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Variation among *Fusarium* spp. the Causal of Potato Tuber Dry Rot in their Pathogenicity and Mycotoxins Production

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The pathogenicity of 33 isolates of *Fusarium* spp. isolated from potato tubers showed varied reactions. Nineteen isolates were pathogenic to tuber slices cv. Spunta and the remaining 14 isolates were non-pathogenic.

Ten isolates from the tested *Fusarium* spp. were chosen, five of them showed avirulent interaction with potato tuber slices and five were virulent, whether they belong to one species or not. The ability of chosen isolates for producing mycotoxins in culture filtrates or in inoculated potato tuber slices was studied. Qualitative and quantitative determination of secreted toxins indicated that the tested isolates produced four mycotoxins which were identified as fusaric acid, fumonisin, zearalenone and T-2 toxin.

Data indicated the presence of significant positive correlation coefficient between fusaric acid production and disease incidence which reached to +0.676 and +0.691 in cultural filtrates and in inoculated slices, respectively. On the other hand, correlation coefficient between T-2 toxin and disease incidence reached -0.071 and -0.668 in cultural filtrates and inoculated potato tuber slices, respectively. There was no relation between fumonisin and zearalenone production and virulence or avirulence state.

Therefore, the effect of fusaric acid in three concentrations on the interaction between avirulent isolates and potato tuber slices was studied. Treating potato tuber slices by fusaric acid prior to inoculation by avirulent isolates changed the interaction from avirulent to virulent state. Fusaric acid increased all figures of disease incidence, *i.e.* infection (%), disease severity (%), weight of rotted tissue and sporulation capacity of the tested isolates.

It was also found that fusaric acid has an inhibitory effect on peroxidase and polyphenol oxidase activity either in potato tuber slices or in extracted protein of treated tubers. Moreover, fusaric acid prevented periderm formation in potato tuber slices. It decreased number of periderm layers from 6±0.8 in non treated slices to 2±0.2 in slices treated with 0.448 and 0.896 µg/ml of fusaric acid.

Keywords: Fumonisin, fusaric acid, periderm formation, peroxidase, polyphenol oxidase, T-2 toxin and zearalenone.

Fusarium dry rot of potatoes is a worldwide economic problem (Nielsen, 1981). The disease may cause greater losses of potatoes than any other post-harvest disease (Powelson *et al.*, 1993).

Crop losses attributed to dry rot have been estimated to an average of 6 to 25% (Chelkowski, 1989). There are many species of *Fusarium* reported to cause dry rot of potato-worldwide (Nielsen, 1981). Some of these *Fusarium* species also produce mycotoxins (Marasas *et al.*, 1984).

It is well established that different *Fusarium* spp. causing dry rot differed in their pathogenicity on potato tubers (Hanson *et al.*, 1996; Ray and Hammerschmidt, 1998; El-Hassan *et al.*, 2004). *Fusarium* spp. have the ability to produce different mycotoxins, *i.e.* trichothecene (T-2 toxin), zearalenone, fumonisin, fusaric acid and other mycotoxins during growth in cultures (Logrieco *et al.*, 1990; Bacon *et al.*, 1996; Voss *et al.*, 1999; Prange *et al.*, 2005 and Jurado *et al.*, 2005). Also, they produced these mycotoxins in potatoes infected naturally with these fungi in the field or in storage (Desjardins and Plattner, 1989; Herrmann *et al.*, 1996; Venter and Steyn, 1998).

This study was planned to investigate the relationship between mycotoxins production and the pathogenicity of different *Fusarium* isolates on potato tuber slices.

Material and Methods

Fungi:

Different *Fusarium* species were isolated from potato tubers show dry rot symptoms collected from different locations in Kalyubia governorate, Egypt. Fungi associated with dry rot tubers were isolated and identified according to Booth (1971). The purified fungi were cultured on PDA medium and incubated at 25°C for 7 days and used for preparing spore suspensions. Spore suspensions were carried out in sterilized distilled water from 8 day old culture and adjusted to 10^5 spore/ml for inoculating potato tuber slices.

Potato tubers and pathogenicity:

Newly harvested and uniform in size potato tubers, cv. Spunta, were kindly obtained from the Committee of Potato Producers, Egypt. Tubers were washed under tap water to remove adhesive soil, then dried and stored at 6-8°C till used. Tubers were taken and left at room temperature for 24h before being used. Tubers were surface sterilized in 0.5 % sodium hypochlorite solution for 10-15 min. and rinsed in sterile water, then cut into slices (10 mm thick) with a sterile knife, after removing the base and the top of tuber. Slices were rinsed in sterile water, placed on wet sterile filter paper in 15 cm Petri dishes and left for 2h before inoculation with the fungal spore suspension. Inoculation of potato tuber slices was carried out by spreading the spore suspension on slice surface till droplet run off. Each tuber slice was inoculated with 0.5 ml spore suspension of each tested Fusarium isolates, and incubated for 4 days at 25±1°C in the dark. Control slices were inoculated with

0.5ml sterile water. The progress of infection was examined daily on 8 replicate slices for each isolate (El-Hassan *et al.*, 2004). The percentage of infection, disease severity, weight of rotted tissue and sporulation capacity were determined.

Disease severity was assessed using the scale described by Sharawy (1988) as follows: 100 % when all the surface of slices was completely covered with infection, 50 % when half of the surface of slices was covered with infection, 25 % when 1/4 of the surface of slices was infected, 12.5 % when 1/8 of the surface of slices was infected and 0 % when no infection occurred. Sporulation capacity of isolates on tuber slices was determined as number of spores / cm^2 of inoculated surface after 4 days from inoculation.

Production of Fusarium toxins in culture filtrates:

Ten isolates of *Fusarium* sp. (five virulent and five avirulent) were tested for their ability to produce mycotoxins (zearalenone, fumonisin, T-2 toxin and fusaric acid).

1. Production of zearalenone, fumonisin and T-2 toxin:

Single disks (0.5cm in diameter) of each *Fusarium* isolate were grown on 50 ml of PDB (potato dextrose broth) in 200 ml Erlenmeyer flasks. Five replicates of each isolate were prepared and incubated at 25±1°C for 21 days after which the toxins were extracted.

2. Production of fusaric acid in culture filtrates:

Production of fusaric acid in culture filtrates was carried out according to the method described by Venter and Steyn (1998). Single plugs (0.5cm in diameter) from the mycelium of *Fusarium* isolates were used to inoculate Erlenmeyer flasks containing 50 ml culture medium. The culture medium consists of the following: 0.74g $MgSO_4.7H_2O$, 0.045g $MnSO_4.4H_2O$, 0.018g $ZnSO_4.7H_2O$, 5.0mg $CuSO_4.5H_2O$, 0.84g $FeSO_4.7H_2O$, 0.11g Na_2EDTA, 0.1g myo-inositol, 2.0mg glycine, 0.5mg nicotinic acid, 0.5mg pyridoxine HCl, 0.1mg thiamine HCl, 10.0g glucose, 2.0g L-asparagine, 1.0g KH_2PO_4 and 5.0µg biotin in 1000ml distilled water. The flasks were incubated at 22±1°C in the dark on an orbital shaker (50 r.p.m.) for 21days after which fusaric acid was extracted.

Extraction of Fusarium toxins from cultural filtrates and inoculating of potato tuber slices:

Mycotoxins of *Fusarium* spp. were extracted from cultural filtrates of virulent or avirulent isolates according to the method described by Mule *et al.* (1997) as follows: 25 ml of culture filtrate was defatted with *n*-hexane (25 ml per extraction for three extractions) and then extracted exhaustively with dichloromethane (15 ml per extraction for three extractions). The organic extracts were collected and evaporated to dryness under reduced pressure at room temperature (24 to 28°C), and the residue was dissolved in 1 ml methanol. Mycotoxins were extracted from (20g) inoculated surface of potato tuber slices (2 mm) in a solution consisted of methanol: NaCl 1% (55:45 v/v) then treated as mentioned before.

Separation and quantitative determination of toxins:

Mycotoxins were determined quantitatively according to the method described by Venter and Steyn (1998) by HPLC (High Performance Liquid Chromatography). A 50 µl sample of methanol was eluted isocratically using a reversed phase of Bondclone 10 C18 column (10 mm, 300×3.9 mm) with 40% methanol and 60 % of an aqueous solution of 0.62 mM Na_2EDTA and 2% H_3PO_4 (Jullien, 1988) at a flow rate of 1ml min^{-1}. The elute was monitored at 254 nm, using a Beckman System Gold Model 166 UV detector. The flow rate was 1 ml min^{-1}. Standard curves were established with pure mycotoxins (Sigma, USA). The column was stored in 100% methanol and equilibrated with eluting buffer 1h before use. Samples were injected as methanol solutions.

Effect of fusaric acid on dry rot incidence:

Potato tubers slices were treated by fusaric acid (Sigma, USA) solution according to the method adopted by Doke *et al.* (1976). Fusaric acid was firstly dissolved in 0.5 ml methanol and the volume was completed by distilled water to give required concentration. The compound was tested in three concentrations 0.224, 0.448 and 0.896 µg/ml, in addition to slices dipped in distilled water amended with the same volume of methanol. Slices were placed on wet sterile filter paper in 15 cm glass Petri dishes, three dishes were used for every particular treatment, every one contained 4 slices. Spore suspension of 3 avirulent isolates FSA2 of *F. sambucinum*, FEQ8 of *F. equiseti* and FOX14 of *F. oxysporum* were used for inoculation of either treated or non-treated slices. Dishes were incubated at 25±1°C in the dark for 4 days. Disease incidence was determined as previously mentioned.

Effect of fusaric acid on periderm formation:

Effect of different concentrations of fusaric acid on the histological aspects (periderm formation) was studied in the upper layer of treated potato tuber slices. Samples were taken for histological studies from potato slices treated with (0, 0.224, 0.448 and 0.896µg/ml) for 2 h and incubated at day light for 7 days. Samples were prepared according to the method described by Miksche (1976). Sections were microtomed at 9-12 microns. Ribbons of serial sections were fixed to slides by means of Albusol adhesive (5ml albumin, 10 ml formalin and 185 ml distilled water), then stained with safranin-light green solutions, and then mounted in Canada balsam. Photomicrographs were obtained by a Nikon camera Type 115 (Nikon FX-35).

Effect of fusaric acid on peroxidase and polyphenol oxidase activities:

1. In potato tuber slices:

The upper layer of potato tuber slices (2 mm) treated by fusaric acid and inoculated or not was collected, then kept under -8°C till use. One g of potato sample was treated by liquid nitrogen and ground with 2 ml sodium phosphate buffer pH (6.5) 0.1 M using a mortar and pestle. Samples were transferred to Eppendorf tubes, and then centrifuged for 20 min at 12000 rpm at 4°C.

Supernatant, containing water-soluble enzymes were stored at -8°C till use. Three replicates were prepared for each treatment. Peroxidase and polyphenol

oxidase activities were determined according to the method of Biles and Martyn (1993) using catechol as a substrate and measuring was carried out every 30 second at 495nm using spectrophotometer model Unico-2100. Peroxidase and polyphenol oxidase activities were expressed as (Δ) change in absorbance of optical density (OD) per gram fresh weight.

2. In extracted protein of untreated tubers:

Potato tubers were peeled, sliced then total protein was extracted by sodium phosphate buffer pH (6.5) 0.1M using a mortar and pestle. Total protein was determined by Coomassie brilliant blue G-250 as described by Asryants *et al.* (1985). The activity of peroxidase and polyphenol oxidase was determined in protein extracts under the influence of different concentrations (0.224, 0.448 and 0.896 µg/ml) of fusaric acid. Peroxidase and polyphenol oxidase activities were determined as mentioned above using catechol as a substrate and measuring was carried out every 30 seconds at 495nm. Peroxidase and polyphenol oxidase activities were expressed as (Δ) change in absorbance of optical density (OD)/mg protein.

Statistical analysis:

All experiments were set up in a complete randomized design. 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

1. Pathogenicity test of Fusarium spp. isolated from potato tubers showing dry rot symptoms:

The pathogenicity test of *Fusarium* spp. isolates were carried out on potato tuber slices cv. Spunta. It is shown from data in Table (1) that *Fusarium* spp. isolates varied in their pathogenicity. They ranged from highly pathogenic isolates, *i.e.* isolates FSA7 and FSA9 of *F. sambucinum*; FSO15, FSO17, FSO21, FSO24, FSO27 and FSO30 of *F. solani*; FOX31 of *F. oxysporum* to completely nonpathogenic isolates, *i.e.* FSA1, FSA2, FSA3, FSA23 and FSA28 of *F. sambucinum*; FOX6, FOX32 and FOX33 of *F. oxysporum*; FCU4 of *F. culmorum*; FEQ8 and FEQ11 of *F. equiseti* and FSE12 of *F. semitectum*.

2. The ability of Fusarium spp. for toxin(s) production:

Five isolates of highly pathogenic *Fusarium* spp. Isolates, *i.e.* FSA7 of *F. sambucinum*, FSO15, FSO21, FSO24 and FSO27 of *F. solani* and five isolates of avirulent isolates, *i.e.* FSA2, FSA3 of *F. sambucinum*, FEQ8 of *F. equiseti* and FOX14, FOX33 of *F. oxysporum* were chosen to study their ability for their mycotoxins production, as clear in Tables (2 & 3).

Toxins produced in cultural filtrates and in inoculated slices by all isolates of *Fusarium* spp. were identified as zearalenone, fumonisin, T-2 toxin and fusaric acid. The isolated toxins were determined quantitively.

The virulent *Fusarium* spp. isolates produced high amounts of fusaric acid in liquid culture filtrates ranging from 0.80µg/ml to 0.31µg/ml and 0.448µg/ml as

Table 1. Variation among *Fusarium* spp. isolates as inducing potato tuber slices rot

Fusarium sp.	Isolate code	Infection (%)	Disease * severity (%)	Weight of rotted tissue (g)	Sporulation capacity (10^5/cm^2)
F. sambucinum	FSA1	0.0^{G}	0.0^{L}	0.0^{J}	0.0^{P}
	FSA2	0.0^{G}	0.0^{L}	0.0^{J}	0.0^{P}
	FSA3	0.0^{G}	0.0^{L}	0.0^{J}	0.0^{P}
	FSA7	100^{A}	100^{A}	21.3^{B}	114.6^{C}
	FSA9	100^{A}	95^{BC}	14.5^{D}	14.5^{LM}
	FSA10	0.0^{G}	0.0^{L}	0.0^{J}	0.0^{P}
	FSA19	83^{B}	62.6^{G}	7.3^{H}	53.6^{H}
	FSA23	0.0^{G}	0.0^{L}	0.0^{J}	0.0^{P}
	FSA26	16.6^{F}	11.2^{J}	1.3^{J}	11.7^{N}
	FSA28	0.0^{G}	0.0^{L}	0.0^{J}	0.0^{P}
	FSA29	66.4^{C}	47^{H}	5.4^{I}	67.2^{G}
F. solani	FSO5	83^{B}	60.7^{G}	6.9^{HI}	14.6^{LM}
	FSO15	100^{A}	97.5^{AB}	19^{C}	91.1^{E}
	FSO17	100^{A}	95.8^{ABC}	23.8^{A}	129.8^{A}
	FSO18	50^{D}	25^{I}	1.5^{J}	13.2^{MN}
	FSO20	16.6^{F}	5.3^{K}	0.3^{J}	9.3^{O}
	FSO21	100^{A}	94.2^{CD}	19.7^{BC}	44^{I}
	FSO24	100^{A}	90.5^{D}	12.3^{EF}	89.4^{E}
	FSO25	83^{B}	50.7^{H}	9.3^{G}	77.9^{F}
	FSO27	100^{A}	90.6^{D}	23.3^{A}	12.8^{MN}
	FSO30	100^{A}	92.2^{CD}	19.3^{C}	16.6^{L}
F. oxysporum	FOX6	0.0^{G}	0.0^{L}	0.0^{J}	0.0^{P}
	FOX13	33.3^{E}	28.6^{I}	1.7^{J}	109.5^{D}
	FOX14	0.0^{G}	0.0^{L}	0.0^{J}	0.0^{P}
	FOX16	33.3^{E}	12.4^{J}	1.4^{J}	23.1^{K}
	FOX31	100^{A}	73.7^{F}	11.1^{FG}	25.7^{J}
	FOX32	0.0^{G}	0.0^{L}	0.0^{J}	0.0^{P}
	FOX33	0.0^{G}	0.0^{L}	0.0^{J}	0.0^{P}
F. culmorum	FCU4	0.0^{G}	0.0^{L}	0.0^{J}	0.0^{P}
	FCU22	83^{B}	82^{E}	13.3^{DE}	121.6^{B}
F. equiseti	FEQ8	0.0^{G}	0.0^{L}	0.0^{J}	0.0^{P}
	FEQ11	0.0^{G}	0.0^{L}	0.0^{J}	0.0^{P}
F. semitectum	FSE12	0.0^{G}	0.0^{L}	0.0^{J}	0.0^{P}

* Measured as percentage of surface colonized by the fungus.
Values with the same letters in each column are not significantly different at P = 0.05.

Table 2. Concentrations of *Fusarium* spp. toxins (µg/ml cultural filtrate) produced in artificial medium by pathogenic and non-pathogenic isolates *in vitro* after 21 days from inoculation

Fusarium sp.	Isolate code	Disease severity (%)	Fusaric acid	Fumon-isin	Zearal-enone	T-2 toxin
F. sambucinum	FSA2	0.0	0.29	0.68	0.0	0.51
F. sambucinum	FSA3	0.0	0.13	0.18	0.03	0.0
F. equiseti	FEQ8	0.0	0.0	0.75	0.35	0.01
F. oxysporum	FOX14	0.0	0.28	0.52	0.0	0.32
F. oxysporum	FOX33	0.0	0.0	0.71	0.31	0.0
Average		0.0	0.140	0.568	0.138	0.168
F. sambucinum	FSA7	100	0.31	0.44	0.04	0.12
F. solani	FSO15	97.5	0.31	0.02	0.0	0.0
F. solani	FSO21	94.2	0.45	0.58	0.05	0.12
F. solani	FSO24	90.5	0.37	0.73	0.0	0.07
F. solani	FSO27	90.6	0.80	0.91	0.07	0.43
Average		94.56	0.448	0.536	0.032	0.148
Correlation coefficient		--	0.676*	-0.097	-0.425	-0.071

* Correlation is significant at the 0.05.

Average. In infected tissues, fusaric acid ranged from 0.82µg/g to 0.0µg/g, and 0.48µg/g fresh weight as average. In case of the avirulent isolates, fusaric acid in culture filtrates ranged from 0.29µg/ml to 0.0µg/ml, and 0.14µg/ml as average and in infected tissues ranged from 0.25µg/g to 0.0µg/g and 0.066µg/g fresh weight as average. Significant correlation between disease incidence and fusaric acid production in culture filtrates and in infected tissues reached + 0.676 and + 0.691, respectively.

No clear difference in production of zearalenone, fumonisin and T-2 toxin between virulent and avirulent *Fusarium* isolates. Virulent isolates produced zearalenone, fumonisin and T-2 toxin reached at average 0.032, 0.536 and 0.148 µg/ml in liquid filtrate and 0.12, 0.262 and 0.16 µg/g fresh weight in infected tissues, respectively. While avirulent isolates produced zearalenone, fumonisin and T-2 toxin reached an average of 0.138, 0.568 and 0.168 µg/ml in liquid filtrate and 0.04, 0.098 and 0.374 µg/g in infected tissues of potato tuber slices, respectively. Concentration of zearalenone was low comparative to other toxins, either in culture filtrate or in infected potato tissues. Correlation coefficient between disease incidence and zearalenone, fumonisin and T-2 toxin production in cultured filtrates and in infected tissues were -0.425, -0.097, -0.071 and +0.337, +0.393, -0.668, respectively. No significant correlation between disease incidence and zearalenone, fumonisin, T-2 toxin production in cultural filtrates. Meanwhile, T-2 toxin gave only the negative significant correlation in infected tissues.

Table 3. Concentrations of *Fusarium* spp. toxins (µg/g fresh weight) produced in potato tuber slices by virulent and avirulent isolates *in vitro* after 4 days from inoculation

Fusarium sp.	Isolate code	Fusaric acid	Fumonisin	Zearalenone	T-2 toxin
Avirulent	--	--	--	--	--
F .sambucinum	FSA2	0.05	0.13	0.18	0.57
F. sambucinum	FSA3	0.0	0.0	0.01	0.30
F. equiseti	FEQ8	0.0	0.0	0.0	0.43
F. oxysporum	FOX14	0.03	0.33	0.0	0.27
F. oxysporum	FOX33	0.25	0.03	0.0	0.30
Average		0.066	0.098	0.038	0.374
Virulent	--	--	--	--	--
F. sambucinum	FSA7	0.35	0.72	0.01	0.18
F. solani	FSO15	0.75	0.0	0.42	0.36
F. solani	FSO21	0.48	0.31	0.02	0.07
F. solani	FSO24	0.0	0.0	0.07	0.03
F. solani	FSO27	0.82	0.28	0.08	0.16
Average		0.480	0.262	0.120	0.160
Correlation coefficient		0.691*	0.393	0.337	-0.668*

* Correlation is significant at the 0.05.

3. Effect of fusaric acid on potato dry rot incidence:

The effect of four concentrations of fusaric acid on infection of potato tuber slices by 3 avirulent isolates (FSA2, FEQ8 and FOX14) was evaluated. Data in Table (4) indicate that fusaric acid at concentrations of 0.448 and 0.896µg/ml change the interaction between the fungus and tuber tissues.

It changed the state from avirulent to virulent. It increased significantly the infection percentage, disease severity, weight of rotted area and sporulation capacity of avirulent isolates, while the lower concentration (0.224µg/ml) of fusaric acid was ineffective in comparative to the control (Table 4 & Fig. 1).

4. Histological studies:

As clear in Figs. (2 & 3), examination of sections from non-treated tuber slices revealed that the periderm was composed of 5-8 layers of bricklike, thin-walled cells, one over the other, without intercellular spaces and with suberized cell walls (Fig. 2A). While sections from treated potato tuber slices by different concentrations (0.224, 0.448 and 0.896 µg/ml) of fusaric acid revealed that the periderm was mainly restricted to the outermost cell layers where fusaric acid prevented periderm formation in treated potato tuber slices. It decreased number of periderm layers from 6±0.8 in non treated slices to 2±0.2 in slices treated with 0.448 and 0.896 µg/ml of fusaric acid as clear in Fig. 2 (C & D) and Fig. (3).

Table 4. Effect of different concentrations of fusaric acid on infection of potato tuber slices cv. Spunta by three avirulent *Fusarium* isolates

Fusarium isolate	Isolate code	Fusaric acid µg/ml	Infection (%)	Disease severity (%)	Weight of rotted tissue (g)	Sporulation capacity (10^5/cm^2)
F. sambucinum	FSA2	0.896	75^{B} *	46^{C}	6.6^{B}	1.8^{C}
		0.448	100^{A}	72^{B}	11.1^{A}	3.8^{A}
		0.224	0.0^{D}	0.0^{G}	0.0^{E}	0.0^{E}
		0.0	0.0^{D}	0.0^{G}	0.0^{E}	0.0^{E}
F. equiseti	FEQ8	0.896	100^{A}	42^{D}	4.5^{C}	2.6^{B}
		0.448	100^{A}	81^{A}	11.2^{A}	4.2^{A}
		0.224	0.0^{D}	0.0^{G}	0.0^{E}	0.0^{E}
		0.0	0.0^{D}	0.0^{G}	0.0^{E}	0.0^{E}
F. oxysporum	FOX14	0.896	50^{C}	30^{F}	3.5^{D}	1.3^{D}
		0.448	50^{C}	37^{E}	4.3^{C}	2.5^{B}
		0.224	0.0^{D}	0.0^{G}	0.0^{E}	0.0^{E}
		0.0	0.0^{D}	0.0^{G}	0.0^{E}	0.0^{E}

* Values with the same letters in each column are not significantly different at P= 0.05.

5. Effect of fusaric acid on soluble peroxidase and polyphenol oxidase activities:

5.1. In potato tuber slices:

Soluble peroxidase and polyphenol oxidase activities extracted from inoculated treated and untreated by fusaric acid tissues were determined using catechol as a substrate. The activity was determined in potato tuber slices treated by different concentrations (0, 0.224, 0.448 and 0.896 µg/ml) of fusaric acid and non-inoculated or inoculated by the avirulent isolate (FEQ8) of *F. equiseti* after 4 days from inoculation. Data of peroxidase and polyphenol oxidase activity are illustrated by Figs. (4 & 5). It's clearly shown from Fig. (4) that peroxidase and polyphenol oxidase activity was varied according to the treatment. Treated potato tuber slices by 0.448 and 0.896 µg/ml of fusaric acid showed low level of activity in comparison to control.

Peroxidase and polyphenol oxidase activity in potato tuber slices treated with fusaric acid and inoculated by avirulent isolate (FEQ8) of *F. equiseti* showed high level of activity with low concentration of fusaric acid and low level of activity was correlated with high concentrations of fusaric acid (Fig. 5).

5.2. In extracted protein of non treated tubers:

Protein containing peroxidase and polyphenol oxidase was extracted from potato tuber tissues. Solution was mixed with different concentrations of fusaric acid, left for one hour then peroxidase and polyphenol oxidase activity was determined spectrophotometrically using catecol as substrate. Data obtained indicated that fusaric acid has an inhibitory effect on the activity of both enzymes (Fig. 6).

Fig.1. Effect of fusaric acid on pathogenicity of different avirulent *Fusarium* isolates on potato tuber slices cv. Spunta. FSA2: *F. sambucinum*, FEQ8: *F. equiseti*, FOX14 *F. oxysporum*.

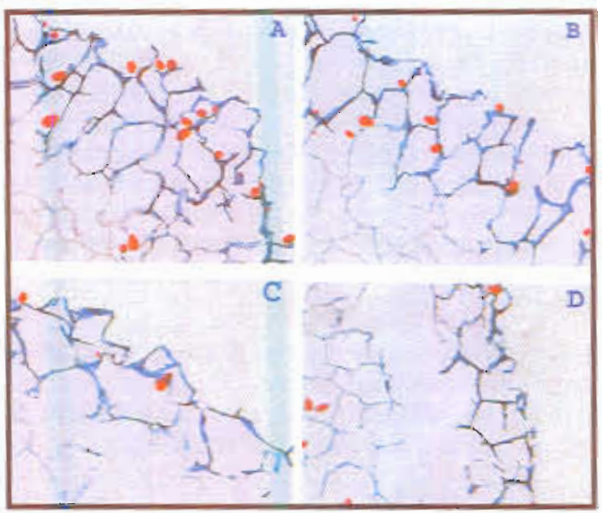

Fig. 2. Periderm formation in tuber tissues of potato cv. Spunta after treatment with different concentrations of fusaric acid. Note that: A= control, B= 0.224µg/ml, C= 0.448µg/ml and D= 0.896µg/ml.

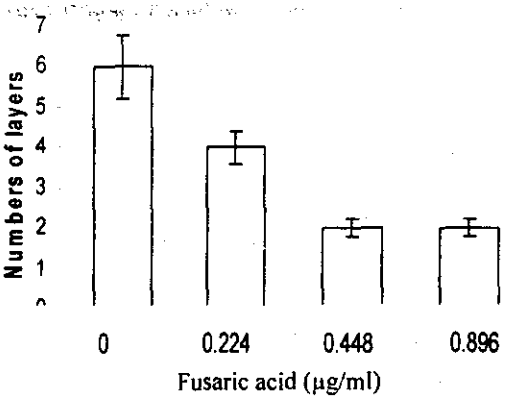

Fig. 3. Number of periderm layers under the influence of fusaric acid treatment.

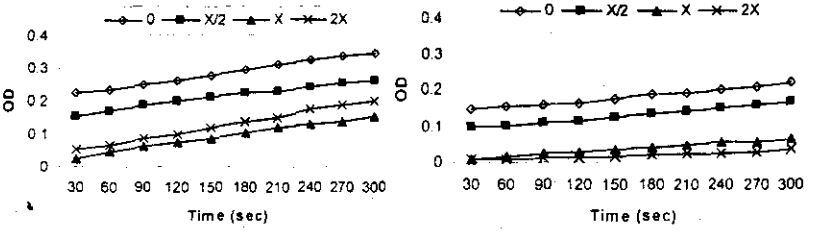

Fig. 4. Effect of different concentrations of fusaric acid on peroxidase activity (left) and polyphenol oxidase (right) in uninoculated potato slices cv. Spunta. X= 0.448 µg/ml.

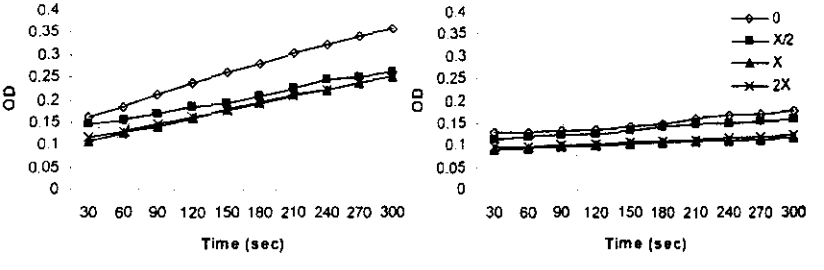

Fig. 5. Effect of different concentrations of fusaric acid on peroxidase activity (left) and polyphenol oxidase (right) in potato slices cv. Spunta inoculated by avirulent isolate (FEQ8) of *F. equiseti*. X= 0.448 µg/ml.

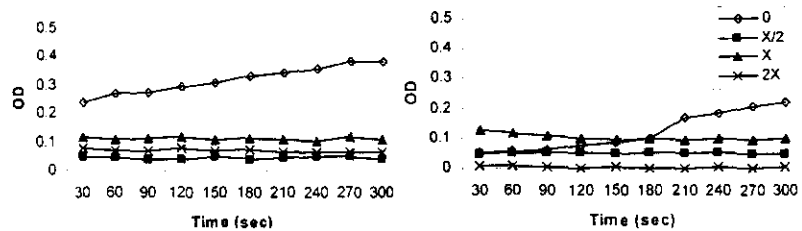

Fig. 6. Effect of different concentrations of fusaric acid on peroxidase (left) and polyphenol oxidase (right) activity in extracted protein of non treated tubers. Data were determined as (Δ) changed in absorbance of optical density (OD) at 495 nm. Every reading is average of three measurements. X= 0.448 µg/ml.

Discussion

Dry rot of potato tubers is considered as one of the devastating diseases infecting tubers during storage. The Disease is caused by different *Fusarium* spp. *Fusarium* spp. isolated from infected tubers varied in their pathogenicity (Ray and Hammerschmidt, 1998 and El-Hassan *et al.*, 2004).

Testing of 33 *Fusarium* spp. isolates isolated from diseased tubers showed the presence of great variation among isolates. Some isolates were highly pathogenic on tuber slices. whereas others were completely avirulent as they caused hypersensitive-like reaction, nevertheless that they belong to different species, for example, isolates FSA7, FSA9 of *F. sambucinum* were highly pathogenic to tuber slices, whereas, isolates FAS1, FSA2, FSA3 which belong to the same species were avirulent to potato slices. The mechanisms underlying this behaviour still unclear. Some studies proposed that mycotoxin produced by *Fusarium* spp. play a key role in this respect (Desjardins and Plattner, 1989; Herrmann *et al.*, 1996 and Venter and Steyn, 1998).

In the present study, it was proved that *Fusarium* isolates produced fusaric acid, zearalenone, fumonisin, T-2 toxin and may be produced other toxins. Data obtained indicated that only fusaric acid plays a considerable role in disease incidence. Correlation coefficient between fusaric acid production in cultural filtrate and disease incidence was +0.676 and in infected tubers was +0.691. In the same time, correlation coefficient between disease incidence and T-2 production in inoculated slices was -0.668. This unexpected result revealed that T-2 toxin may play a role in prevention of disease incidence.

The role of fusaric acid as a factor of pathogenicity was studied. This compound was tested in three concentrations. Treating potato tuber slices by either of tested concentrations changed the interaction between *Fusarium* isolates and potato tuber slices from avirulent to virulent.

In many studies it was found that enhancement of polyphenol oxidase and peroxidase is a marker of disease resistance (Graskova *et al.*, 2004; Valentines *et al.*, 2005 and Armas *et al.*, 2007). Data obtained indicated that the activities of both enzymes were decreased owing to the treatment of tuber slices by fusaric acid especially 0.448 and 0.896µg/ml concentrations in uninoculated tissues. The same trend of enzymes activities was observed in treated inoculated slices. The results attributed the occurrence of disease partially to fusaric acid production by virulent isolates due to its inhibitory effect on peroxidase and polyphenol oxidase activities.

Periderm formation is a factor of resistance of tubers against post harvest disease (Ray and Hammerschmidt, 1998 and El-Hassan *et al.*, 2004). Fusaric acid decreased number of periderm layers (Figs. 2 & 3), and may affect subsequently the chemical components associated with periderm formation, *i.e.* steroid glycoalkaloides (Weltring *et al.*, 1997).

As a conclusion, fusaric acid was found to be one of the factors of pathogenicity and other toxins, *i.e.* T-2 toxin may be a factor of inducing hypersensitive like the reaction associated with the behaviour of avirulence.

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التباين في القدرة المرضية لعزلات الفيوزاريم المسببة للعفن الجاف على درنات البطاطس وعلاقتها بإنتاج التوكسينات

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يعتبر العفن الجاف من أخطر الأمراض الفطرية التي تصيب البطاطس أثناء التخزين. يهدف هذا البحث إلى دراسة الفرق بين عزلات الفيوزاريم المختلفة في قدرتها المرضية وعلاقة ذلك بإنتاجها لأنواع مختلفة من التوكسينات مثل zearalenone, fumonisin, T-2 toxin, fusaric acid ودور هذه التوكسينات في حدوث وتطور مرض العفن الجاف.

تم دراسة قدرة خمس عزلات ممرضة وأخرى غير ممرضة على إنتاج التوكسينات السابقة الذكر على بيئات صناعية وعلى شرائح درنات البطاطس. حيث اختلفت العزلات في قدرتها على إنتاج التوكسينات على البيئات المختلفة، ولم يوجد ارتباط معنوي بين القدرة المرضية للعزلات وبين قدرتها على إنتاج التوكسينات باستثناء حمض الفيوزاريك حيث كان هناك ارتباطاً معنوياً بين القدرة المرضية للعزلات وقدرتها على إنتاج حمض الفيوزاريك سواء على البيئة الصناعية أو على شرائح درنات البطاطس، مما يعني أن لهذا التوكسين دور في حدوث العفن الجاف على درنات البطاطس.

لتأكيد دور حمض الفيوزاريك في حدوث وتطور العفن الجاف، تم معاملة شرائح درنات بطاطس بتركيزات مختلفة من حمض الفيوزاريك ثم حقنت بثلاث عزلات غير ممرضة، أظهرت النتائج تحول العزلات الغير ممرضة إلى ممرضة وظهور حالة المرض مما يؤكد دور هذا التوكسين في حدوث المرض.

درست بعض التغيرات المرافقة لحالة حدوث المرض أثناء معاملة شرائح البطاطس بحمض الفيوزاريك، فقد وجد نقص في نشاط كلا من إنزيمي البيروكسيديز والبولي فينول اوكسيديز وسرعة تكوين طبقة البريدرم في أنسجة درنات البطاطس المعاملة بالتركيزات السابقة من حمض الفيوزاريك سواء الغير معدية أو المعدية بالعزلة الغير ممرضة من الفيوزاريوم، مقارنة بالشاهد، وهذا النقص ارتبط طرديا بالتركيز المستخدم من حمض الفيوزاريك.