.
16. Mall OP. Studies on wilt disease of coriander with special reference to variability of the pathogen. Indian Phytopathology. 1968; 21:379-388.
17. Manoranjitham SK, Rabindram R, Doraiswamy S. Management of seed-borne pathogens and wilt disease of coriander. Madras Agricultural Journal. 2003; 90:4-6.
18. Mathur BN, Prasad N. Studies on wilt disease of cumin caused by *Fusarium oxysporum* f.sp. c*umini*. Indian Journal of Agricultural Sciences. 1964; 34(2):131-137.
19. Narula SC, Joshi PS. *Fusarium oxysporum Sch*. f.sp. *coriandrii.* Science and Culture. 1963; 29:206.
20. Peter KV, Shylaja MR. Introduction to herbs and spices: definitions, trade and applications. In: The Handbook of herbs and spices 2nd edition, Woodhead Publishing. 2012; 1:1-24.
21. Prakasam V, Vedamuthu PGB, Khader MA, Jeyarajan R. Screening coriander lines for wilt resistance. South Indian Horticulture. 1987; 35:258-259.
22. Prasad N, Patel PN. *Fusarium* wilt of cumin in Gujarat state, Indian Plant Disease Reporter. 1963; 47(6):528-531.
23. Rajan FS, Vedamuthu PGB, Khader MK, Jeyarajan R. Screening coriander lines against grain mould disease. South Indian Horticulture. 1990; 38:168-169.
24. Singh SK, Singh B, Singh VB, Reena. Morphological, cultural and pathogenic variability among the isolates of *Fusarium oxysporum* f. sp. ciceri causing wilt of chick pea. Annals of Plant Protection Science. 2011; 19(1):155-158.
25. Srivastava US. Effect of interaction of factors on wilt of coriander caused by *Fusarium oxysporum* f.sp. corianderii, Kulkarni, Nikam & Joshi. Indian Journal of Agricultural Science. 1972; 42:618-620.
26. Stevenson EC. Diseases of drug and related plants at the plant industry station, Beltsville, Maryland. Plant Disease Reporter. 1943; 27:700-703.
27. Waalwijk C, Koning JRA, Baayen RP. Discordant groupings of *Fusarium* spp. from sections *Elegans*, *Liseola* and *Dlaminia* based on ribosomal ITS1 and ITS2 sequences. Mycologia. 1996; 88:361-368.