

Pathogenicity of Some *Fusarium* Species Associated with Superficial Blemishes of Potato Tubers

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Received 19 April 2012, revised 10 September 2012, accepted 18 September 2012

Abstract

As an organ for reserve and propagation, the tuber grows underground and is in contact with soil-borne microorganisms, making it potentially exposed to blemishes. Therefore, the objective of this study was the possibility of using some modern methods of molecular diagnostics and detection of the presence of fungal contaminants in potato blemishes in Al-Qasim (Saudi Arabia). Polygonal lesions were the most observed blemish type in the collected samples. One hundred and sixty isolates were recovered from different types of blemishes obtained in this study. *Fusarium*, *Penicillium*, *Ilyonectria*, *Alternaria* and *Rhizoctonia* were the most common genera collected from different blemish types. Using ITS region sequencing, all collected fungi were identified at the species level. All *Fusarium* strains collected during this study were used to detect their pathogenicity against potato tubers. This is the first comprehensive report on the identification of major pathogenic fungi isolated from potato tuber blemishes in Saudi Arabia.

Key words: *Fusarium* spp., Dry rot, ITS regions, potato tuber blemishes in Saudi Arabia

Introduction

There are literally hundreds of potato producers in the Kingdom of Saudi Arabia (KSA) comprising potato production units of all sizes and capabilities, growing many varieties for sale mainly to the wholesale markets. Over 75% of the total potato production in the Kingdom comes from small farmers distributed in different regions, and who supply local wholesale markets. Worldwide about 40 soil-borne diseases affect potato and cause severe damage especially on tubers, the economically most important part of the plant. Soil-borne diseases affecting potato crop can be divided into two groups depending on symptoms: symptoms damaging tubers and those damaging other parts of the plant (Gudmestad *et al.*, 2007). Among diseases affecting tubers, symptoms can be divided into three categories: galls, blemishes and rots. Blemishes affect only the tuber skin, but they became economically important since consumers' habits have changed and tubers are washed before selling (Fiers *et al.*, 2010).

Blemishes or superficial alterations affect only the tuber skin, without affecting the taste or the nutritional properties. However, they have a negative cosmetic

effect on the tubers and destroy the integrity of the natural barrier of the skin, forming an entry point for pathogenic microorganisms (Fiers *et al.*, 2010). Moreover, it has been shown that skin visual appearance is the most important factor influencing consumer behavior in fresh potato purchase. Economical data about such potential losses are difficult to estimate, but all potato sectors, *i.e.* seed, ware, and processing are affected. Potato tubers can show a large range of superficial blemishes. These blemishes may result from a pathogen attack or unfavourable environmental factors. When their causes are known, and the Koch's postulates have been fulfilled, these blemishes are called typical blemishes (Fiers *et al.*, 2010). By contrast, the blemishes for which the causal agent has not been clearly identified are called atypical blemishes (Fiers *et al.*, 2010). Atypical blemishes frequently observed in ware potato production are the atypical corky blemishes, especially polygonal lesions and corky spots (Fiers *et al.*, 2010).

Several *Fusarium* spp. cause potato (*Solanum tuberosum* L.) seed piece decay, plant wilt and tuber dry rot in storage, leading to losses in crop yield and quality (Secor and Salas, 2001). *Fusarium* dry rot is caused by several species including *Fusarium sambucinum* Fuckel,

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Fusarium culmorum (W.G. Smith) Sacc. and *Fusarium oxysporum* Schlecht, under field and storage conditions (Boyd, 1972; Schisler and Slininger, 1994). Crop losses attributed to the dry rot have been estimated to average 6%, with losses up to 25% reported (Chelkowski, 1989). In addition to destroying tuber tissues, *Fusarium* spp. can produce toxins that have been implicated in mycotoxicoses of humans and animals (Senter *et al.*, 1991; Schisler *et al.*, 1997). *F. sambucinum*, *Fusarium solani* (Mart.) Sacc., *F. culmorum* and *F. oxysporum* are reported as common causes of dry rot of potatoes in Turkey, resulting in partial or almost complete loss of stored commercial potatoes varieties (Eken *et al.*, 2000). Several *Fusarium* spp. cause potato seed piece decay, plant wilt and tuber dry rot in storage, leading to losses in crop yield and quality (Secor and Salas, 2001). In North America and parts of Europe, *Fusarium sambucinum* Fuckel is considered to be the most significant causal agent of tuber dry rot (Tivoli *et al.*, 1986; Secor and Salas, 2001), whereas in the UK, *F. coeruleum* (Libert) Sacc. is more prevalent (Hide *et al.*, 1992). In some instances, *F. avenaceum* (Fr.) Sacc. has also been implicated as the causal agent of tuber dry rot (Hide *et al.*, 1992; Satyaprasad *et al.*, 1997).

Rapid detection of plant parasitic pathogens enables to set up adapted control measures and to avoid disease expansion and yield losses, even if the infestation level is low. Molecular biology based-diagnosis and detection methods are expected to complement classical diagnosis. The most developed detection methods are based on polymerase chain reaction (PCR), which amplify DNA regions specific of the pathogen of interest. Intraspecific identifications such as fingerprinting methods – Restriction Fragment Length Polymorphism (RFLP) or Amplified Fragment Length Polymorphism (AFLP) – is used for more accurate identification of pathovars or races of bacteria, fungi or nematodes (Abeln *et al.*, 2002; Cullen *et al.*, 2007; Flores-Gonzalez *et al.*, 2008; Pitman *et al.*, 2008). Although not translated into proteins, the ITS (internal transcribed spacers) coding regions have a critical role in the development of functional rRNA, with sequence variations among species showing promise as signature regions for molecular assays (Gardes and Bruns, 1993). A widely used approach is to conduct PCR using oligonucleotide primers specific for the conserved flanking regions of the internally transcribed spacers (ITS) of the fungal rRNA gene (White *et al.*, 1990). Our group used ITS region sequencing for identification of several fungal species including, *Trichoderma* (Gherbawy *et al.*, 2004), *Pythium* (Gherbawy *et al.*, 2005), *Mucor* and *Rhizopus*, (Gherbawy and Hussien, 2010). Using ITS region sequences, Fiers *et al.* (2010) studied diversity of microorganisms associated with atypical superficial blemishes of potato tubers in France. They reported that the most

represented fungi belonged to the genera *Fusarium*, *Rhizoctonia*, *Alternaria*, *Penicillium* and *Clonostachys*.

As far as we know there is no literature concerning mycobiota of potato blemishes in Saudi Arabia, therefore; this study aimed to isolate fungi associated with potato superficial blemishes, identify the fungal communities which could be associated with blemishes and detect their pathogenicity potentials against potato tubers.

Experimental

Materials and Methods

Plant material. The potato tubers were collected from different markets in Al-Qasim region during the period from January to December 2011. Blemishes were observed and classified according to Fiers *et al.* (2010). The tubers were stored in paper bags at 4°C during several weeks until the start of the experiment.

Isolation of fungi. As previously published by Fiers *et al.* (2010) as follows; tubers were washed under running tap water, and air dried. A 6 mm diameter and 5 mm deep pieces were excised with a cork borer from the affected area of each tuber. The tubers surface was sterilized in 1% bleach for 15 s and rinsed three times in sterile water. Each tuber explant was dried on sterile paper and plated on potato dextrose agar (PDA). After 5 days of incubation at room temperature under natural light, fungal colonies developing from the plant material were identified by microscopic observations and purified at least twice by serial transfers on PDA.

Molecular identification of fungal isolates. For DNA extraction, all the collected fungal isolates were inoculated in tubes on PDA slants. Two ml of potato dextrose broth (PDB) poured into PDA tubes and vortexed to disperse the spores, and the spores-PDB mix poured into flasks containing 100 ml of PDB. Flasks were incubated at room temperature without shaking for 2 to 3 days. The mycelium was harvested by filtration, frozen at –80°C during 30 min, lyophilized and stored at –80°C. The mycelium was ground in liquid nitrogen in a sterile mortar to obtain a mycelium powder. The DNA extracted from 20 mg of mycelium powder using DNeasy plant mini kit. The DNA quantity and quality checked by electrophoresis on a 0.8% agarose gel revealed with ethidium bromide and visualized by UV trans-illumination. The internal transcribed spacer (ITS) region of the ribosomal DNA (rDNA) was amplified by PCR with the primers ITS1-F (CTTGGTCATTTAGAGGAAGTAA) and ITS4 (TCCTCCGCTTATTGATATGC) (Gardes and Bruns, 1993; White *et al.*, 1990). PCR amplifications were performed in a final volume of 50 µl by mixing 2 µl of DNA

with 0.5 μ M of each primer, 150 μ M of dNTP, 6 U of Taq DNA polymerase and PCR reaction buffer. Amplification conducted in a thermal cycler with an initial denaturation of 3 min at 94°C, followed by 35 cycles of 1 min at 94°C, 1 min at 50°C, 1 min at 72°C, and a final extension of 10 min at 72°C. Aliquots of PCR products checked by electrophoresis on a 1% agarose gel revealed with ethidium bromide and visualized by UV trans-illumination. The PCR products sequenced using primers ITS1-F and ITS4. For each PCR product, sequences from both strands assembled to produce a consensus sequence.

Identifications based on sequences. The sequence results were blasted against GenBank to identify the isolated fungi to species level.

Pathogenicity test for the selected strains. The procedure was performed according to Peters *et al.* (2008). The healthy potato tubers (*Solanum tuberosum* L.) were used in this experiment. Initially, tubers appearing healthy and uniform in size (100–120 g) were selected and washed to remove excess soil, surface sterilized in 0.5% sodium hypochlorite solution for 10 min and rinsed in 3 changes of sterile distilled water (Lui and Kushalappa, 2002; Lui *et al.*, 2005) and then air dried. Then the tubers wounded with a cork borer with a diameter of 5 mm to a depth of 5 mm (Choiseul *et al.*, 2007; Peters *et al.*, 2008). An agar plug (5 mm diameter) containing active mycelium of *Fusarium* and *Gibberella* isolates extracted from the margin of a 3-day-old cultures grown on one fourth strength PDA and placed into the wound, which was subsequently sealed with the excised plug of tuber tissue. Three tubers used for each fungal strain. All the wounded potato tubers were wrapped in black polyethylene bags (Manici and Cerato, 1994; Lui and Kushalappa, 2002) and incubated in the dark at 20°C for 3 weeks. As a control, tubers were inoculated with an agar plug (one fourth strength PDA) only. Following incubation, tubers were cut longitudinally from the point of inoculation and the depth of internal necrosis was measured using electronic calipers. The depth of wound response in controls was also recorded for comparison. For each isolate where disease was expressed, a small tissue sample (10 \times 5 \times 3 mm) from an individual tuber was taken from the margin of the internal necrotic region with a sterile scalpel, surface sterilized in 0.6% sodium hypochlorite for 15 s, rinsed twice in sterile distilled water, and blotted dry on sterile filter paper. Tissue pieces were then plated onto one fourth strength PDA amended with tetracycline (0.05 g/l) and streptomycin sulfate (0.1 g/l). Petri dishes were incubated in the dark at 22°C for 4–7 days. Following incubation, hyphal tips from the margins of actively growing isolates were removed with a sterile probe and plated onto one fourth strength PDA to generate pure cultures (Peters *et al.*, 2008).

Statistical analysis. Data were subjected to analysis of variance (ANOVA) using the Statistical Analysis System (SAS Institute, Inc., 1996). Means were separated by Duncan's multiple range test at $P < 0.05$ level.

Results and Discussion

The collected tubers samples showed 4 different blemish types namely: corky cracks, enlarged lenticels, netted scab and polygonal lesions (Table I). Polygonal lesions were the most frequently observed blemish type (Table I). This result is in agreement with Fiers *et al.* (2010), as they recorded several blemish types in potato tubers. They reported that the polygonal lesions were the most recorded blemish type.

One hundred and sixty one fungal strains were collected from the different blemish types (Table I). Eighty four strains were associated with polygonal lesions, while 50 strains were associated with enlarged lenticels. On the other hand, 14 and 13 strains were associated with corky cracks and netted scab, respectively. Sequences from the internal transcribed spacer (ITS) region of the nuclear ribosomal DNA have been commonly used for the identification of fungi (Köljalg *et al.*, 2005; Naumann *et al.*, 2007; Nilsson *et al.*, 2008; Gherbawy and Hussien, 2010; Gherbawy *et al.*, 2010). Using ITS1 and ITS4 primers, the isolated strains produced amplicons ranging from 460 to 549 bp. *Fusarium* and *Gibberella* species produced amplicon sizes ranging from 496 to 522 bp. Mirhendi *et al.* (2010) reported that the amplicon sizes of ITS region sequences in *Fusarium* species using ITS1 and ITS4 primers ranged from 535 to 566 bp. The sequence result of each strain was blasted against GenBank to confirm the identification. The accession numbers of the collected strains are shown in Table II.

Thirty one species belonged to 19 genera were collected from the different types of blemish and identified from their ITS sequence (Table II). *Fusarium*, *Penicillium*, *Ilyonectria*, *Alternaria* and *Rhizoctonia* were the most common genera. They were represented by *Fusarium oxysporum*, *Penicillium brevicompactum*, *P. chrysogenum*, *Ilyonectria radicolica*, *Alternaria alternata* and *Rhizoctonia solani*. Fiers *et al.* (2010) reported that the most represented genera from different blemish types were *Fusarium* (80 strains), *Rhizoctonia* (68 strains), *Alternaria* (46 strains), *Penicillium* (33 strains), and *Clonostachys* (27 strains). The fungus *R. solani* was cited as well as being responsible for the formation of polygonal lesions. Woodhall *et al.* (2007) reported *Rhizoctonia solani* AG3 as causal agents for blemish in potato tubers. The fungus growing on the tuber surface would delay the growth of the underlying tissues, resulting in deformed tubers. The pattern of the lesions on the skin would be related to the

Table I
Fungal species isolated from the different blemishes

Genera and species	Blemishes				Total
	Polygonal lesions	Corky cracks	Enlarged lenticels	Netted scab	
<i>Alternaria</i>	13	–	3	3	19
<i>A. alternata</i>	10	–	3	3	16
<i>A. arborescens</i>	1	–	–	–	1
<i>A. longissima</i>	2	–	–	–	2
<i>Aspergillus</i>	5	–	–	–	5
<i>A. flavus</i>	2	–	–	–	2
<i>A. niger</i>	3	–	–	–	3
<i>Bionectria ochroleuca</i>	4	1	1	–	6
<i>Boeremia exigua</i>	1	–	1	1	3
<i>Chaetomium piluliferum</i>	3	–	–	–	3
<i>Cladosporium cladosporioides</i>	1	–	2	–	3
<i>Cylindrocarpon olidum</i>	2	–	–	–	2
<i>Davidiella tassiana</i>	–	1	–	–	1
<i>Fusarium</i>	12	6	10	2	30
<i>F. oxysporum</i>	10	6	4	2	22
<i>F. redolens</i>	2	–	2	–	4
<i>F. solani</i>	–	–	4	–	4
<i>Gibberella</i>	1	–	1	–	2
<i>G. pulicaris</i>	1	–	–	–	1
<i>G. zeae</i>	–	–	1	–	1
<i>Gliomastix marmorum</i>	1	–	–	–	1
<i>Ilyonectriaradicicola</i>	11	3	12	–	26
<i>Mucor</i>	2	–	4	2	8
<i>M. circenelloides</i>	1	–	4	1	6
<i>M. hiemalis</i>	1	–	–	1	2
<i>Penicillium</i>	11	3	12	3	29
<i>P. brevicompactum</i>	7	–	2	3	12
<i>P. chrysogenum</i>	2	–	10	–	12
<i>P. purpurogenum</i>	1	–	–	–	1
<i>P. swiecickii</i>	1	3	–	–	4
<i>Rhizoctonia solani</i>	6	–	4	1	11
<i>Rhizopus oryzae</i>	3	–	–	–	3
<i>Trichoderma</i>	5	–	–	1	6
<i>T. tomentosum</i>	2	–	–	–	2
<i>T. velutinum</i>	3	–	–	1	4
<i>Ulocladium</i>	2	–	–	–	2
<i>U. capsicum</i>	1	–	–	–	1
<i>U. atrum</i>	1	–	–	–	1
<i>Verticillium dahliae</i>	1	–	–	–	1
Total	84	14	50	13	161

pattern of the branching hyphae of the fungus. Some of these hyphae may still be present on newly harvested tubers (Tsrer., 2010). *Fusarium oxysporum* is one of the most frequently found *Fusarium* species on potato where it is more common in the wet and warm climate

of Italy (Manici and Cerato, 1994). Also, Manici and Cerato (1994) and Chehri *et al.* (2011) isolated *Fusarium oxysporum* as the one of the most common species isolated from potato tubers. Salami and Popoola (2007) isolated five pathogenic fungi *viz: Botryodiplodia theo-*

Table II
Fungal strains collected from potato blemishes, their accession numbers and percentage of similarities with other GeneBank fungal species based on ITS region sequencing results.

No	Strains	Accession numbers	Gene bank strains	Identities	Given names
1	KAUF1	HE649372	<i>Alternaria alternata</i> JN604114	527/532 (99%)	<i>Alternaria alternata</i>
2	KAUF2	HE649373	<i>Alternaria arborescens</i> EU314991	455/460 (99%)	<i>Alternaria arborescens</i>
3	KAUF3	HE649374	<i>Alternaria longissima</i> DQ865104	523/530 (99%)	<i>Alternaria longissima</i>
4	KAUF4	HE649375	<i>Aspergillus flavus</i> JN252115	515/525 (98%)	<i>Aspergillus flavus</i>
5	KAUF5	HE649376	<i>Aspergillus niger</i> JF710186	511/511 (100%)	<i>Aspergillus niger</i>
6	KAUF6	HE649377	<i>Chaetomium piluliferum</i> AB625587	521/531 (98%)	<i>Chaetomium piluliferum</i>
7	KAUF7	HE649378	<i>Cladosporium cladosporioides</i> JN565298	525/534 (98%)	<i>Cladosporium cladosporioides</i>
8	KAUF8	HE649379	<i>Davidiella tassiana</i> GU566258	499/509 (98%)	<i>Davidiella tassiana</i>
9	KAUF9	HE649380	<i>Bionectria ochroleuca</i> FJ025201	533/543 (98%)	<i>Bionectria ochroleuca</i>
10	KAUF10	HE649381	<i>Cylindrocarpon olidum</i> GU198183	493/503 (98%)	<i>Cylindrocarpon olidum</i>
11	KAUF11	HE649382	<i>Gibberella zeae</i> JN942838	512/522 (98%)	<i>Gibberella zeae</i>
12	KAUF12	HE649383	<i>Fusarium oxysporum</i> JF807394	506/516 (98%)	<i>Fusarium oxysporum</i>
13	KAUF13	HE649384	<i>Fusarium redolens</i> DQ093672	486/496 (98%)	<i>Fusarium redolens</i>
14	KAUF14	HE649385	<i>Gibberella pulicaris</i> JN942835	500/509 (98%)	<i>Gibberella pulicaris</i>
15	KAUF15	HE649386	<i>Fusarium solani</i> JN168971	500/510 (98%)	<i>Fusarium solani</i>
16	KAUF16	HE649387	<i>Gliomastix murorum</i> HQ115690	520/530 (98%)	<i>Gliomastix murorum</i>
17	KAUF17	HE649388	<i>Mucor hiemalis</i> HM172832	523/531 (98%)	<i>Mucor hiemalis</i>
18	KAUF18	HE649389	<i>Mucor circinelloides</i> JN368455	520/531 (98%)	<i>Mucor circinelloides</i>
19	KAUF19	HE649390	<i>Neonectria radicola</i> HQ840391	515/524 (98%)	<i>Ilyonectriaradicola</i>
20	KAUF20	HE649391	<i>Penicillium brevicompactum</i> FJ884116	504/513 (98%)	<i>Penicillium brevicompactum</i>
21	KAUF21	HE649392	<i>Penicillium chrysogenum</i> JF731276	539/549 (98%)	<i>Penicillium chrysogenum</i>
22	KAUF22	HE649393	<i>Penicillium purpurogenum</i> HQ392494	523/532 (98%)	<i>Penicillium purpurogenum</i>
23	KAUF23	HE649394	<i>Penicillium swiecickii</i> GU441580	499/508 (98%)	<i>Penicillium swiecickii</i>
24	KAUF24	HE649395	<i>Phoma exigua</i> EU520200	504/514 (98%)	<i>Boeremia exigua</i>
25	KAUF25	HE649396	<i>Rhizoctonia solani</i> JN545836	461/470 (98%)	<i>Rhizoctonia solani</i>
26	KAUF26	HE649397	<i>Rhizopus oryzae</i> JN943054	524/534 (98%)	<i>Rhizopus oryzae</i>
27	KAUF27	HE649398	<i>Trichoderma tomentosum</i> JN628069	497/506 (98%)	<i>Trichoderma tomentosum</i>
28	KAUF28	HE649399	<i>Trichoderma velutinum</i> HQ115666	524/533 (98%)	<i>Trichoderma velutinum</i>
29	KAUF29	HE649400	<i>Ulocladium capsicum</i> AY762940	516/526 (98%)	<i>Ulocladium capsicum</i>
30	KAUF30	HE649401	<i>Ulocladium atrum</i> AY625072	535/544 (98%)	<i>Ulocladium atrum</i>
31	KAUF31	HE649402	<i>Verticillium dahliae</i> HQ206719	482/492 (98%)	<i>Verticillium dahliae</i>

bromae, *Fusarium redolens*, *Fusarium oxysporum*, *Penicillium* sp. and *Rhizopus oryzae* associated with post harvest storage rot of root-tubers. In Turkey, Demirci *et al.* (2011) isolated several fungal species from potato tubers namely: *Acremonium strictum*, *Alternaria alternata*, *Chaetomium* sp., *Cladosporium cladosporioides*, *Fusarium equiseti*, *F. oxysporum*, *F. solani*, *F. verticillioides*, *Gliocladium catenulatum*, *G. viride*, *Paecilomyces* sp., *P. marquandii*, *P. sulphurellus*, *Penicillium camemberti*, *P. expansum*, *P. frequentans*, *P. nigricans*, *P. olsonii*, *P. phialosporum*, *Plectosporium tabacinum*, *Sporothrix* sp., *Sporothrix schenckii*, *Trichoderma harzianum* and *Verticillium dahliae*. Lydia *et al.* (2012) isolated *Alternaria alternata* and *A. solani* from potato leaves with lesions from the Pacific Northwest over several growing

seasons. Cwalina-Ambroziak (2012) isolated 31 species of filamentous fungi, non-sporulating fungi, and yeast like fungi from stems of three potato cultivar in Poland and *Alternaria alternata* and *Colletotrichum coccodes* were the predominant species. The results of a field survey of potato fungal diseases for two successive years in the six main potato producing regions in the Kingdom of Saudi Arabia proved the presence of fifteen fungal genera (Al-Kherb *et al.*, 1996). They isolated the following fungal genera, in descending order: *Fusarium*, *Alternaria*, *Rhizoctonia*, *Stemphylium*, *Cladosporium*, *Helminthosporium*, *Colletotrichum*, *Phytophthora*, *Pythium*, *Macrophomina*, *Phoma*, *Rosellinia*, *Verticillium*, *Cercospora* and *Sclerotium*. They reported that *Rhizoctonia solani* was the most frequent on

tubers, while *Fusarium* spp. were the most frequent on roots and stem bases.

Pathogenicity. During this work, five species of *Fusarium* and *Gibberella* were collected. These were: *Fusarium oxysporum* (4 strains), *F. redolens* (2), *F. solani* (1), *Gibberellapulicaris* = *Fusarium sambucinum* (1) and *Gibberella zeae* = *Fusarium graminearum* (1). Dry rot of potato caused by *Fusarium* species is a common and commercially serious disease of potato tubers found in all production areas of the world (Nasr-Esfahani, 1998; Lui and Kushalappa, 2002; Slininger *et al.*, 2004; Cullen *et al.*, 2005; Chehri *et al.* 2011). Gashango *et al.* (2012) surveyed the seed potato tubers in Michigan seed production storage facilities during 2009 and 2010. They identified *Fusarium* spp. associated with tuber dry rot symptoms. They reported that, *F. oxysporum* was the most commonly isolated species (30.3%), followed by *F. equiseti* (19.3%). *F. sambucinum* and *F. avenaceum* were the third most prevalent (each at 13.6%). The pathogenicity of the collected *Fusarium* isolates against potato tubers was studied. Variable degrees of pathogenicity were recorded with the collected *Fusarium* isolates (Table III). They ranged from highly pathogenic isolates: *Gibberellapulicaris* (KAUF14) = *Fusarium sambucinum*, *Gibberella zeae* (KAUF11) = *Fusarium graminearum* and *Fusarium solani* KAUF15). These results were in agreement with the results published by El-Hassan *et al.* (2007). They reported that *Fusarium* spp. isolates varied in their pathogenicity against potato tubers. They ranged from highly pathogenic like two isolates of *Fusarium sambucinum*, 6 isolates of *F. solani* and one isolate of *F. oxysporum* to totally

nonpathogenic isolates like 5 isolates of *F. sambucinum*, 3 isolates of *F. oxysporum*, one isolates of *F. culmorum*, 2 isolates of *F. equiseti* and one isolate of *F. semitectum*. Peters *et al.* (2008) recorded that *Fusarium sambucinum*, *Fusarium graminearum* and *Fusarium graminearum* were listed among the most pathogenic fungi to potato tubers. *Fusarium sambucinum* is the most common *Fusarium* dry rot pathogen in North America and Europe, although other *Fusarium* spp. have been also reported (Secor and Salas, 2001; Peters *et al.*, 2008; Delgado *et al.*, 2010). The natural occurrence of *Fusarium* dry rot caused by *F. graminearum* (teleomorph *Gibberella zeae*) was reported in 2005 in naturally infected, commercially stored potato tubers in North Dakota and Minnesota (Ali *et al.*, 2005). Estrada *et al.* (2010) surveyed the stored potato tubers affected by *Fusarium* dry rot (FDR) from commercial storage facilities during 2004–05. They determined that 58% of the *Fusarium* spp. isolated was *F. sambucinum*, and 42% was *F. graminearum*.

Fusarium redolens represented by two strains (KAUF13.1 and KAUF13.2) showed moderate pathogenicity according to the measurement of depth of necrosis. *Fusarium oxysporum* strains KAUF12.1, KAUF12.2, KAUF12.3 and KAUF12.4 did not show any pathogenicity against tubers (Table 3). Salami and Popoola (2007) reported that *Fusarium redolens* and *Botryodiplodia theobromae* were the most aggressive and virulent to potato tubers, followed by *Rhizopus oryzae* and *Fusarium oxysporum* while *Penicillium* species was the least pathogenic. Also, Peters *et al.* (2008) tested 3 strains of *F. oxysporum* isolated from potato against potato tuber, all strains were non-pathogenic. A study done by Song *et al.* (2008) also cited that *F. oxysporum* is the predominant species causing dry rot. Chehri *et al.* (2011) in Malaysia studied the occurrence and pathogenicity of *Fusarium* spp. on potato tubers. Their results of the pathogenicity test revealed that *F. solani* isolates FSO4, FSO12, FSO18 and *F. oxysporum* isolates FOX4 and FOX16 were strongly pathogenic to the inoculated potato tubers.

In conclusion, polygonal lesions were the most frequently observed blemish type in Saudi potato tubers. *Fusarium* was the most prevalent genus among fungal genera associated with potato tuber blemish in Saudi Arabia. The collected *Fusarium* species showed variable degrees of pathogenicity against potato tubers.

Acknowledgement

This work was supported by a grant (Contract No. 1431-363-430) from King Abdulaziz University, Kingdom of Saudi Arabia. The authors are very grateful for the deanship of scientific research at King Abdulaziz and for the financial support they received. The authors express their gratitude to prof. dr Gary Strobel (Department of Plant Science, Montana State University) for English revision of the manuscript.

Table III
Pathogenicity, as measured by mean depth of necrosis (in mm), of 9 isolates of *Fusarium* and *Gibberella* spp. following wound inoculation of potato tubers.

<i>Fusarium</i> and <i>Gibberella</i> species	Strain	Depth of necrosis (mm)	Pathogenicity rating ^b
Control ^a	–	6.5 ^c	NP
<i>Fusarium oxysporum</i>	KAUF12.1	7.6 ^c	NP
<i>Fusarium oxysporum</i>	KAUF12.2	7.6 ^c	NP
<i>Fusarium oxysporum</i>	KAUF12.3	8.2 ^c	NP
<i>Fusarium oxysporum</i>	KAUF12.4	7.6 ^c	NP
<i>Fusarium redolens</i>	KAUF13.1	12.0 ^d	P
<i>Fusarium redolens</i>	KAUF13.2	12.1 ^d	P
<i>Fusarium solani</i>	KAUF15	16.5 ^d	P
<i>Gibberellapulicaris</i>	KAUF14	23.2 ^e	P
<i>Gibberella zeae</i>	KAUF11	18.2 ^d	P

^a Control tubers were wounded and inoculated with an agar plug (5 mm diameter) of potato dextrose only (no fungal mycelium).

^b Pathogenicity rating: NP = non pathogenic and P = pathogenic. Values with the same letters in each column are not significantly different at P = 0.05.

Literature

- Abeln E.C.A., A.M. Stax, J. de Gruyter and H.A. van der Aa. 2002. Genetic differentiation of *Phoma exigua* varieties by means of AFLP fingerprints. *Mycol. Res.* 106: 419–427.
- Al-Kherb S.M., Z.M. Abul-Hayja and I.M. Al-Shahwan. 1996. Potato Fungal Diseases in the Kingdom of Saudi Arabia. *Journal of King Saud University. Agricultural Sciences* 8: 147–156.
- Ali S., V.V. Rivera and G.A. Secor. 2005. First report of *Fusarium graminearum* causing dry rot of potato in North Dakota. *Plant. Dis.* 89: 105.
- Boyd A.K.W. 1972. Potato Storage Diseases. *Rev. Plant Pathol.* 51: 297–321.
- Chehri K., N.F. Mohamed, B. Salleh and Z. Latiffah. 2011. Occurrence and pathogenicity of *Fusarium* spp. on the potato tubers in Malaysia. *African J. Agric. Res.* 6: 3706–3712.
- Chelkowski J. 1989. *Toxinogenic of Fusarium species causing dry rot of potato tubers*. In: Chelkowski J. (Ed.), *Fusarium Mycotoxin, Taxonomy and Pathogenicity*. Elsevier Publishing Co., New York, pp. 435–440.
- Choiseul J., L. Allen, S.F. Carnegie. 2007. Fungi causing dry tuber rots of seed potatoes in storage in Scotland. *Potato Res.* 49: 241–253.
- Cullen D.W., I.K. Toth, N. Boonham, K. Walsh, I. Barker and A.K. Lees. 2007. Development and validation of conventional and quantitative polymerase chain reaction assays for the detection of storage rot potato pathogens, *Phytophthora erythroseptica*, *Pythium ultimum* and *Phoma foveata*. *Journal of Phytopathology*. 155: 309–315.
- Cullen D.W., I.K. Toth, Y. Pitkin, N. Boonham, K. Walsh, I. Barker, and A.K. Lees. 2005. Use of quantitative molecular diagnostic assays to investigate *Fusarium* dry rot in potato stocks and soil. *Phytopathology* 95: 1462–1471.
- Cwalina-Ambroziak B. 2012. The effect of foliar fertilizers on mycelial growth of select pathogenic fungi under *in vitro* conditions. *Pol. J. Environ. Stud.* 21: 589–594.
- Delgado J.A., P.B. Schwarz, J. Gillespie, V.V. Rivera-Varas and G.A. Secor. 2010. Trichothecene mycotoxins associated with potato dry rot caused by *Fusarium graminearum*. *Phytopathology* 100: 290–296.
- Demirci E., E. Dane and C. Eken. 2011. *In vitro* antagonistic activity of fungi isolated from sclerotia on potato tubers against *Rhizoctonia solani*. *Turk. J. Biol.* 35: 457–462.
- El-Hassan K.I., M.G. El-Saman, A.A. Mosa and M.H. Mostafa. 2007. Variation among *Fusarium* spp. the causal of potato tuber dry rot in their pathogenicity and mycotoxins production. *Egypt. J. Phytopathol.* 35: 53–68.
- Eken C., E. Demirci and F. Sahin. 2000. Pathogenicity of the fungi determined on tubers from potato storages in Erzurum, Turkey. *Journal of Turkish Phytopathology* 29: 61–69.
- Estrada R., N.C. Gudmestad, V.V. Rivera and G.A. Secor. 2010. *Fusarium graminearum* as a dry rot pathogen of potato: Prevalence, aggressiveness and factors affecting etiology. *Plant. Pathol.* 59: 1114–1112.
- Fiers M., C. Chatot, V. Edel-Hermann, Y. Le Hingrat, A. Yanougo Konate, N. Gautheron, E. Guillery, C. Alabouvette and C. Steinberg. 2010. Diversity of microorganisms associated with atypical superficial blemishes of potato tubers and pathogenicity assessment. *Eur. J. Plant Pathol.* 128:353–371.
- Flores-Gonzalez R., I. Velasco and F. Montes. 2008. Detection and characterization of *Streptomyces* causing potato common scab in Western Europe. *Plant Pathology*. 57:162–169.
- Gachango E., L. Hanson, A. Rojas, J. Hao and W. Kirk. 2012. *Fusarium* spp. Causing dry rot of seed potato tubers in Michigan and their sensitivity to fungicides. *Plant Disease* <http://dx.doi.org/10.1094/PDIS-11-11-0932-RE>
- Gardes M. and T.D. Bruns. 1993. ITS primers with enhanced specificity for Basidiomycetes – Application to the identification of mycorrhizae and rusts. *Molecular Ecology*. 2:113–118.
- Gherbawy Y. and N. Hussien. 2010. Molecular characterization of *Mucor circinelloides* and *Rhizopus stolonifer* strains isolated from some Saudi fruits. *Journal of Foodborne pathogens and disease* 7: 137–142.
- Gherbawy Y., H.M.A. Abdelzaker, J. Meens and H. El-Hariry. 2005. Morphological and molecular identification of some closely related *Pythium* species in Egypt. *Archives of Phytopathology and Plant Protection* 38: 193–208.
- Gherbawy Y., I. Druzhinina, G.M. Shaban, M. Wuczkowski, M. Yaser, M.A. El-Naghy, H. Prillinger and C.P. Kubicek. 2004. *Trichoderma* populations from alkaline agricultural soil in the Nile valley, Egypt, display an unusual poor biodiversity. *Mycological progress* 3: 211–218.
- Gherbawy Y., C. Kesselboth, H. Elhariry and K. Hoffmann. 2010. Molecular barcoding of microscopic fungi with emphasis on the mucoralean genera *Mucor* and *Rhizopus* (2010): In: Gherbawy Y. and Voigt K. (eds.) *Molecular identification of fungi*. Springer Verlag, Berlin, Heidelberg, New York. p. 213–250.
- Gudmestad N.C., R.J. Taylor and J.S. Pasche. 2007. Management of soilborne diseases of potato. *Australasian Plant Pathology*. 36: 109–115.
- Hide G.A., P.J. Read and S.M. Hall. 1992. Resistance to thiabendazole in *Fusarium* species isolated from potato tubers affected by dry rot. *Plant Pathol.* 41:745–748.
- Köljalg U., K.H. Larsson, K. Abarenkov, R.H. Nilsson, I.J. Alexander, U. Eberhardt, S. Erland, K. Høiland, R. Kjølner and E. Larsson. 2005. UNITE: a database providing web-based methods for the molecular identification of ectomycorrhizal fungi. *New Phytologist* 166:1063–1068.
- Lui L.H. and A.C. Kushalappa. 2002. Response surface models to predict potato tuber infection by *F. sambucinum* from duration of wetness and temperature, and dry rot lesion expansion from storage time and temperature. *Int. J. Food Microbiol.* 76, 19–25.
- Lui L.H., A. Vikram, Y. Abu-Nada, A.C. Kushalappa, G.S.V. Raghavan and K. Al Mughrabi. 2005. Volatile metabolic profiling for discrimination of potato tubers inoculated with dry and soft rot pathogen. *Am. J. Potato Res.* 82:1–8.
- Lydia S.T., D.A. Johnson and T.F. Cummings. 2012. The effects of wounding and potato cultivar on pathogenicity and aggressiveness of *Alternaria solani* and *A. alternata* 39–45. Proceedings of the Washington-Oregon Potato Conference January 24–26, 2012. Kennewick, Washington. <http://www.potatoes.com/IPMStuff/PDFs/Proceedings2012.pdf>
- Manici L.M. and C. Cerato. 1994. Pathogenicity of *Fusarium oxysporum* f. sp. *tuberosi* isolates from tubers and potato plants. *Potato Res.* 37: 129–134.
- Mirhendi H., A. Ghiasian, H.F. Vismer, M.R. Asgary, N. Jalalizand, M.C. Arendrup and K. Makimura. 2010. Preliminary identification and typing of pathogenic and toxigenic *Fusarium* species using restriction digestion of ITS1–5.8S rDNA–ITS2 region. *Iranian J. Publ. Health.* 39: 35–44.
- Nasr-Esfahani M. 1998. *Fusarium* species associated with dry rot of potato tubers in Esfahan. *Iran. J. Plant Pathol.* 34: 225–232.
- Nauman A., M. Navarro-González, O. Sánchez-Hernández, P.J. Hoegger and U. Kües. 2007. Correct identification of wood-inhabiting fungi by ITS analysis. *Current Trends in Biotechnology and Pharmacy* 1: 41–61.
- Nilsson R.H., E. Kristiansson, M. Ryberg, N. Hallenberg and K.H. Larsson. 2008. Intraspecific ITS variability in the kingdom Fungi as expressed in the international sequence databases and its implications for molecular species identification. *Evolutionary Bioinformatics* 4: 193–201.

- Peters J.C., A.K. Lees, D.W. Cullen, L. Sullivan, G.P. Stroud and A.C. Cunnington. 2008. Characterization of *Fusarium* spp. responsible for causing dry rot of potato in Great Britain. *Plant Pathol.* 57: 262–271.
- Pitman A.R., P.J. Wright, M.D. Galbraith and S.A. Harrow. 2008. Biochemical and genetic diversity of pectolytic enterobacteria causing soft rot disease of potatoes in New Zealand. *Australasian Plant Pathology* 37: 559–568.
- Salami A.O. and O.O. Popoola. 2007. Thermal control of some post-harvest rot pathogens of Irish potato (*Solanum tuberosum* L.). *Journal of Agricultural Sciences* 52: 17–31.
- SAS Institute, Inc. 1996. SAS/STAT User's Guide, Version 6, 12th ed. Vol. 2, 846 pp. SAS Institute, Inc. Cary, North Carolina, USA.
- Satyaprasad K., G.L. Bateman and P.J. Read. 1997. Variation in pathogenicity on potato tubers and sensitivity to thiabendazole of the dry rot fungus *Fusarium avenaceum*. *Potato Res.* 40: 357–366.
- Schisler D.A. and P.J. Slininger. 1994. Selection and performance of bacterial strains for biologically controlling *Fusarium* dry rot of potatoes incited by *Giberella pulicaris*. *Plant Disease* 78: 251–255.
- Schisler D.A., P.J. Slininger and R.J. Bothast. 1997. Effects of antagonists cell concentration and two-strain mixtures on biological control of *Fusarium* dry rot of potatoes. *Phytopathology* 87: 177–183.
- Secor G.A. and B. Salas. 2001. *Fusarium* dry rot and fusarium wilt. In: *Compendium of potato diseases*, eds. W.R. Stevenson, R. Loria, G.D. Franc, and D.P. Weingartner, 23–25. St. Paul, MN, USA: APS Press.
- Senter L.H., D.R. Sanson, D.G. Corley, M.S. Tempesta, A.A. Rottinghaus and G.E. Rottinghaus. 1991. Cytotoxicity of trichothecene mycotoxins isolated from *Fusarium sporotrichioides* (MC-72083) and *Fusarium sambucinum* in baby hamster kidney (BHK-21) cells. *Mycopathologia* 113:127–131.
- Slininger P.J., K.D. Burkhead and A.D. Schisler. 2004. Antifungal and sprout regulatory bioactivities of phenylacetic acid, indole-3-acetic acid and tyrosol isolated from the potato dry rot suppressive Bacterium *Enterobacter cloacae* S11: T:07. *J. Ind. Microbiol. Biotechnol.* 31: 517–524.
- Song H., H. Lee, J. Jeong, H. Park and C. Lee. 2008. Diversity in beauvericin and enniatins H, I and MK1688 by *Fusarium oxysporum* isolated from potato. *Int. J. Food Microbiol.* 122: 296–301.
- Tivoli B., A. Deltour, D. Molet, P. Bedin and B. Jouan. 1986. Isolation of thiabendazole resistant strains of *Fusarium roseum* var. *sambucinum* from potato tubers. *Agronomie* 6: 219–224.
- Tsror L. 2010. Biology, epidemiology and management of *Rhizoctonia solani* on Potato. *J. Phytopathol.* 158: 649–658.
- White T.J., T. Bruns, S. Lee and J. Taylor. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis M.A., Gelfand D.H., Sninsky J.J. and White T.J., eds. *PCR protocols. A Guide to Methods and Applications*. San Diego: Academic Press: ed. 315–322.
- Woodhall J.W., A.K. Lees, S.G. Edwards and P. Jenkinson. 2007. Characterization of *Rhizoctonia solani* from potato in Great Britain. *Plant Pathol.* 56: 286–295.