



Identification of *Fusarium* spp. Associated with Potato Tubers in Upper Egypt by Morphological and Molecular Characters

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Authors' contributions

This work was carried out in collaboration among all authors. Author YAG designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors MAH and EGAED managed the analyses of the study. Authors NAH and SAA managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/AJBGMB/2019/v2i330062

Editor(s):

(1) Dr. Theocharis Koufakis, 1st Department of Internal Medicine, Aristotle University, Thessaloniki, Greece.

Reviewers:

(1) Natalia Stasyuk, All-Russian Research Institute of Phytopathology, Russia.

(2) Rostand Romeo Chamedjeu, Pan African University and Jomo Kenyatta University of Agriculture and Technology, Kenya.
Complete Peer review History: <http://www.sdiarticle4.com/review-history/51810>

Original Research Article

Received 01 August 2019
Accepted 03 October 2019
Published 14 October 2019

ABSTRACT

Potato (*Solanum tuberosum* L.) is an important crop which holds promise for food security considering the global population growth rate. *Fusarium* dry rot is one of the most significant diseases of potato. To build up strategies for the control of this disease, it ought to be made primarily a correct diagnosis and identification of the pathogen. A total of 504 *Fusarium* isolates were recovered from potato tubers collected from Upper Egypt. *Fusarium* isolates were identified based on morphology and partial DNA sequencing of β -tubulin (TUB) genes. 62.5% of the isolates were identified as *F. sambucinum*, followed *F. oxysporum* (57.5%), then *F. verticillioides* (56.25%) and *F. incarnatum* (47.5%). All the tested *Fusarium* species were able to produce amylase. The pathogenicity of the isolates was tested by inoculation of healthy potato tubers; all of the tested isolates were pathogenic to healthy potato tubers. *F. sambucinum* had a highly virulent effect.

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Keywords: Potato (*Solanum tuberosum* L.); *Fusarium* spp.; pathogenicity; amylase.

1. INTRODUCTION

Potato (*Solanum tuberosum* L.) is a species of the Solanaceae family and an important crop is used in the diet through the worldwide [1]. Egypt is one of the biggest producers and exporters of potatoes in Africa [2]. In Egypt, the potato has a critical position among all vegetable crops, where about 20% of total region starch for vegetable production is cultivated with potato [3]. Moreover, approximately 4.5 million tons of potatoes are achieved from the cultivation of 268,077 hectares. Moreover, there is a normal generation of over 4.5 million tons with total cultivation of potatoes achieved 268,077 hectares [4]. This yield is financially fundamental to Egypt and any disrupting influences in its generation impact seriously its local and all the more significantly export impact. During their seasonal plantations (summer, winter Nile and fall), potato plants are subjected to different pathogens and insect pests which cause broad misfortune in Egyptian quantitative and qualitative potato yield [5,6].

Fusarium dry rot is a noteworthy post-harvest disease of potato tubers [7]. This infection is scattered worldwide and happens wherever potatoes are developed [8]. Storage losses related with *Fusarium* dry rot have been surveyed to extend from 6 to 25%, and occasionally losses as extraordinary as 60% have been accounted [1]. Potato dry rot is caused by different species of *Fusarium* spp. which are found in many soils where potatoes are cultivated and can survive as chlamydospores free in the soil for very long periods [9,10]. The infection influences tubers causing withered plants in the field. Tubers become contaminated through wounds during harvest, handling and transport but the symptoms become obvious after 2 to 3 months of storage [11]. Starting symptoms of *Fusarium* dry rot look like shallow dark depressions on the outside of the tuber, which can develop and become wrinkled in concentric rings as the underlying dead tissue desiccates [10,12]. Dry rot in potato diminishes the collect yield as well as taint the tubers with mycotoxins that cause cyto, geno, neuro and hepatotoxic effects in animals and people and thus threaten health when eaten directly (in *Fusarium*-infected plants) or indirectly (in the milk or meat of animals that have been fed with infected feed [1].

Fusarium spp. infect potato tubers change contingent upon the time of the survey and

geographic area. *Fusarium sambucinum* and *Fusarium solani* are the most common recognized pathogenic species in Egypt [13,14]. *Fusarium sambucinum* was the species most much of the time secluded from potato tubers among eight species reported in northeastern USA in 1992–1993 [15], but also more recently in China [7,16,17] reported that *F. oxysporum* was the main factor responsible for potato dry rot in Michigan, USA and South Korea.

Fast identification of plant-parasitic pathogens empowers to set up adjusted control measures and to maintain a strategic distance from disease extension and yield losses, regardless of whether the invasion level is low. For *Fusarium* species, molecular identification is usually applied to identify *Fusarium* isolates that have comparable morphological qualities. For example, many species in a species complex such as *Fusarium fujikuroi* species complex (FFSC), *Fusarium solani* species complex (FSSC) and *Fusarium oxysporum* species complex (FOSC) produce similar colony appearance and macroconidial features. So, molecular identification is used to differentiate species in a species complex [18,19, 20].

By conducting phylogenetic analysis, the DNA sequence can be used to distinguish between *Fusarium* species that show similar morphological characteristics as well as to distinguish isolates in a species complex [18,21, 22]. For molecular identification and phylogenetic analysis of *Fusarium* species, β -tubulin, TEF1- α genes and ITS region are widely used as the genes and region are recommended by many researchers [23,24,25,26]. The β -tubulin encoding gene is among the most prominent diagnostic genes for *Fusarium* species detection and this gene has been used to differentiate between fungi at all levels [27,28]. In the current study, the beta (β)-tubulin gene was used to identify and differentiate among *Fusarium* species.

Phytopathogenic fungi secrete hydrolytic enzymes such as pectinases, cellulases, xylanases and proteases which are involved in penetration and colonization of host plant tissues during infection [29]. An enzyme capable of degrading plant cell wall polysaccharides is considered to play an important role in pathogenesis [30,31]. Gashgari and Gherbawy [32] reported that *Fusarium* spp. showed variation in pathogenicity level.

2. MATERIALS AND METHODS

2.1 Collection of Samples

A total of eighty samples of potato tubers collected from four Governorates in Upper Egypt (Aswan, Luxor, Qena and Sohag), twenty samples from each. Each sample was put in a sterile polythene bag sealed and transferred to the mycological laboratory. Samples were kept in a cool place during storage (3-5°C) till fungal analysis.

2.2 Isolation and Morphological Identification of *Fusarium* spp.

The collected potato tubers were cleaned under running tap water and air-dried. Potato tubers were cut into small fragments of approximately equal 1.0~1.5 cm size. The potato fragments were surface-sterilized with 0.1% sodium hypochlorite (NaOCl) for one minute, next washed three times with sterile distilled water, then dried with sterilized filter paper after that were placed in Petri plates (four pieces in each Petri dish) containing PDA medium supplement with dichloran, 0.002 g/L. Rose Bengal, 0.025 g/L and chloramphenicol, 0.1 g/L were added as bacteriostatic agents [33] and finally incubated at 25°C for 5-7 days. For pure cultures single spore of *Fusarium* colonies were placed firstly into Petri dishes with a PDA medium, followed by inoculation of it in slants containing PDA medium and preserved at 4°C for further studies. The growth rates and pigment production of *Fusarium* spp. were observed by transferring all strains on PDA plates and incubated at 25°C for 10-14 days for microscopic examinations [34] by using the morphological key [9].

2.3 Molecular Identification of *Fusarium* Isolates

2.3.1 DNA extraction

Fungal isolates were grown in 250 ml flasks containing 50 ml Potato Dextrose Broth (PDB) for 2-3 days using a rotary shaker (120-150 rpm) at 25°C. The mycelium was collected by filtration and grind to a fine powder with liquid nitrogen. In an Eppendorf tube (1.5 ml) fifty milligrams of the powder was put in it, then mixed with 0.7 ml of 2 x CTAB buffer and vortex for 2 min. Eppendorf tubes were incubated at 65°C for 60-80 min, then mixed briefly with 0.7 ml of chloroform. After that centrifugation at 15.000 rpm for 10 min was carried out, the supernatant was transferred to a

new Eppendorf tube mixed with 0.6 ml isopropanol and chilled to 20°C, followed by another centrifugation step at 15.000 rpm for 5 min. The supernatant was removed and the remaining pellet was washed twice with 1 ml of 70% ethanol, followed by another centrifugation for 3 min at maximum speed 15.000 rpm, after that dried and dissolved in 0.1 ml TE (10 mM Tris, 1 mM EDTA, pH 7.5) buffer [35]. The DNA quality was observed by electrophoresis on a 1.4% agarose gel provided with ethidium bromide and visualized by UV trans-illumination.

2.3.2 PCR amplification and sequencing

Part of the β -tubulin gene amplification was carried out by using the primers Bt2a and Bt2b [36]. The gene amplification reaction was employed in a total volume of 25 μ L, the tube having 5 μ L of the PCR Master Mix (Jena Biosynthesis) (buffer, dNTP, Taq DNA polymerase, 2 mM MgCl₂), 1 μ L of the template DNA, 0.5 μ L of each primer and 18 μ L of PCR water. The amplification reaction was performed in a thermal cycler (Flexigene, Techne, Cambridge, UK). PCR cycles for β -tubulin: initial denaturation at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 64°C for 30 s, extension at 72°C for 1 min and a final extension at 72°C for 5 min. PCR product was examined by 1.4% agarose gel, stained with ethidium bromide and visualized with UV trans-illuminator. Amplified products were purified, quantified and sequenced MacroGen Company (South Korea).

2.3.3 Phylogenetic analysis

Sequencing data were aligned and clustered by Mega 6.0 [37]. The *Acremonium* genus is closely related to *Fusarium*. Therefore, the phylogenetic tree was rooted with *Acremonium sclerotigenum* (KC987128). The phylogenetic construction was done using the neighbour-joining (NJ) algorithm, with bootstrap values calculated from 1,000 replicate runs, using the software routines included in the MEGA 6 software.

2.3.4 Screening of *Fusarium* isolates for amylase production

Fifteen isolates of *Fusarium* were screened for their abilities to produce extracellular α -amylase. Isolates were cultured on solid starch yeast extract agar (SYE) medium with a composition (in g/L) of soluble starch, 5.0; Bacto-yeast extract, 2.0; KH₂ PO₄, 1.0; MgSO₄. 7 H₂O, 0.5 and agar, 15 [38]. Cultures were incubated at

28°C for 6 days. The inoculums were obtained by using a sterile cork borer (10 mm diameter). One sterile 100 ml Erlenmeyer flask containing 50 ml of the broth SYE was prepared for each fungal species. Cultures were incubated at 28°C without shaking for 7 days after that the mycelium was collected by filtration. The amyolytic activity of *Fusarium* species was determined by using the filtrates as the method of the society of American Bacteriologists (1957). Briefly, 0.1 ml of a culture filtrate were put into 10 mm pores which were cut in SYE agar plates. After 24 h of incubation at 28°C, plates were immersed with iodine solution (KI, 15 g; I₂, 3 g per liter of distilled water). The formation of a clear zone around cavities indicates amylases production. The mean diameter of clear zones (in mm) of the triplicates for each isolate was recorded.

2.3.5 Pathogenicity test of the selected strains

The healthy potato tubers (*Solanum tuberosum* L.) were used in this test. Initially, tubers look like healthy and as the same in the size (100–120 g) were used and washed from the soil, surface-sterilized in 50% sodium hypochlorite solution for 10 min and put in 3 times of sterile distilled water [39,40] and then dried under laminar flow. With a cork borer the tubers wounded with a diameter of 5 mm to a depth of 5 mm [41,42]. An agar plug (5 mm diameter) having active mycelium of *Fusarium* sp. cut from the edge of 3-day-old cultures grown on the quarter of the quantity of PDA and put into the wound, which covered with the plug of tuber tissue. For each fungal strain, two tubers were used. All the wounded potato tubers were put in black polyethylene bags [39,43] and incubated in the dark at 20°C for 21 days. As a control, tubers were inoculated with an agar plug only. Following incubation, tubers

were cut longitudinally from the point of inoculation and the depth of internal necrosis was measured using electronic callipers. Re-isolations on PDA medium were employed from all isolates. The depth of wound response in controls was also measured for comparison.

2.4 Statistical Analysis

Results data were subjected to analysis of variance (ANOVA) using the Statistical Analysis System [44]. Means were separated by Duncan's multiple range test at $P < 0.05$ level.

3. RESULTS

3.1 Morphological and Molecular Characteristics of *Fusarium* spp.

A total of 504 isolates of *Fusarium* were isolated from potato tubers on plates of PDA media at 25°C which collected from four Governorates in Upper Egypt (Aswan, Luxor, Qena and Sohag). Fifty hundred and four isolates of *Fusarium* were isolated from eighty samples of potato tubers that were marketed in Upper Egypt. Isolation and identification media was PDA at 25°C.

Based on morphological characteristic aerial mycelia of all isolates were white at the initial stage, while the colonies became off-white, pale pink, violet, purple, orange and grey in the later stages and microscopically examination (shape, form, separation, size of microconidia, macroconidia, phialides and chlamydo spores), so four species were identified [9], namely *F. sambucinum* with the highest frequency (62.5%), followed by *F. oxysporum* (57.5%), then *F. verticillioides* (56.25%) and *F. incarnatum* (47.5%) presented in Table 1.

Table 1. Total counts (TC), (calculated per 960 segments), percentage counts (%C, calculated per total fungal), percentage frequency (%F, calculated per 80 samples), number of cases of isolation (NCI, out of 80) and the relative importance values (RIV) of *Fusarium* spp. recovered from potato tuber on PDA at 25°C

<i>Fusarium</i> Species	TC	%C	NCI	%F	RIV
<i>F. incarnatum</i>	133	26.39	38	47.5	73.89
<i>F. oxysporum</i>	143	28.37	46	57.5	85.87
<i>F. sambucinum</i>	118	23.41	50	62.5	85.91
<i>F. verticillioides</i>	110	21.83	45	56.25	78.08
Gross total count	504	100			

3.2 Molecular Characterization of *Fusarium* Species

β -tubulin was successfully amplified from fifteen *Fusarium* isolates (a single band of 350 bp) recovered from the potato tubers samples. All the sequences for β -tubulin were deposited in the GenBank and their accession numbers are indicated in the Table 3. The β -tubulin sequences of all isolates were subjected to the GenBank database using BLAST search and the results were recorded as the most closely related sequences with a high percentage of homology.

The phylogenetic trees generated from analyses of β -tubulin (Fig. 1) demonstrated that isolates recovered from different locations of Egypt with similarly named species clustered together forming well distinct, two major clusters (Clusters A and B), Cluster A contained clade 1 and 2 while Cluster B contained clade 3 and 4. The high-resolution β -tubulin dataset (Fig. 1) separated *F. oxysporum* species complex into five groups, *F. incarnatum* into two groups, *F. sambucinum* into one group and also and *F. verticillioides* into one group.

Phylogenetic analysis has been performed on the present 15 *Fusarium* spp. nucleotide sequences with other eleven *Fusarium* spp. published in the GenBank and the results of this analysis were shown in Fig. 1. Phylogenetic tree was generated from 26 sequences including 17 *F. oxysporum* species complex, 3 *F. verticillioides*, 2 *F. sambucinum* and 3 *F.*

incarnatum in addition to the out-group sequence *Acremonium sclerotigenum* (KC987128). Phylogenetic analysis of this dataset resulted in the *Fusarium* spp. clustered together in two clusters to the exclusion of outgroup taxa. First clade separated *F. oxysporum* species complex into five groups. First group SVUFo16 *F. oxysporum* cluster as a base for all other *Fusarium oxysporum* species complex. The second group comprised 5 isolates (SVUFo12, SVUFo13, SVUFo14, SVUFo15 and SVUFo17) of *F. oxysporum* species complex clustered together in one cluster. SVUFo8 strain (Third group) clustered with the other FOSC (KY189919.1, MH979021.1, KU938935.1 and MH485132.1) obtained from GenBank. Fourth group include strains SVUFo9 and SVUFo11, these two isolates can be considered as *Fusarium oxysporum* f. sp. *vasinfectum*. SVUFo10 strain (fifth group) forming a distinct clade with different FOSC sequences obtained from NCBI. The tree showed a well-supported relationship (98% bootstrap) between *F. verticillioides* (LS423017.1) obtained from GenBank and two isolates (SVUFv105 and SVUFv106) that based on morphological features were identified as *F. verticillioides* in the second clade. Third and fourth clade isolates were closer to each other than to the other clades with strong support 99% bootstrap value. SVUFsa18 *F. sambucinum* clustered with the isolate of KP674308.1 (third clade). Other strain (SVUFi3 and SVUFi6) formed clear grouping with *F. incarnatum* strains (fourth clade).

Table 2. Screening of *Fusarium* spp. for α -amylase production

No. of isolate	Code of isolate	Morphological identification	Molecular identification	Amylase activity (Clear zone, mm)
1	SVUFi3	<i>F. incarnatum</i>	<i>Fusarium incarnatum</i>	11±0
2	SVUFi6	<i>F. incarnatum</i>	<i>F. incarnatum</i>	11±1
3	SVUFo8	<i>F. oxysporum</i>	<i>F. nirenbergiae</i>	10±0
4	SVUFo9	<i>F. oxysporum</i>	<i>F. oxysporum</i> f. sp. <i>vasinfectum</i>	10±0
5	SVUFo10	<i>F. oxysporum</i>	<i>F. glycines</i>	12±0
6	SVUFo11	<i>F. oxysporum</i>	<i>F. oxysporum</i> f. sp. <i>vasinfectum</i>	12±1
7	SVUFo12	<i>F. oxysporum</i>	<i>F. oxysporum</i> f. sp. <i>Dianthi</i>	10±0
8	SVUFo13	<i>F. oxysporum</i>	<i>F. oxysporum</i> f. sp. <i>vasinfectum</i>	9±2
9	SVUFo14	<i>F. oxysporum</i>	<i>F. oxysporum</i>	11±1
10	SVUFo15	<i>F. oxysporum</i>	<i>F. oxysporum</i> f. sp. <i>vasinfectum</i>	10±0
11	SVUFo16	<i>F. oxysporum</i>	<i>F. oxysporum</i>	10±0
12	SVUFo17	<i>F. oxysporum</i>	<i>F. oxysporum</i> f. sp. <i>vasinfectum</i>	11±1.73
13	SVUFsa18	<i>F. sambucinum</i>	<i>F. sambucinum</i>	16±2
14	SVUFv105	<i>F. verticillioides</i>	<i>F. verticillioides</i>	11±1
15	SVUFv106	<i>F. verticillioides</i>	<i>F. verticillioides</i>	12±1

Table 3. Origin of sample collection, code, accession numbers, name of the pathogen identified and measurement of lesion expansion and rank of the tubers' condition 21 days after incubation from each sample

No. of isolate	Code of isolate	Molecular identification	Origin	Measurement (mm)	Pathogenicity/virulence	Accession number
0	-	Control	-	8	A	-
1	SVUFI3	<i>Fusarium incarnatum</i>	Aswan	15 ±0	B	MH763578
2	SVUFI6	<i>F. incarnatum</i>	Qena	15±0	B	MH763581
3	SVUFO8	<i>F. nirenbergiae</i>	Aswan	15±0	B	MH757406
4	SVUFO9	<i>F. oxysporum f. sp. vasinfectum</i>	Sohag	15±0	B	MH757407
5	SVUFO10	<i>F. glycines</i>	Luxor	12.5±1	B	MH757408
6	SVUFO11	<i>F. oxysporum f. sp. vasinfectum</i>	Luxor	15±0	B	MH757409
7	SVUFO12	<i>F. oxysporum f. sp. Dianthi</i>	Sohag	15±0	B	MH757410
8	SVUFO13	<i>F. oxysporum f. sp. vasinfectum</i>	Qena	13.8±1.26	B	MH763571
9	SVUFO14	<i>F. oxysporum</i>	Luxor	15±0	B	MH763572
10	SVUFO15	<i>F. oxysporum f. sp. vasinfectum</i>	Aswan	15±0	B	MH763573
11	SVUFO16	<i>F. oxysporum</i>	Qena	13.8±1.26	B	MH763574
12	SVUFO17	<i>F. oxysporum f. sp. vasinfectum</i>	Aswan	15±0	B	MH763575
13	SVUFsa18	<i>F. sambucinum</i>	Luxor	30±0	B	MH763582
14	SVUFv105	<i>F. verticillioides</i>	Luxor	17±1	B	MH810144
15	SVUFv106	<i>F. verticillioides</i>	Aswan	17.7±2.5	B	MH810145

Means with different letters are significantly different from each other ($p < 0.05$); A: healthy, no visible symptoms (nonvirulent) and B (high virulent)

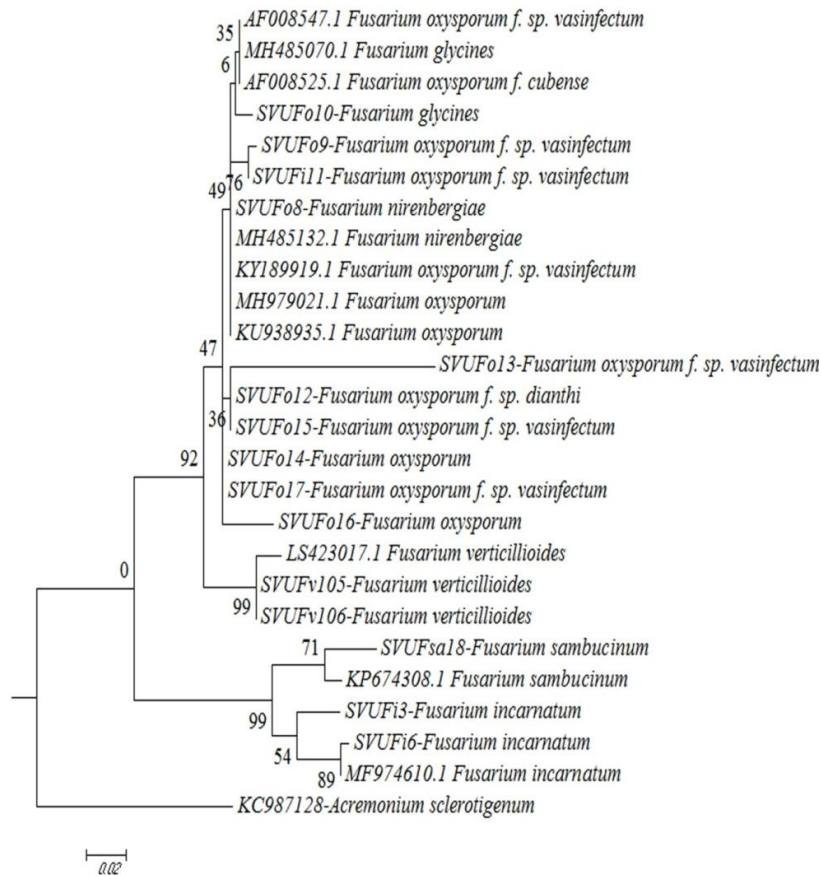


Fig. 1. Phylogenetic tree of *Fusarium* isolates came from potato tubers based on β - tubulin sequence data. The number above branches indicate bootstrap values

3.3 Screening of *Fusarium* spp. for α -Amylase Production

Fifteen *Fusarium* spp. isolates were selected from five hundred and four isolates which were also sequenced during this study to be screened for production of α -amylase qualitative assay which depending on the colour change of Iodine indicator from blue to colourless in the culture of *Fusarium* spp. All isolates showing a positive result for α -amylase production. All results recorded in Table 2. Each sample was done in three replicates. The selected 15 isolates based on morphological and molecular features were identified as *Fusarium incarnatum* 2, *F. oxysporum* species complex 10, *F. sambucinum* 1 and *F. verticillioides* 2. It was observed that all the tested *Fusarium* isolates were significantly in amylase production with high levels, more than ≥ 9 mm and *Fusarium sambucinum* (SVUa18) was able to produce 16 mm of amylase. Other isolates of *Fusarium* spp., *F. incarnatum* (SVUi3

and SVUi6), *F. oxysporum* species complex (SVUo10, SVUo11, SVUo14 and SVUo17), and *F. verticillioides* (SVUv105 and SVUv106) produce α -amylase at levels ranging from 11 – 14 mm as the mean value measuring by the ruler. The resulted data showed that *F. oxysporum* species complex isolates including SVUo8, SVUo9, SVUo12, SVUo13, SVUo13, SVUo15 and SVUo16 were less α -amylase producers (≤ 10 mm). According to (Table 2, Fig. 2 & photo 1) the levels of production of α -amylase enzyme different associated with isolates of *F. oxysporum* species complex.

3.4 Pathogenicity of *Fusarium* Species

All of the fifteen isolates were evaluated for their pathogenicity on healthy potato tubers. Measurements consisted into sizing the lesions in the pathogenicity tests. The results revealed that all the 15 isolates caused discolouration, necrosis and lesion on the tubers. Tubers

inoculated with isolate (SVUsa18) of *F. sambucinum* showed a mean lesion size of 30 mm, the highest average lesion size among all tested isolates. Also, the results revealed that *F. verticillioides* isolates (SVUv105 and SVUv106) caused lesions of 17:20 mm in the inoculated tubers. Whereas tubers inoculated with isolates

F. incarnatum (SVUi3 and SVUi6), *F. oxysporum* species complex (SVUo8, SVUo9, SVUo10, SVUo11, SVUo12, SVUo13, SVUo14, SVUo15, SVUo16 and SVUo17) were pathogenic to inoculated potato tubers and caused lesions of (10:16 mm) in the tubers (Table 3, Fig. 3 and photo 2).

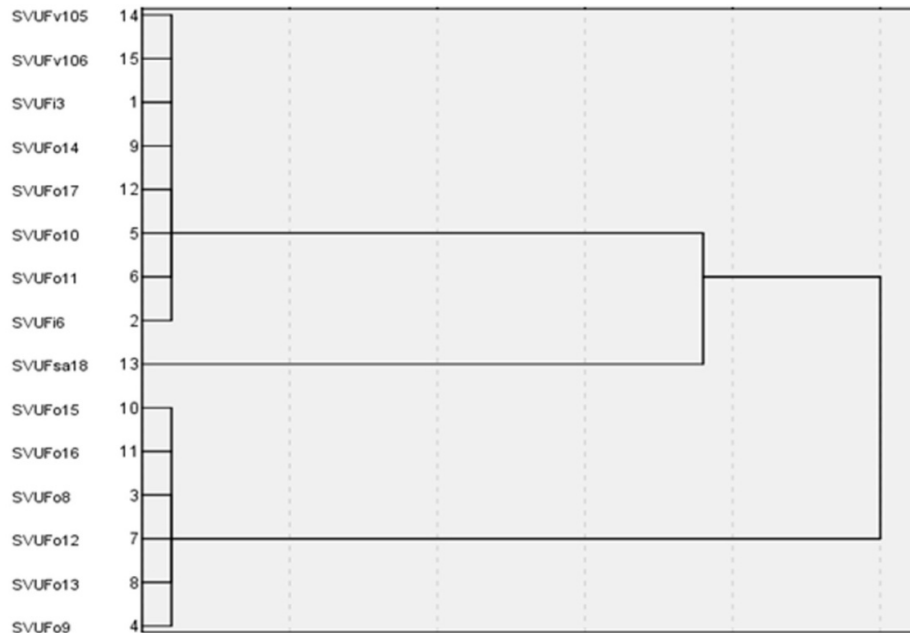


Fig. 2. Dendrogram showing relationships among 15 isolates of *Fusarium* spp. based on α -amylase production

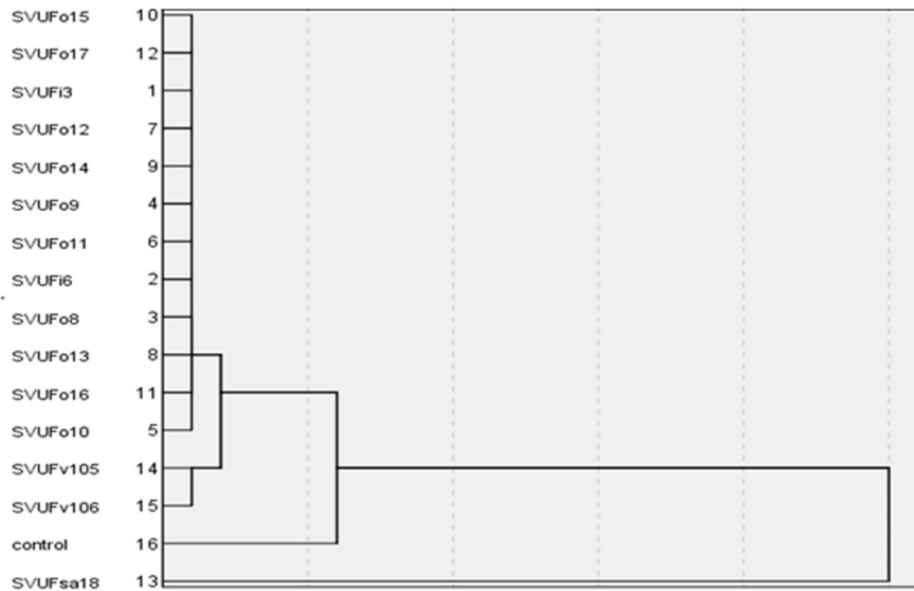


Fig. 3. Dendrogram showing relationships among 15 isolates of *Fusarium* spp. Based on pathogenicity test

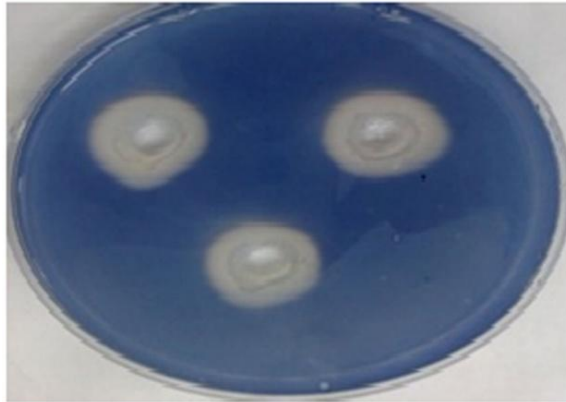


Photo. 1. Clear zone representing amylase activity produced by *Fusarium* spp.

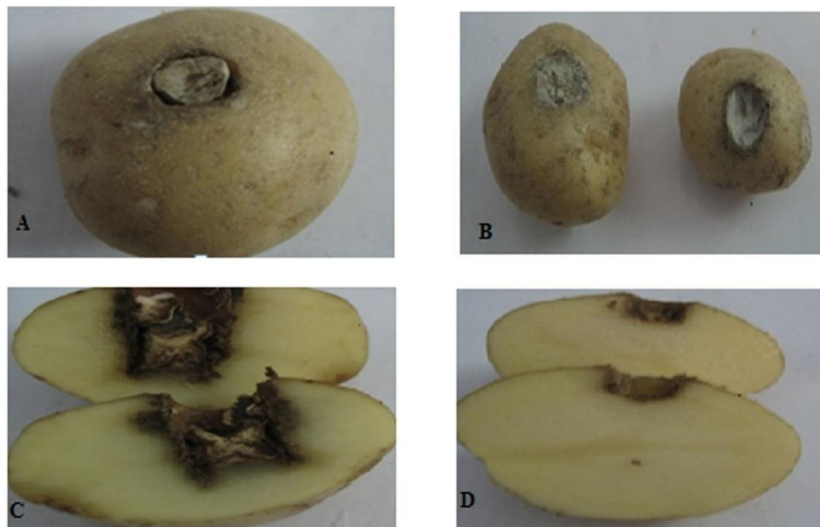


Photo. 2. External (A and B) and internal (C and D) symptoms of dry rot occasioned by *Fusarium* spp. on potato tubers after 21 days of incubation

Based on Duncan's test, species existed into one of the two groups. The most pathogenic species, *F. sambucinum* formed separate branch, while the less pathogenic group comprised *F. incarnatum*, *F. oxysporum* species complex and *F. verticillioides* (Table 2) & (Fig. 2).

According to Tables 2 and 3, and Figs. 2 and 3, *Fusarium sambucinum* showed the highest level of both α -amylase production and average lesion size in pathogenicity test among all tested isolates.

4. DISCUSSION

This study is a comprehensive research for identification and genetic diversity of *Fusarium* spp.,

affecting potato tubers in Upper Egypt. In this study, four *Fusarium* species were associated with potato tubers collected from the markets in Upper Egypt. The most abundant species were *F. sambucinum* and *F. oxysporum*. This result is in agreement with several authors [13,45,46] who reported that *F. sambucinum* and *F. oxysporum* were the most frequently *Fusarium* species associated with potato dry rot in Egypt. According to Gashgari and Gherbawy [32] isolated *Fusarium oxysporum*, *F. redolens*, *F. solani*, *F. sambucinum* and *F. graminearum* from Saudi potato tubers. However, the genus *Fusarium* is complex and morphological differences may be difficult to observe. Therefore, the DNA analysis is needed for accurate identification and characterization of the

species. β -tubulin sequences are frequently used to identify fungal species, and vast numbers of these sequences are already available in databases. However, as reported by Ayoubi and Soleimani [47]. The examination of fifteen *Fusarium* isolates resulting phylogenetic relationships in agreement with previous studies [7,26,48,49]. The isolates of the same species were grouped in the resulting tree, supporting their right distinguishing proof. and our examination in agreement with the past study [1] who isolated 10 *Fusarium* species related with dry rot in potatoes and identified them molecularly by ITS, *tef-1 α* and β -tubulin which were analysed to determine the relationships among the species and to give data regarding the intraspecific variation. From phylogenetic examination, *F. oxysporum* isolates were clustered in several groups which showed intraspecific variations. *F. oxysporum* is viewed as a species complex and has reported being genetically heterogeneous morph species by several authors [19,47, 50]. This species is also considered as a monophyletic group and showed a diverse complex of evolutionary lineages [47]. The intraspecific variation may be due to their role as soil inhabitants in which the species can be highly variable in their growth characteristics on different substrates [51] and in ecological characteristics [52].

In this study, all the *Fusarium* species were significant in producing amylase, especially in the isolate *F. sambucinum* SVUsa18 (mean value 16 mm), this result in agreeing with [53] they conducted that all tested *Fusarium* species were capable of producing amylase. Aydođdu et al. [54] reported that *F. oxysporum*, *F. poae* and *Gibberella fujikuroi* produced amylase with moderate activity. *Gibberella fujikuroi* and *Fusarium* spp. on medium adjusted to pH 4.0-5.0 showed high rates of amyolytic activity [55]. According to Gawade et al. [56] reported that phytopathogenic fungi secrete a broad range of enzymes which are helped pathogen in penetration during infection. According to Delgado et al. [57] confirmed that extracellular enzymes activity determining the virulence of the fungal pathogen. So, amylases are required by the pathogen *Fusarium* spp. to rot potato tubers, especially during stages of pathogenesis.

In the present study testing fifteen isolates of *Fusarium* spp. isolated from potato tubers showing the presence of great variation among isolates. Some isolates are highly pathogenic on potato tubers, for example, (SVUsa18) of *F.*

sambucinum had a mean lesion size of 30 mm. Similar results have also been reported by other researchers Stefańczyk [1], O'Donnell and Cigelnik [46]. Where other researchers recorded that *Fusarium sambucinum* was listed among 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 Estrada et al. [58] and Estrada et al. [59]. Gashgari and Gherbawy [32] reported that *Fusarium* spp. isolates varied in their pathogenicity against Saudi potato tubers. They ranged from highly pathogenic like isolates: *Fusarium sambucinum* (KAUF14), *Fusarium graminearum* (KUAF11) and *Fusarium solani* (KAUF15) and moderate pathogenic like two isolates of *Fusarium redolens* (KAUF13.1 and KAUF13.2). Jawed et al. [60] surveyed the stored potato tubers affected by *Fusarium* dry rot from commercial storage facilities conducted during 2004–2005 determined that 58% of the *Fusarium* spp. isolated was *F. sambucinum*.

Fusarium oxysporum represented with (10 strains), *F. incarnatum* (2 strains) and *F. verticillioides* (2 strains) showed pathogenicity according to the measurement of lesion size (10-20 mm). SAS [61] reported that pathogenicity test of *F. oxysporum* confirmed the virulent nature of the pathogen and produced the similar symptoms of potato dry rot. Bayona et al. [62] confirmed the pathogenicity of two *F. oxysporum* isolates associated with potato dry rot in Colombia. Gashgari and Gherbawy [32] recorded that *Fusarium oxysporum* strains (KAUF12.1, KAUF12.2, KAUF12.3 and KAUF12.4) isolated from Saudi potato tubers did not show any pathogenicity against tubers. Chehri et al. [63] in Malaysia studied the occurrence and pathogenicity of *Fusarium* spp. on the potato tubers. Inoculation with both induced moderate dry rot. Mode of spread is by planting infected tubers or by contaminated, as the pathogen is soil-borne, airborne or carried in plant residue [64].

The observed differences in pathogenicity between the tested *Fusarium* isolates could be attributed to the differences in their aggressiveness that expressed as various given dry rot lesion caused by individual *Fusarium* spp., which supported this assumption [65]. Moreover, several factors may explain why different *Fusarium* species were identified in the present survey compared to that implemented by Du et al. [7], including use of different rotation

patterns and different potato cultivars, as well as different environmental and/or edaphic conditions. Some of the *Fusarium* spp. have isolated from potato tubers are known to be important cereal pathogens and growing such cereal host species before or next to potatoes should be avoided to limit the risk of dry rot of potato tubers [1].

5. CONCLUSION

Based on morphological, microscopic characteristic and molecular identification by sequencing of β -tubulin gene, we proved the presence of *F. sambucinum*, *F. oxysporum* species complex, *F. incarnatum* and *F. verticillioides* species on potato tubers in Upper Egypt. *F. sambucinum* had the highest frequency (62.5%) and demonstrated the most aggressive properties and amylase production, with presented strong relationships between pathogenicity and α -enzyme production. *Fusarium oxysporum* complex has comprised different species so, we are needed more studies and primer designs to accurate differentiation of this complex group.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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