Microbiology Research Journal International

23(4): 1-16, 2018; Article no.MRJI.40746
ISSN: 2456-7043
(Past name: British Microbiology Research Journal, Past ISSN: 2231-0886, NLM ID: 101608140)

Prevalence of *Fusarium* Species Associated with Peach Decline in Tunisian Nurseries

Sabrine Mannai[1], Najwa Benfradj[1], Najet Horrigue-Raouani[1] and Naima Boughalleb-M'Hamdi[1*]

[1]Department of Biological Sciences and Plant Protection, High Institute of Agronomy of Chott Mariem, University of Sousse, 4042 Sousse, UR13AGR03, Tunisia.

Authors' contributions

This work was carried out in collaboration between all authors. Author SM designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors NB and NHR managed the analyses of the study. Author NBMH managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/MRJI/2018/40746
Editor(s):
(1) Giuseppe Blaiotta, Professor, Department of Agriculture, Division of "Grape and Wine Sciences", University of Naples Federico II, Via Universita' 100 – Palazzo Mascabruno 80055 Portici, Italy.
Reviewers:
(1) R. Mahalakshmi, India.
(2) Hassani Abdelkrim, Ibn Khaldoun University, Algeria.
Complete Peer review History: http://www.sciencedomain.org/review-history/24245

Original Research Article

Received 20th January 2018
Accepted 6th April 2018
Published 20th April 2018

ABSTRACT

Aims: To survey nurseries and diagnosis of the young peach seedlings decline, to investigate the *Fusarium* species associated with the decline of peach in Tunisian nurseries using morphological and molecular tools and determine the pathogenicity of the most predominant species on peach seedlings.
Place and Duration of Study: Tunisian peach nurseries and Department of Biological Sciences and Plant Protection, Higher Institute of Agronomy of Chott Mariem, 4042, Sousse, Tunisia, between November 2012 and July 2014.
Methodology: The surveys were conducted in peach nurseries. Five root samples were taken from each vigor of each peach variety. The isolation and the morphological identification were done on PDA medium. The molecular identification was done using ITS1 and ITS4. Pathogenicity tests were made for the dominant species which are *F. oxysporum* (25 isolates) and *F. solani* (8 isolates).
Results: The isolation was done on PDA medium and morphological and molecular identification (using ITS1 and ITS4) revealed the presence of 62 isolates of *Fusarium oxysporum*, 32 isolates of

**Corresponding author: E-mail: n.boughalleb@laposte.net;*

F. solani, 7 isolates of *F. equiseti*, 4 isolates of *F. proliferatum* and 2 isolates of *F. chlamydosporum*. *F. oxysporum* and *F. solani* were isolated from roots from all surveyed nurseries. *F. equiseti* were isolated from roots from nurseries in Chebika, Zaghouan and Monastir. *F. proliferatum* was recovered from roots from nurseries in the Chebika and Zaghouan regions. *F. chlamydosporum* were recovered only from roots in nurseries at Monastir region. Pathogenicity of *Fusarium oxysporum* and *Fusarium solani* was evaluated by using two varieties of peach, Carnival and Royal Glory grafted onto the Garnem rootstock (*Prunus dulcis* x hybrid clonal of *Prunus persica*) planted in inoculated soil. Symptoms of peach decline namely browning of the apical vegetative part, height reduction and collar rot. These two *Fusarium* species were more virulent on Carnival than Royal Glory. *Fusarium solani* induced root and collar rot symptoms whereas *F. oxysporum* induced necrotic roots symptom, browning and height reduction.

Conclusion: This finding showed that *Fusarium oxysporum* and *F. solani* were the most dominant species. They were virulent to peach seedlings.

Keywords: Fusarium spp.*; nursery; pathogenicity; peach; PCR; surveys.*

1. INTRODUCTION

Peach decline, responsible of seedlings root and collar rot in nurseries, is one of the most destructive diseases causing a dramatic reduction in plant growth [1,2,3]. The symptoms of peach decline included roots browning, and shoot stunting [4,5]. The internal necrosis of the plants was not readily evident in many trees, especially those with a dark brown bark [6]. The difficulty in the early identification of the infected seedlings led to the disease transfer to the orchards, resulting in further disease development and death of newly planted trees in the field [6]. The causes of this disease may differ among regions and various abiotic and biotic factors have been attributed to the appearance of peach decline symptoms [7]. Previous studies reported the primary role of many soil-borne fungi called “root rot fungal complex” in this disease worldwide [7,8,9,10].

The causal agents of peach decline can survive in the rhizosphere and in the seedlings roots within 1–2 years after the plantation of orchard’s tree. Thus, it can later cause trees decline [11].

This soil-borne disease was reported in fruit trees-growing areas worldwide such as Europe [5,9], North America [12,13], Australia [14] and South-Africa [15]. Several investigations showed that *Fusarium* spp., such as *F. equiseti*, *F. moniliforme*, *F. oxysporum* and *F. solani* were frequently isolated from peach orchards showing replant symptoms in Canada [16] and in United States [17,18]. However, *Fusarium solani* and *F. oxysporum* were the most predominant species isolated from stem and root lesions associated with decline of young peach seedlings [19].

The aims of this study were to (i) survey nurseries and diagnosis of the young peach seedlings decline, (ii) investigate the *Fusarium* species associated with the decline of peach in Tunisian nurseries using morphological and molecular tools and (iii) determine the pathogenicity of the most predominant species (*Fusarium oxysporum* and *Fusarium solani*) on peach seedlings.

2. MATERIALS AND METHODS

2.1 Disease Survey and Samples Collecting

Six nurseries of peach, located in 3 different areas of peach production in Tunisia, Chebika1, Chebika2 and Chebika3 (Kairouan), Manzelnour and Ouardanin from Monastir government and Zaghouan (Zaghouan) were surveyed from October 2012 to December 2013 (Fig.1). From each nursery, five samples of roots per each vigor/variety of peach seedlings, aged from 9 to 18 months, grafted onto the Garnem rootstock or Bitter almond were randomly sampled regardless of the symptoms. The vigor index (IV) has been divided into four levels according to the height of each seedling scion (x): IV1 ($x \leq 25$ cm), IV2 (25 cm $< x \leq 50$ cm), IV3 (50 cm $< x \leq 100$ cm) and IV4 ($x > 100$ cm).

2.2 Pathogen Isolation from Infected Peach Seedlings

A total of 85 samples of peach seedlings were collected from the nurseries according to the vigor. Samples of roots were washed under tap water to remove adhering soil and cut aseptically into small pieces of 3 to 5 mm in length, followed

Fig. 1. Surveyed peach nurseries location in Tunisia (☆: indicates the peach nurseries location)

by dipping in a solution of sodium hypochlorite (3%) for 3 to 4 min. Then, these pieces were rinsed in sterile distilled water and air dried in a laminar flow hood. When completely dried, samples were plated onto PDA medium (Potato-Dextrose-Agar) amended with 100 µg ml^{-1} of streptomycin. The plates were then incubated in the dark at 25°C, and checked daily for colony growth. Colonies that developed from the root segments were then transferred to PDA plates and purified by single-spore method using Water Agar (2%) medium. The pure isolates were preserved in1ml of distilled sterile water with glycerol (20%) in 1.5 ml tubes and stored at -20°C.

2.3 Morphological Identification of Isolates

The identification of the collected isolates was performed after 7 days of incubation of each colony on PDA medium at 25°C, based on morphological criteria as described by Leslie and Summerell [20].

2.4 DNA Extraction and Polymerase Chain Reaction

Two isolates of *F. oxysporum*, two of *F. solani* and 9 isolates of *Fusarium* spp. have been used for the molecular characterization.

The extraction of genomic DNA of each isolate has been made according to the protocol of Möller et al. [21] with some modifications. Thus, 0.1 g of 6-days-old mycelia of each isolate grown on PDA medium was ground grinding in liquid nitrogen. Then, powdered mycelium was put into a microtube (1.5 ml) containing 500 µl of TES (100 mM Tris, pH 8.0, 10 mM EDTA, 2% SDS), 140 µl of NaCl (1.4 M) and 65 µl of CTAB (10%). After incubation for 60 min at 60°C with occasional gentle mixing, 700 µl of chloroform has been added to the microtube, mixed gently and incubated for 30 min on ice, then centrifuged for 10 min at 13000 rpm. Supernatant obtained were transferred to another 1.5 ml tube containing 225 µl of ammonium acetate (5M) and mix gently; then placed on ice for 30 min. The microtube was centrifuged for 5 min at 13000 rpm. Obtained supernatant was transferred to a fresh microtube containing 510 µl of cold isopropanol and incubated for 20 min at -20°C. Microtubes were then centrifuged immediately for 10 min at 13000 rpm. Finally, the supernatant were aspirated off and pellet obtained were washed with cold ethanol (70%). After drying each pellet was dissolve in about 100 µl TE.

The ITS region was amplified with universal primers ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') [22]. PCR was performed in a 50 µl of volume reaction containing 2 µl of DNA (50 ng/µl), 0.5 µl

of Taq polymerase (5U/µl), 3 µl of $MgCl_2$ (1.25 mM), with 5 µl of PCR buffer (10x), 5 µl of dNTP (1.25 mM), 5µl of each of 5 µM forward (ITS1) and reverse (ITS4) primers and 24.5 µl of sterile distilled water. Cycling conditions of PCR were started by a denaturation at 95°C for 3 min, followed by 35 cycles at 95°C for 30 s, then at 57°C for 30 s, and at 72°C for 1 min, with a final elongation at 72°C for 1 min. The PCR product was analyzed by electrophoresis in a 1% agarose gel.

PCR products were purified and sequenced at Biotools society (Monastir, Tunisia). The identity of isolates has been realized using BLAST (Basic Local Alignment Search Tool) analysis. The rDNA-ITS sequence of isolates were compared with known sequences of *Fusarium* spp. obtained from the GenBank (http://www.ncbi.nlm.nih.gov) (Table 1). Then, sequences have been deposited in the GenBank.

Then, the frequency of recovery of each species was evaluated in each vigor index group found in each nursery.

2.5 Pathogenicity Tests

Pathogenicity tests were made for the two dominant species *F. oxysporum* (25 isolates) and *F. solani* (8 isolates). Isolates were selected from different nurseries randomly regardless of the vigor. These pathogenicity tests were conducted using seedlings of two varieties of peach carnival and royal glory grafted onto the rootstock Garnem (*Prunus dulcis* x hybride clonal of *Prunus persica*) (18-months-old). These seedlings were grown in a glass house, in plastic pots (23 cm diameter x 23 cm deep) containing a potting mix (50% sterilized soil, 25% sterilized peat and 25% sand), at a temperature ranging from 20°C to 25°C and a relative humidity between 60% and 70%.

To prepare the inoculum, bottles containing 200 g of sterile wheat seeds has been inoculated with 10 mycelial discs (8 mm diam.) of each *Fusarium* isolate grown on PDA medium for two weeks [23]. As control, wheat seeds were inoculated with discs of PDA medium. Then, these bottles were incubated in darkness at 25°C, for 15 days and shaken every two days to ensure seeds colonization. In June 23, 2015, the inoculation of peach seedling-soil was made by adding the prepared mixt to the soil at a rate of 1% (v/v). Then the soils has been incubated in plastic bags for 24 h at 20°C–25°C in darkness prior to planting as mentioned by Tewoldemedhin et al. [15]. The experiment was conducted as a complete randomized block design, and each isolate was tested using three peach seedlings. The symptoms of decline appear gradually by the desiccation and browning of the apical part first and down to reach the whole plant.

Table 1. Species selected from GenBank included in this study showing accession numbers of the isolates

Species	Strain number	Origin	Host
Fusarium equiseti	JF773646	Mexico	*Taxus globosa*
	FJ441009	-	Mushroom
	KC427030		Soil
	JQ690085	China	Melon
	KJ677236	Mexico	*Jatropha curcas*
	HQ339990	India	-
	KT211524	-	Cassava
Fusarium solani	KM235740	China	*Solanum lycopersicum*
	KY617066	South Africa	*Pelargonium Sidoides*
Fusarium oxysporum	KY810792	Brazil	Black-wattle minicutting
	KC282839	Tunisia	Clementine/Sour orange
Fusarium chlamydosporum	KM076600	-	*Trianthema portulacastrum*
	EU520242	China	-
Fusarium proliferatum	KF986684	India	Ginger rhizosphere soil
	FJ040179	-	*Oryza sativa*
	MF687307	China	-
	MF471668	China	*Clivia miniata*

Symptoms of peach decline were evaluated after six months of the inoculation of peach seedlings by *Fusarium* species. For the evaluation of disease severity, peach seedlings were removed in December, 23, 2015, from the potting bags and washed under running water to remove excess potting mix adhering to roots. Then, for each seedling the height, root weight and root rot were noted.

Root rot was rated onto a 0–5 scale (0=no obvious symptoms; 1=moderate discoloration of root tissue; 2=moderate discoloration of tissue with some lesion; 3=extensive discoloration of tissue; 4= extensive discoloration of tissue with girdling lesions; and 5= dead plant) [15]. Re-isolation was made from all discolored fibrous roots of seedlings to confirm the pathogenicity of the tested isolates.

2.6 Statistical Analyses

Data were subjected to a one-way analysis of variance (ANOVA) using Statistical Package for the Social Sciences software (SPSS), version 20.0. Means of the values were separated using the Student–Newman–Keuls (S-N-K) test to identify significant differences at $P \leq 0.05$.

3. RESULTS

3.1 Surveys of Nurseries and Diagnosis of Young Peach Tree Decline

The results of the surveys highlighted the presence of peach decline. Infected peach seedlings showed symptoms of drying and browning of the apical part of the scion and/ or browning at the collar which will eventually result in a complete decline and death of the plant. The uprooting showed root browning which was observed in all nurseries and on the two rootstocks, Garnem (*Prunus dulcis* x hybride clonal of *Prunus persica*) and bitter almond (*Prunus dulcis*) (Fig. 2). There is no relation between the symptoms and the vigor index.

The rootstock Garnem was found in Ouardanin, Zaghouan, Chebika1, Chebika2 and Chebika3 nurseries. However, the rootstock bitter almond was found in Menzel nour, Zaghouan and Chebika3 nurseries (Table 2).

3.2 Isolation, Morphological and Molecular Identification of the Collected Isolates

On the basis of morphological identification the following numbers of cultures were isolated during the surveys: *Fusarium oxysporum* 62 (Fig. 3), *Fusarium solani* 32 (Fig. 4) and 9 isolates of *Fusarium* spp. (Table 3).

Fusarium oxysporum was the dominant species isolated from rootstocks from all nurseries, followed by *Fusarium solani*. The highest percent of the isolation of *F. oxysporum* (100%) was obtained from roots of the rootstock Garnem found in Chebika1 nursery while the highest percent of the isolation of *F. solani* (60%) was found in roots of the rootstock Bitter almond localized in Zaghouan region. The percent of other species were low or null in all nurseries (Table 4).

Fig. 2. Symptoms of drying and browning of a) the apical part of Royal Glory scion, b) browning of collar of the peach rootstock Garnem, c) roots and d) total decline of Royal glory scion/ Garnem rootstock

Table 2. Characteristics of rootstocks collected from surveyed nurseries

Nurseries	Location	Rootstocks	Samples number	Age (Months)	Years
Manzelnour	Monastir	Bitter almond	15	18	2013
Ouardanin	Monastir	Garnem	5	17	2013
Zaghouan	Zaghouan	Bitter almond	5	9	2012
		Garnem	5	10	
Chebika2	Kairouan	Garnem	20	18	2012
Chebika1	Kairouan	Garnem	15	10	2013
Chebika3	Kairouan	Bitter almond	5	10	2012
		Garnem	15	12	

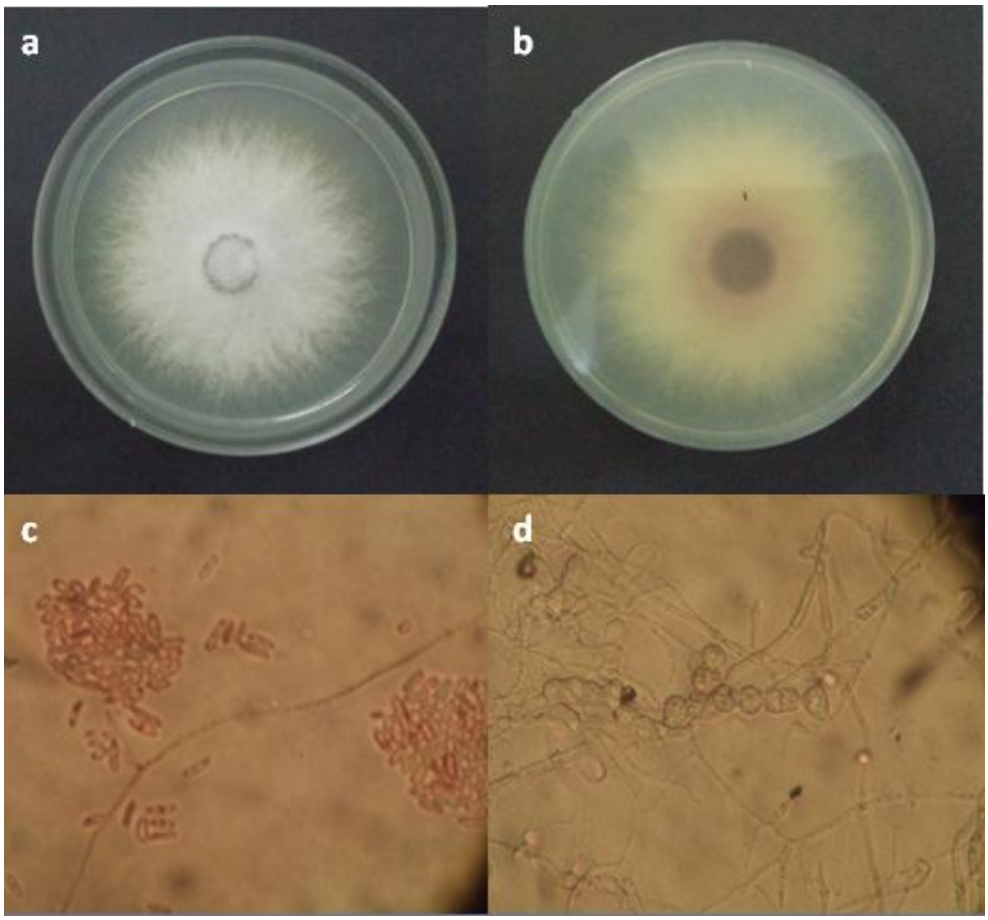

Fig. 3. Morphological characteristics of *Fusarium oxysporum:* mycelium colony on PDA medium (a, b), microconidia (c), clamydospores and short phyalides (d)

The results of the morphological identification have been confirmed by the molecular analysis of rDNA ITS sequences of *F. oxysporum* and *F. solani* isolates. The average of two isolates of rDNA ITS sequences were 497 base pairs for *F. oxysporum*, 532 base pairs for *F. solani*.

For the other nine isolates of *Fusarium* spp., the molecular analysis of rDNA ITS sequences gave three other species of *Fusarium*: five *Fusarium equiseti* isolates, two *Fusarium chlamydosporum* isolates and two *F. proliferatum* isolates. The average of rDNA ITS sequences were 543 base pairs for *F. proliferatum*, 498 base pairs for *F. equiseti* and 497 base pairs for *F. chlamydosporum*.

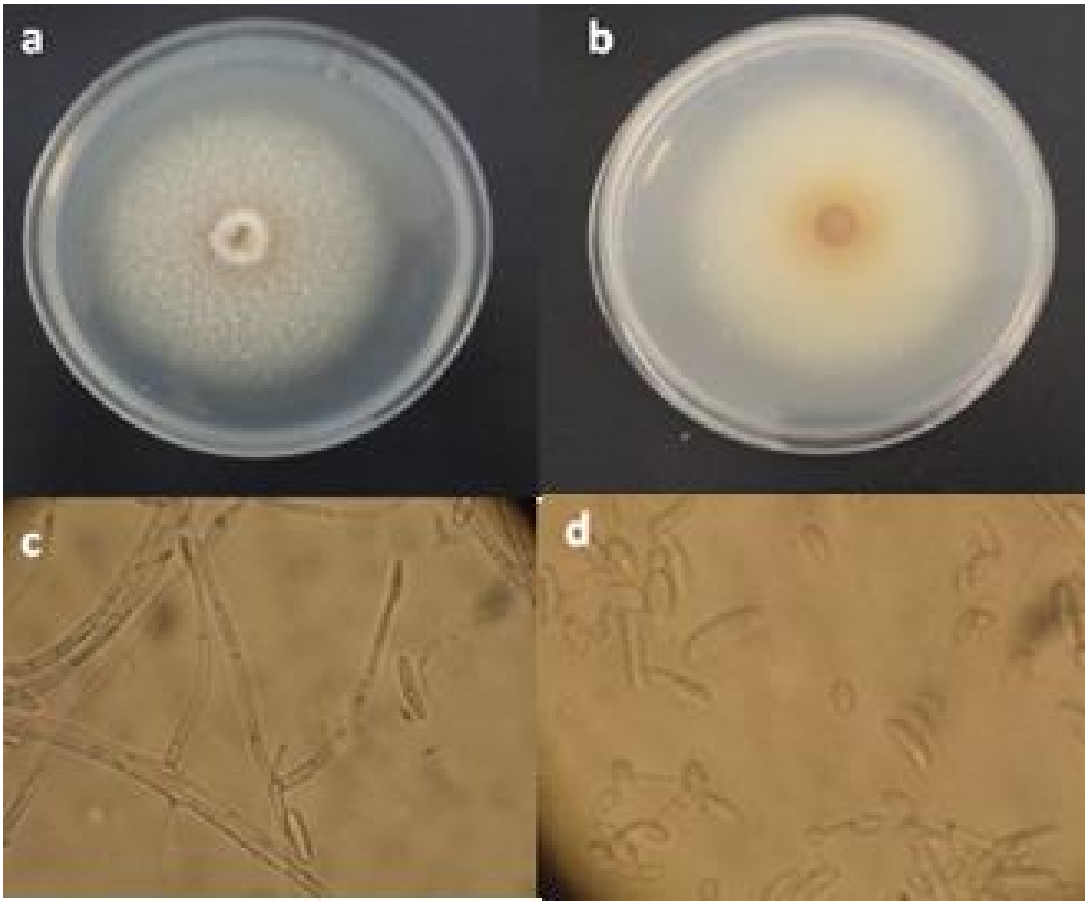

Fig. 4. Morphological characteristics of *Fusarium solani:* mycelium colony on PDA medium (a, b), long phyalides and microconidia (c, d)

Table 3. Origin of *Fusarium oxysporum* and *F. solani* isolates used in the pathogenicity tests and all isolates species of *F. equiseti, F. chlamydosporum* and *F. proliferatum* isolates collected from different nurseries

Codes	Species	Nurseries	Locations	Years	GenBank accession number
Fo1	*Fusarium oxysporum*	Chebika2	Kairouan	2012	-
Fo2	*Fusarium oxysporum*	Chebika2	Kairouan	2012	-
Fo3	*Fusarium oxysporum*	Zaghouan	Zaghouan	2013	-
Fo4	*Fusarium oxysporum*	Chebika1	Kairouan	2013	-
Fo5	*Fusarium oxysporum*	Chebika1	Kairouan	2013	-
Fo6	*Fusarium oxysporum*	Zaghouan	Zaghouan	2013	-
Fo7	*Fusarium oxysporum*	Chebika3	Kairouan	2012	-
Fo8	*Fusarium oxysporum*	Chebika1	Kairouan	2013	-
Fo9	*Fusarium oxysporum*	Zaghouan	Zaghouan	2013	-
Fo10	*Fusarium oxysporum*	Zaghouan	Zaghouan	2013	-
Fo11	*Fusarium oxysporum*	Zaghouan	Zaghouan	2013	-
Fo12	*Fusarium oxysporum*	Chebika1	Kairouan	2013	-
Fo13	*Fusarium oxysporum*	Chebika1	Kairouan	2013	-
Fo14	*Fusarium oxysporum*	Chebika1	Kairouan	2012	-
Fo15	*Fusarium oxysporum*	Chebika2	Kairouan	2012	-

Codes	Species	Nurseries	Locations	Years	GenBank accession number
Fo16	*Fusarium oxysporum*	Chebika1	Kairouan	2013	MF993096
Fo17	*Fusarium oxysporum*	Manzelnour	Monastir	2013	-
Fo18	*Fusarium oxysporum*	Chebika3	Kairouan	2012	-
Fo19	*Fusarium oxysporum*	Chebika3	Kairouan	2012	-
Fo20	*Fusarium oxysporum*	Zaghouan	Zaghouan	2013	-
Fo21	*Fusarium oxysporum*	Manzelnour	Monastir	2013	-
Fo22	*Fusarium oxysporum*	Zaghouan	Zaghouan	2013	MF993097
Fo23	*Fusarium oxysporum*	Chebika3	Kairouan	2012	-
Fo24	*Fusarium oxysporum*	Chebika1	Kairouan	2012	-
Fo25	*Fusarium oxysporum*	Ouardanin	Monastir	2013	-
F75	*Fusarium solani*	Zaghouan	Zaghouan	2013	-
F67	*Fusarium solani*	Chebika1	Kairouan	2012	-
F92	*Fusarium solani*	Zaghouan	Zaghouan	2013	-
F191	*Fusarium solani*	Chebika1	Kairouan	2013	-
F149	*Fusarium solani*	Manzelnour	Monastir	2013	-
F171	*Fusarium solani*	Zaghouan	Zaghouan	2013	MF993093
F48	*Fusarium solani*	Chebika3	Kairouan	2012	MF993094
F150	*Fusarium solani*	Manzelnour	Monastir	2013	-
Fe2	*Fusarium equiseti*	ZAghouan	Zaghouan	2013	MF993081
Fe4	*Fusarium equiseti*	Ouardanin	Monastir	2013	MF993088
Fe5	*Fusarium equiseti*	Manzelnour	Monastir	2013	MF993089
Fe6	*Fusarium equiseti*	Chebika3	Kairouan	2012	MF993090
Fe7	*Fusarium equiseti*	Chebika1	Kairouan	2012	MF993091
Fc1	*Fusarium chlamydosporum*	Ouardanin	Monastir	2013	MF993100
Fc2	*Fusarium chlamydosporum*	Manzelnour	Monastir	2013	MF993101
Fp1	*Fusarium proliferatum*	Chebika2	Kairouan	2012	MF993104
Fp2	*Fusarium proliferatum*	Zaghouan	Zaghouan	2013	MF993106

A BLAST search of the rDNA ITS sequences of *Fusarium oxysporum* and *Fusarium solani* gave 99% of similarity with ITS sequences of species from GenBank. Indeed, there is a similarity of 99% between our isolates of *F. solani* MF993093 and MF993094 with KM235740 and KY617066 respectively. Also, the two isolates of *F. oxysporum* MF993096 and MF993097 were similar to KY810792 and KC282839 respectively by 99%.

The BLAST alignment with ITS sequences of isolates from GenBank revealed 99% of similarity for the two isolates Fp1(MF993104) and Fp2 (MF993106) with the *F. proliferatum* isolates KF986684 and FJ040179 respectively, from GenBank.

The BLAST search of rDNA ITS sequences of *Fusarium* spp. with ITS sequences of *Fusarium* isolates from GenBank gave 99% of similarity for Fe2 (MF993081), Fe4 (MF993088), Fe5 (MF993089) and Fe7 (MF993091) with the GenBank *F. equiseti* isolates FJ441009, JQ690085, KJ677236 and KT211524 respectively. 100% of similarity for Fe6 isolate has been found with the *F. equiseti* isolate KT211524. However, in the case of Fc1 and Fc2, the BLAST search of rDNA ITS sequences gave a similarity of 96% for Fc1 isolate with *F. chlamydosporum* KM076600 isolate and *F. equiseti* KU377478 isolate. For Fc2 isolate, 99% of similarity showed with the *Fusarium chlamydosporum* EU520242 isolate, *Fusarium verticillioides* isolate KX553874 and *F. equiseti* isolate KY318493.

3.3 Vigor Index

The study of peach seedlings vigor index showed the existence of the four levels. However, no difference has been noted between the percent of isolation of each *Fusarium* species found and each vigor index (Table 5).

Table 4. Percent of *Fusarium* species isolation from collected rootstocks samples

Nurseries	Rootstocks	Plants number	Isolation percent of each identified species				
			F. oxysporum	***F. solani***	***F. equiseti***	***F. chlamydosporum***	***F. proliferatum***
Chebika1	Garnem	15	100	50	15	0	10
Chebika2	Garnem	20	90	30	0	0	10
	Garnem	15	60	30	0	0	0
Chebika3	Bitter almond	5	70	50	10	0	0
	Garnem	5	60	30	0	0	10
Zaghouan	Bitter almond	5	80	60	10	0	0
Ouardanin	Garnem	5	50	20	5	10	0
Manzelnour	Bitter almond	15	80	50	10	10	0

Table 5. Percent of *Fusarium* species isolation in each vigor index group

Nurseries	Rootstocks	*Fusarium* spp.	Vigor index			
			IV1	**IV2**	**IV3**	**IV4**
Chebika1	Garnem	*F. oxysporum*	90.00±10.61[a]	89.00±10.25[a]	88.00±8.37[a]	-
		F. solani	55.00±11.18[a]	45.00±11.18[a]	50.00±13.69[a]	-
		F. equiseti	4.00±4.18[a]	3.00±4.47[a]	7.00±6.71[a]	-
		F. proliferatum	2.00±4.47[a]	3.00±4.47[a]	6.00±6.52[a]	-
Chebika2	Garnem	*F. oxysporum*	89.00±11.40[a*]	85.00±11.73[a]	82.00±13.04[a]	85.00±11.73[a]
		F.solani	25.00±10.00[a]	27.00±6.71[a]	28.00±9.08[a]	25.00±5.00[a]
		F. proliferatum	6.00±4.18[a]	5.00±3.54[a]	5.00±3.54[a]	6.00±4.18[a]
Chebika3	Garnem	*F. oxysporum*	53.00±8.37[a]	50.00±15.41[a]	49.00±8.94[a]	-
		F. solani	26.00±6.52[a]	32.00±12.55[a]	30.00±12.75[a]	-
	Bitter almond	*F. oxysporum*	-	-	70±11.00	-
		F. solani	-	-	50±13.69	-
		F. equiseti	-	-	10±5.00	-
Manzel nour	Bitter almond	*F. oxysporum*	-	82.00±7.58[a]	84.00±10.84[a]	79.00±12.45[a]
		F. solani	-	45.00±10.00[a]	45.00±5.00[a]	50.00±7.91[a]
		F. equiseti	-	7.00±5.70[a]	5.00±3.54[a]	7.00±4.47[a]
		F.chlamydospor-um	-	3.00±4.47[a]	4.00±4.18[a]	3.00±4.47[a]
Ouardanin	Garnem	*F. oxysporum*	-	-	-	50±15.00
		F. solani	-	-	-	20±10.00

Nurseries	Rootstocks	*Fusarium* spp.	Vigor index			
			IV1	IV2	IV3	IV4
		F. equiseti	-	-	-	5±4.47
		F.chlamydospor-um	-	-	-	10±3.53
Zaghouan	Garnem	*F. oxysporum*	-	60±5.00	-	-
		F. solani	-	30±12.75	-	-
		F. proliferatum	-	10±4.47	-	-
	Bitter almond	*F. oxysporum*	-	80±10.00	-	-
		F. solani	-	60±7.58	-	-
		F. equiseti	-	10±5.00	-	-

(*) means ± standard error in the line followed by the same letter are not significantly different according to SNK test at P < 0.05.
(-) No plants in this group.

3.4 Pathogenicity Tests

After 6 months of the inoculation of the peach rootstock Garnem by 8 isolates of *Fusarium solani* symptoms of root and collar rot have been observed (Fig. 5). These symptoms were not found in the roots of the un-inoculated control peach seedlings. Thus, these isolates revealed to be pathogens. However, no significant difference was noted between aggressiveness of these isolates. In case of *F. solani*, the average index of root rot was ranged from 1.5 for F75 isolate to 3.25 for F48 isolate (Table 6). Concerning the roots weight, all treatments did not affect this parameter. A reduction of the height for cv Carnival plants was registered particularly when inoculated by F48 isolate of *F. solani* (30.64%).

For *Fusarium oxysporum*, a cut at the stem of Garnem rootstock inoculated by *Fusarium oxysporum* isolates exhibited a browning. These symptoms were not found in the roots of the un-inoculated control trees.

In addition, Fo6, Fo10, Fo22, Fo23, Fo5 and Fo19 isolates induced drying of the apical portion of the scion on carnival variety. For Royal variety, only Fo22 isolate induced causing this type of symptom. The analysis of variance of the necrosis index on peach plants roots (Garnem) revealed a significant difference between isolates (Fig. 6). The value of this index varied from 3 (Fo16) to 5 (Fo24) (Table 7). Concerning the roots weight, the seedlings inoculated by the different *Fusarium* spp. isolates showed no effect on this parameter. The majority of isolates reduced the height plants cv carnival except FO5 and FO20 isolates. On cv Royal glory, only the isolates FO5, FO10, FO15, FO19, FO20 reduced the plants height. The fungal species were re-isolated from roots tissues from all of the inoculated peach seedlings and confirmed the Koch's postulates.

Fig. 5. Symptoms of root rot generated by *Fusarium solani* isolates on peach plants recorded six months after inoculation: (a) control, (b) desiccation of the branches of royal glory and (d) carnival variety inoculated with the isolate F48; Collar canker and browning of the roots and the stem of the Garnem rootstock caused by the isolates (c) F48, (e) F191 and (f) F171

Table 6. Disease severity, plants heights, and root fresh weights of peach seedlings after 6 months of inoculation by *Fusarium solani*

Isolates	Parameters evaluated			
	Roots weights (g)	Roots rots	Heights of peach varieties (cm)	
			Carnival	Royal
Control	32.32±9.80[a]*	1.25 ±0.5[a]	86.50 ±4.50[c]	84.50 ±12.50[a]
F75	50.41±11.06[a]	1.50 ±0.58[ab]	74.80 ±3.30[a-c]	99.50 ±0.50[a]
F67	39.49±18.05[a]	2.25 ±0.5[ab]	73.00 ±8.00[a-c]	98.00 ±4.00[a]
F92	48.83 ±24.41[a]	2.75 ±0.96[ab]	81.50 ±6.76[bc]	95.50 ±2.78[a]
F191	45.04 ±11.45[a]	2.50 ±0.58[ab]	74.33 ±4.51[a-c]	91.00 ±3.00[a]
F149	44.58 ±28.80[a]	1.75 ±0.96[ab]	73.00 ±5.00[a-c]	90.33 ±6.01[a]
F171	32.71 ±7.94[a]	2.75 ±0.50[ab]	69.16 ±0.29[ab]	87.13 ±8.80[a]
F48	33.49±18.13[a]	3.25 ±1.26[b]	60.00 ±11.00[a]	83.73 ±26.12[a]
F150	27.77 ±7.43[a]	2.50 ±0.58[ab]	81.16 ±0.76[bc]	75.86 ±6.80[a]

(*) means ± standard error in the column followed by the same letter are not significantly different according to SNK test at P ≤ 0.05

Table 7. Disease severity, plants heights, and root fresh weights of peach seedlings after 6 months of inoculation by *Fusarium oxysporum*

Codes	Roots weights (g)	Roots rots	Heights of peach varieties (cm)	
			Carnival	Royal
Control	32.32±9.80[a]*	1±0.50[a]	86.33±4.51[cd]	84.33±12.50[a-c]
Fo1	56.41±20.56[a]	1.25±0.50[a]	80.23±4.35[b-d]	93.50±0.50[a-c]
Fo2	62.57±37.39[a]	1.25±0.50[ab]	77.00±7.00[a-d]	89.20±8.70[a-c]
Fo3	53.2±10.54[a]	2.5±0.58[ab]	84.00±12.53[cd]	100.56±2.11[a-c]
Fo4	53.64±11.88[a]	2.5±0.58[ab]	72.36±14.46[a-d]	112.60±5.92 [c]
Fo5	33.49±2.24[a]	2.25±0.50[ab]	86.83±0.29 [cd]	80.16±4.25[ab]
Fo6	52.24±17.50[a]	2.25±0.50[ab]	82.00±13.53[b-d]	95.33±12.06[a-c]
Fo7	47.48±20.58[a]	2.00±0.00 [ab]	72.00±0.00[a-d]	91.83±1.76[a-c]
Fo8	48.06±9.88[a]	2.00±0.82[ab]	69.33±4.51[a-d]	90.00±2.00[a-c]
Fo9	60.89±26.04[a]	1.75±0.50[ab]	76.66±1.53[a-d]	99.43±0.40[a-c]
Fo10	40.50±8.19[a]	2.00±0.82[ab]	81.56±5.17[b-d]	74.63±10.96[a]
Fo11	54.55±24.19[a]	1.50±0.58[a]	74.33±11.56[a-d]	103.83±1.76[bc]
Fo12	33.82±14.76[a]	2.00±0.82[ab]	70.50±2.29[a-d]	84.50±15.76[a-c]
Fo13	45.85±25.88[a]	2.00±0.82[ab]	63.23±5.65[a-d]	88.30 ±14.45[a-c]
Fo14	38.42±18.40[a]	2.50±0.58[ab]	77.66±3.51[a-d]	99.13±15.20[a-c]
Fo15	40.58±6.89[a]	2.25±0.50[ab]	82.00±4.00[b-d]	76.33±2.52[ab]
Fo16	38.31± 18.42[a]	3.00±0.00 [b]	69.33±8.50[a-d]	92.46±10.31[a-c]
Fo17	46.75±14.28[a]	2.25±0.50[ab]	54.60±7.41[a]	91.16±15.25[a-c]
Fo18	47.01± 18.63[a]	2.75±0.50[ab]	71.33±14.75[a-d]	101.33±6.51[a-c]
Fo19	40.67±13.03[a]	2.50±0.58[ab]	75.00±5.00[a-d]	78.33±14.01[ab]
Fo20	39.68±5.14 [a]	2.00±0.00 [ab]	88.63±4.46[d]	74.16±0.76[a]
fo21	28.44±16.16[a]	2.25±0.50[ab]	73.50±15.31[a-d]	89.00±3.00[a-c]
Fo22	33.78±5.75[a]	2.25±0.50[ab]	61.83±14.78[a-c]	87.46±0.06[a-c]
Fo23	52.38±17.18[a]	2.00±0.82[ab]	65.46±0.50[a-d]	85.33±1.53[a-c]
Fo24	38.57±16.90[a]	3.00±0.82[b]	56.76±9.15[ab]	94.83±11.50[a-c]
Fo25	55.80±7.91[a]	1.50±0.58[a]	81.16 ±6.25[b-d]	87.00± 16.00[a-c]
Fo19	40.67±13.03[a]	2.50±0.58[ab]	75.00±5.00[a-d]	78.33±14.01[ab]
Fo20	39.68±5.14 [a]	2.00±0.00 [ab]	88.63±4.46[d]	74.16±0.76[a]
fo21	28.44±16.16[a]	2.25±0.50[ab]	73.50±15.31[a-d]	89.00±3.00[a-c]
Fo22	33.78±5.75[a]	2.25±0.50[ab]	61.83±14.78[a-c]	87.46±0.06[a-c]
Fo23	52.38±17.18[a]	2.00±0.82[ab]	65.46±0.50[a-d]	85.33±1.53[a-c]
Fo24	38.57±16.90[a]	3.00±0.82[b]	56.76±9.15[ab]	94.83±11.50[a-c]
Fo25	55.80±7.91[a]	1.50±0.58[a]	81.16 ±6.25[b-d]	87.00± 16.00[a-c]

(*) means ± standard error in the column followed by the same letter are not significantly different according to SNK test at P ≤ 0.05

Fig. 6. Browning generated by the isolates Fo24 (a), Fo1 (c) of *Fusarium oxysporum* on the peach rootstock Garnem and dryness of the apical part of branch generated by the isolate Fo22 on the carnival variety (b)

4. DISCUSSION

This study showed that *Fusarium solani*, *F. oxysporum*, *F. equiseti*, *F. proliferatum* and *F. chlamydosporum* are associated with peach rootstocks in Tunisian nurseries. These species were also isolated from peach orchards in different countries [17,16]. Most of these species, except *F. oxysporum* and *F. solani*, were not present in all nurseries and consisted of no more than 9 isolates. Thus, *Fusarium oxysporum* and *F. solani* were found with the highest percent of the total *Fusarium* population. This finding is in agreement with previous investigations [24,15]. Therefore, given that these two species were more abundant; their role was investigated further. Our results showed that *F. oxysporum* and *F. solani* were virulent to the rootstock Garnem and the two Carnival and Royal of peach varieties grafted on Garnem rootstock. Indeed, some *F. solani* isolates reduced significantly the height of cv Carnival grafted on Garnem and induced seedlings root rot. Similar studies showed that these species caused necrosis of peach feeder roots in greenhouse tests and decreased shoot growth and plant height [18]. *F. solani* has been also reported as the causal agent of many cankers and wilt diseases of forest tree nurseries, such as seed deterioration, damping-off, cankers, and root rot of both conifers and hardwoods [25]. The nurseries surveys showed that the rotation crop was used in all nurseries prospected. This way could reduce the disease severity. Previous studies showed that the diversity of root fungal flora has been found to be negatively correlated with disease incidence of crops, because crop rotation increases the diversity of root soil fungi and reduces the inoculum of soil-borne pathogens selected by monoculture [26,27]. The findings of the present investigation showed that *Fusarium oxysporum* isolates generated symptoms of root rot, browning without any effect on seedling growth. This result could be explained by the synergism of these species that could reduce the incidence and their effect on plant development. This specie is responsible of necrosis of peach feeder roots in greenhouse tests and caused a reduction of shoot growth and plant height [18]. Some of the most abundant rhizosphere inhabiting *Fusarium* spp., such as *F. oxysporum* and *F. compactum*, showed phytotoxicity of culture filtrates suggesting that this genus may be responsible for plant growth reduction through a series of toxic metabolites including fusaric acid, enniatins and equisetin [28]. The phytotoxicity of this genus may be due either to an environmentally induced shift towards a high production of fusaric acid, by enhancing phytotoxicity through additive effect with other occurring metabolites [29,30], or to changes in host susceptibility caused by biotic or abiotic stress [31]. Although, it will be important to target *Fusarium* species in management strategies against nurseries peach decline in Tunisia.

For the *Fusarium equiseti, Fusarium proliferatum* and *Fusarium chlamydosporum* found in this study, there is no previous study about their

pathogenicity on peach seedling. In addition, the BLAST alignment of rDNA ITS sequences of some isolates with ITS sequences of *Fusarium* isolates from GenBank gave the same similarity percent with more than one species. Thus, it will be important to use other loci like translation elongation factor 1_ (EF-1_), the largest subunit of RNA polymerase (RPB1), and the second largest subunit of RNA polymerase (RPB2), to identify these isolates [32,33]. Then, they should have been subject to pathogenicity test.

Furthermore, in nurseries, seedlings can be associated and attacked by more than one soil borne pathogen [34]. Thus, although, it will be important to target *Fusarium* species in management strategies, the study of other genera like *Phytophthora* and *Pythium* which can cause the decline of several fruit trees species like peach, apple and apricot [35,17,36,7,37,13] should be important. In addition, some other genera have low virulence such as the genera *Cylindrocarpon* [38,39,40], should not be neglected. These genera may either increase the severity of damage caused by *Fusarium.* Amongst themselves, they cause damage especially in association with root lesion nematodes or when plants are under stress [41]. For this reason, the identification and pathogenicity tests of the *Pythium* and *Phytophthora* species associated to peach seedling in tunisian nurseries is will be done in further study.

5. CONCLUSION

This finding showed that *Fusarium oxysporum* and *F. solani* were isolated with the highest percent of the total *Fusarium* population. These two species were virulent to the rootstock Garnem and the two Carnival and Royal of peach varieties. The carnival variety was more susceptible than Royal glory to these species. Thus, all *Fusarium solani* isolates induced symptoms of root and collar rot. A reduction of the height for cv Carnival plants was registered particularly when inoculated by *F. solani* F48 isolate. The majority of *Fusarium oxysporum* isolates reduced the height plants and provoked drying of the apical portion of the scion on carnival variety. While, some isolates reduced the plants height and only one isolate induced this type of symptom on Royal variety. A cut at the stem of Garnem rootstock inoculated by *Fusarium oxysporum* isolates exhibited the browning symptom. The identification and pathogenicity tests of other *Fusarium* species and other soil-borne genera will be subjected to further studies.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Haygood RA, Graves CH, Ridings WH. *Phytophthora* root rot and stem canker of peach trees in Mississippi. Plant Dis. 1986; 70:866-868.
2. Stylianidis DC, Chitzanidis A, Theochari-Athanasiou I. Evaluation of resistance to *Phytophthora* spp. and *Rhizoctonia solani* in stone fruit rootstocks. Options Méditerranéennes. 1985;85:73–78.
3. Yang J, Ruegger PM, McKenry MV, Becker JO, Borneman J. Correlations between root-associated microorganisms and peach replant disease symptoms in a California soil. PLoS ONE. 2012;7(10): e46420.
4. Caruso FL, Neubauer BF, Begin MD. A histological study of apple roots affected by replant disease. Can J Bot. 1988;67: 742-749.
5. Hoestra H. Replant disease of apple in the Netherlands. Meded. Landbouwhogesh. Wageningen. 1968;68:13.
6. Marek SM, Yaghmour MA, Bostock RM. *Fusarium* spp., *Cylindrocarpon* spp., and environmental stress in the etiology of a canker disease of cold-stored fruit and nut tree seedlings in California. Plant Dis. 2013;97:259-270.
7. Mazzola M. Elucidation of the microbial complex having a causal role in the development of apple replant disease in Washington. Phytopathology. 1998; 88:930-938.
8. Browne GT, Connell JH, Schneider SM. Almond replant disease and its management with alternative pre-plant soil fumigation treatments and rootstocks. Plant Dis.2006;90:869–876.
9. Manici LM, Ciavatta C, Kelderer M, Erschbaumer G. Replant problems in South Tyrol: Role of fungal pathogens and microbial population in conventional and organic apple orchards. Plant Soil. 2003; 256:315–324.
10. Mazzola M, Manici LM. Apple replant disease: Role of microbial ecology in

cause and control. Annu Rev Phytopathol. 2012;50:45–65.
11. Mazzola M. Transformation of soil microbial community structure and *Rhizoctonia*-suppressive potential in response to apple roots. Phytopathology. 1999;89:920-927.
12. Willett M, Smith TJ, Peterson AB, Hinman H, Stevens RG, Ley T, et al. 1994. Growing profitable apple orchards in replant sites: Wilcox WF, Ellis MA. *Phytophthora* root and crown rots of peach trees in the eastern Great Lakes region. Plant Dis. 1988;73:794–798.
13. Bent E, Loffredo A, Yang J, McKenry MV, Jorn Ole Becker JO, Borneman J. Investigations into peach seedling stunting caused by a replant soil. FEMS Microbiol Ecol. 2009;68:192–200.
14. Dullahide S, Stirling G, Nikulin A, Stirling A. The role of nematodes, fungi, bacteria and abiotic factors in etiology of apple replant disorders in the granite belt of Queebsl. Aust J Exp Agric. 1994;34:1177-1182.
15. Tewoldemedhin YT, Mazzola M, Botha WJ, Spies CFJ, McLeod A. Characterization of fungi (*Fusarium* and *Rhizoctonia*) and oomycetes (*Phytophthora* and *Pythium*) associated with apple orchards in South Africa. Eur J Plant Pathol. 2011;130:215–229.
16. Wensley RN. The peach replant problem in Ontario: IV. Fungi associated with replant failure and their importance in fumigated and nonfumigated soils. Can J Bot. 1956; 34:967–981.
17. Hine RB. Role of fungi in peach replant problem. Plant Dis. 1961;45:462-465.
18. Nyczepir AP, Pusey PL. Association of *Criconemellaxenoplax* and *Fusarium* spp. with root necrosis and growth of peach. SON. 1986;18:217-220.
19. Rodriguez RP, Hernández E. Mucor foliar spot and mycoflorain stem and root lesions of peach. J AGR U PUERTO RICO. 2004; 88:155-160.
20. Leslie JF, Summerell BA. The *Fusarium* laboratory manual. Oxford, UK: Blackwell Publishing Ltd. 2006;388.
21. Möller EM, Bahnweg G, Sandermann H, Geiger HH. A simple and efficient protocol for isolation of high molecular weight DNA from filamentous fungi, fruit bodies, and infected plant tissues. Nucleic Acids Res. 1992;22:6115-6116.
22. Ristaino JB, Madritch M, Trout CL, Parra G. PCR amplification of ribosomal DNA for species identification in the plant pathogen genus *Phytophthora*. Appl Environ Microbiol. 1998;64:948–954.
23. Strauss J, Labuschagne N. Pathogenicity of *Fusarium solani* isolates on citrus roots and evaluation of different inoculum types. APS. 1995;9:48–52.
24. Manici LM, Kelderer M, Franke-Whittle IH, Rühmer T, Baab GF, Nicoletti F, et al. Relationship between root-endophytic microbial communities and replant disease in specialized apple growing areas in Europe. Appl Soil Ecol. 2013;72:207–214.
25. Bloomberg WJ. Diseases caused by *Fusarium* in forest nurseries. In: Nelson PE, Tousson TA, Cook RJ. (Eds) *Fusarium*: diseases, biology, and taxonomy. Pennsylvania State University Press, University Park. 1981;178-187.
26. Manici LM, Caputo F. Fungal community diversity and soil health in intensive potato cropping systems of the east Po valley, northern Italy. Ann Appl Biol. 2009;155: 245–258.
27. Nitta T. Diversity of root fungal floras: Its implication on soil-borne diseases and crop growth. JARQ. 1991;25:6–11.
28. Manici LM, Caputo F, Saccà ML. Secondary metabolites released into the rhizosphere by *Fusarium oxysporum* and *Fusarium* spp. as underestimated component of nonspecific replant disease. Plant Soil. 2017;415:85–98.
29. Bacon CW, Porter JK, Norred WP, Leslie JF. Production of fusaric acid by *Fusarium* species. Appl Environ Microbiol. 1996; 62:4039–4043.
30. D'Mello JPF, Placinta CM, Macdonald AMC. *Fusarium* mycotoxins: A review of global implications for animal health, welfare and productivity. Anim Feed Sci Technol. 1999;80:183–205.
31. Fisher PJ, Petrini O. Fungal saprobes and pathogens as endophytes of rice (*Oryza sativa* L.). New Phytologist. 1992; 120:137–143.
32. O'Donnell K, Sutton DA, Rinaldi MG, Sarver BAJ, Balajee SA, Schroers HJ, et al. An Internet-accessible DNA sequence database for identifying fusaria from human and animal infections. J Clin Microbiol. 2010;48:3708–3718.
33. O'Donnell K, Rooney AP, Proctor RH, Brown DW, McCormick SP, Ward TJ, et al. Phylogenetic analyses of RPB1 and RPB2 support a middle cretaceous origin for a clade comprising all agriculturally and

medically important fusaria. Fungal Genet Biol. 2013;52:20–31.
34. Gilbert GS. Rain forest plant diseases: The canopy – Understory connection. Selbyana. 1995;15:75-77.
35. Wilcox WF. *Phytophthora* root and crown rots. Disease Identification Sheet. 1992;7.
36. Sewell GWF. Effects of *Pythium* species on the growth of apple and their possible causal role in apple replant disease. Ann Appl Biol. 1981;97:31-42.
37. Mazzola M, Andrews PK, Reganold JP, Lacvesque CA. Frequency, virulence, and metalaxyl sensitivity of *Pythium* spp. isolated from apple roots under conventional and organic production systems. Plant Dis. 2002;86:669-675.
38. Axelrood PE, Chapman WK, Seifert KA, Trotter DB, Shrimpton G. Cylindrocarpon and *Fusarium* root colonization of douglas-fir seedlings from British Columbia reforestation sites. Can J Forest Res. 1998;28:1198-1206.
39. Bhat RG, Schmidt LS, Browne GT. Quantification of *Cylindrocarpon* sp. in roots of almond and peach trees from orchards affected by *Prunus* replant disease. (Abstr.) Phytopathology. 2011; 101:S15.
40. Tewoldemedhin YT, Mazzola M, Mostert L, McLeod A. *Cylindrocarpon* species associated with apple tree roots in South Africa and their quantification using real-time PCR. Eur J Plant Pathol. 2010; 129(4):637-651.
41. Unestam T, Beyer-Ericson L, Strand M. Involvement of *Cylindrocarpon destructans* in root death of *Pinus sylvestris* seedlings: Pathogenic behavior and predisposing factors. Scand J Forest Res. 1989;4: 521-536.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history/24245