Morphology, pathogenicity and molecular identification of *Fusarium solani* species complex (FSSC) associated with potato tubers

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Research Article

Keywords: Potato, FSSC, β-tubulin, TEF-1α, amylase, pathogenicity.

Posted Date: January 21st, 2021

DOI: https://doi.org/10.21203/rs.3.rs-147809/v1

Version of Record: A version of this preprint was published at Journal of Basic Microbiology on November 11th, 2021. See the published version at https://doi.org/10.1002/jobm.202100393.

Abstract

Potato (Solanum tuberosum L.) is among the top five crops growing worldwide following cereals, wheat, rice, corn and barley due to its high carbohydrate content and adaptability. Potatoes are particularly valued in developing countries as a rich source of starch, vitamins C and B6 and essential amino acids. Fusarium solani species complex (FSSC) is common pathogen of potato, causing dry rot in the Upper Egypt. In this study were isolated and identified FSSC from potato tubers based on the morphological and molecular characteristics. 187 isolates of Fusarium solani were obtained from potato tubers collected from different regions in the Upper Egypt. Based on the morphological characters, sequence data from β-tubulin and translation elongation factor (TEF-1α) genes, all of the selected FSSC isolates were divided into three major groups (F. keratoplasticum, F. falciforme and F. solani). All the tested FSSC were able to produce amylases. All of the isolates were evaluated for their pathogenicity on healthy potato tubers; which showed pathogenic effect, lesion sizes were quite variable. F. solani (SVUFs73) had a highly virulent effect.

Introduction

Potato (*Solanum tuberosum* L.) is one of the world's most significant food crops, and the second most significant vegetable crop after tomato. Egypt is one of the biggest producers and exporters of potatoes in Africa [1]. In recent decade, different pathogens causing potato tuber infections, particularly fungi of genus *Fusarium*, have become widely spread in Egypt [2,3]**.** In Egypt, potato has a significant situation among all vegetable crops, where about 20% of whole area dedicated for vegetable production is cultivated with potato [4]. Dry rot caused by *Fusarium* species is a significant potato disease worldwide, which causes post-harvest rotting and seed piece rot after planting [5]. *Fusarium* dry rot of seed tubers can reduce crop establishment by affecting the development of potato sprouts, resulting in poor emergence and reduced plant stands with weakened plants [6,7]. Storage damages associated with *Fusarium* dry rot have been reported to range from 6 to 25%, and occasionally losses as great as 60% have been estimated [8,9]. Many species of this genus are pathogens affecting different economically important crops [10,11,12]. It was shown that potato dry rot was caused by more than thirteen different *Fusarium* species [13,5]. *Fusarium* spp. which infect potato tubers differ depending on time of the survey and geographic location. *Fusarium solani* are the most common pathogenic species in Egypt [14,15].

Rapid identification of plant fungal pathogens enables to set up adjusted control measures and to maintain a strategic distance from disease expansion and yield losses, regardless of whether the invasion level is low. For *Fusarium* species, molecular identification is usually applied to identify them that have comparable morphological qualities. For example, many species in a species complex such as *Fusarium solani* species complex (FSSC) produce similar colony appearance and macroconidial features. So, molecular identification is used to differ between species in a species complex [16, 17, 18]**.** DNA sequence can be used to distinguish between *Fusarium* species that show similar morphological characters as well as to differentiate isolates in a species complex [19]**.** For molecular characterization and

phylogenetic analysis of *Fusarium* species, β–tubulin and TEF1-α genes are widely used as the genes and regions are reported by many investigators [18, 20]. Fungi secreted Hydrolytic enzymes such as cellulases, pectinases, proteases and xylanases, which are played critical roles in successful host colonization by degradation of the cell wall [21].

This work aimed to characterize of *F. solani* species complex isolated from potato tuber and molecular diagnosis by β-tubulin and TEF1-α genes. Also, evaluate the abilities of *F. solani* species complex for α-amylase production and test the pathogenicity of *F. solani* against potato tubers.

Methods

Isolation and morphological identification of *Fusarium solani*

Many isolates of *F. solani* were isolated from eighty samples of potato tubers (forty were healthy and the other forty were infected by moulds) were collected from four Governorates in Upper Egypt (Aswan, Luxor, Qena and Sohag). For pure cultures single spore of *Fusarium* colonies were inoculated firstly into petri dishes contained PDA medium, followed by inoculation of it in slants containing PDA medium and maintained at 4°C for further studies [22]. Colony morphology and microscopic examination were used for classical identification [23].

Molecular identification of *Fusarium* isolates

DNA extraction

Fusarium solani isolates were cultured in 250 ml flasks containing 50 ml Potato Dextrose Broth (PDB) for 2-3 days using a rotary shaker for 25°C at 120-150 rpm. The mycelium was collected by filtration and ground to fine powder in liquid nitrogen. In an Eppendorf tube (1.5 ml) fifty milligrams of the powder was put in it, then mixed with 0.7 ml 2 x CTAB buffer and vortex for 2min. Eppendorf tubes were incubated at 65^{o}C for 60-80 min, then 0.7 ml of chloroform was added and mixed briefly. After centrifugation at 15.000 rpm for 10 min, the supernatant was transmitted to a new tube mixed with 0.6 ml isopropanol and chilled to 20^{o}C, followed by another centrifugation step at15.000 rpm for 5 min at maximum speed. The supernatant was discarded and the resting pellet was washed twice with 1 ml of 70% ethanol, followed by another centrifugation for 3 min at maximum speed 15.000, after that dried and dissolved in 0.1 ml TE (10 mM Tris, 1 mM EDTA, pH 7.5) buffer (Moeller et al. 1992). The DNA quantity and quality checked by electrophoresis on a 1.4% agarose gel revealed with ethidium bromide and visualized by UV trans-illumination.

PCR amplification and sequencing

The primers used to amplify β-tubulin were Bt2a and Bt2b as described by Glass and Donaldson (1995). The primers used to amplify translation elongation factor-1α (TEF-1α) were EF1 and EF2 modified from [26].

PCR reaction for both genes was carried out in PCR tubes containing 5 µL of the master mix (buffer, dNTP, Taq DNA polymerase, 2 mM $MgCl_2$) 1µL of the template DNA, 0.5µL of both forward and reverse primers and the volume was completed to 25 µL with PCR water. Amplification was performed in a thermal cycler (Flexigene, Techne, Cambridge, UK).

Polymerase chain reaction (PCR) cycles for β-tubulin were as follows: initial denaturation at 94 ºC for 1 min, 30 cycles of denaturation at 94 ºC for 30 s, annealing at 54 ºC for 30 s, extension at 72 ºC for 1 min and final extension at 72 ºC for 5 min [25]. PCR cycles for TEF-1α, the cycle started with initial denaturation at 94 ºC for 5 min, 40 cycles of denaturation at 94 ºC for 1 min, annealing at 58 ºC for 1 min, extension at 72 ºC for 2 min and final extension at 72 ºC for 10 min [27]. PCR product was observed in a 1.4% agarose gel, stained with ethidium bromide and visualized with UV transilluminator. Amplified products were purified, quantified and sequencing in Macrogen (South Korea).

Phylogenetic analysis

Sequences of β-tubulin and TEF-1α were edited by using chromas program and aligned using Clustal X included in MEGA version 6.0 [28]. Phylogenetic analysis was conducted using combined dataset of β-tubulin and TEF-1α sequences. The *Acremonium* genus is closely related to *Fusarium*. Therefore, phylogenetic tree was rooted with *Acremonium sclerotigenum* (KC987128 and KT878381). The phylogenetic reconstruction was done using the neighbor joining (NJ) algorithm, with bootstrap values calculated from 1,000 replicate runs, using the software routines included in the MEGA software.

Screening of *Fusarium solani* isolates for amylase production

Eighty eight isolates of *Fusarium solani* were screened for their abilities to produce extracellular α-amylase. Isolates were grown on solid starch yeast extract agar (SYE) medium with a composition (in g /L) of soluble starch, 5.0; Bacto-yeast extract, 2.0; KH_2PO_4, 1.0; $MgSO_4$. 7 H_2O, 0.5 and agar, 15 [29]. Cultures were incubated at 28°C for 6 days. Using a sterile cork borer (10 mm diameter), the inoculums was obtained. For each fungal isolate, one sterile 100 ml Erlenmeyer flask containing 50 ml of the liquid SYE was prepared. Cultures were incubated at 28°C without shaking for 7 days after which the mycelium was harvested by filtration. Aliquots of 0.1 ml of a culture filtrate were pipetted into 10 mm cavities which were made in SYE plates. After 24 h incubation at 28°C, plates were flooded with iodine solution (KI, 15 g; I_2, 3 g per liter of distilled water). A zone without blue indicates the production of amylase. In case of positive strains, the average diameter of clear zones (in mm) of the triplicates for each isolate was recorded.

Pathogenicity test of the selected isolates

The healthy potato tubers (*Solanum tuberosum* L.) were used in this experiment. Initially, tubers appearing healthy and similar in the size (100–120 g) were selected and washed to remove excess soil, surface sterilized in 50% sodium hypochlorite solution for 10 min and rinsed in 3 times of sterile distilled water (Lui and Kushalappa 2002; Lui et al. 2005) and then dried under laminar flow. Then the tubers

wounded with a cork borer with a diameter of 5 mm to a depth of 5 mm [32, 33]. An agar plug (5 mm diameter) containing active mycelium of *Fusarium solani* isolates extracted from the margin of a 3-day-old cultures grown on quarter of the quantity of PDA and placed into the wound, which was subsequently sealed with the excised plug of tuber tissue. Two tubers used for each fungal strain. All the wounded potato tubers were wrapped in black polyethylene bags [34, 30] and incubated in the dark at 20°C for 3 weeks. 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 calipers. Re-isolations on PDA medium were attempted from all isolates. The depth of wound response in controls was also recorded for comparison. Tubers were cut through the inoculation points, and the degree of rot was estimated.

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

Morphological characterization of *F. solani* species complex

187 isolates of *Fusarium solani* were examined microscopically (Table 1). On PDA medium, aerial mycelia of all isolates were white at the initial stage, while the colonies became off-white, violet, purple and gray in the later stages. Single isolates of *F. solani* produced oval to kidney shape, microconidia measuring 9.7-23.4 x 2.9-5.6 µm. Macroconidia had 3-5 septa ranging 28.5-50.6 x 3.1-6.2 µm (Table 2& 3). On the same medium, chlamydospores were observed that appeared singly or in pairs and conidiophores were long monophialides.

The hierarchical analysis according to morphological characters of FSSC

The dendrogram was generated from 88 FSSC, the first group (16 strains) and the second group (72 strains) according to morphological properties (Figure 1).

From the hierarchical analysis, (Figure 1) the isolates which had the same morphological properties were clustered together. The dendrogram divided into two clades.

The first clade comprised SVUFf1, SVUFf2, SVUFs24, 28, 34, 57, 63, 71, 74, 75, 76, 77, 81, 85, 88 and 104, these isolates had the same colony color (Off-white to pale cream) and the shape of macroconidia are falcate shape with pointed apexes. The second clade included two sub-clades, the first sub-clade comprised 1 isolate of SVUFs87, this isolate was shorter than isolates in this clade and the second sub-clade comprised 71 isolates (SVUFs19, 20, 21, 22, 23, 25, 26, 27, 29, 30, 31, 32, 33, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 58, 59, 60, 61, 62, 64, 65, 66, 67, 68, 69, 70, 72, 73, 78, 79, 80, 82, 83, 84, 86, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102 and 103), these isolates

had long macroconidia with fusiform and thick wall (the apical cell was blunt while the basal cell was foot-shaped) (Figure 1) (Table 2).

Molecular characterization of *Fusarium solani* by amplification of β-tubulin and TEF-1α genes

To confirm the morphological identification of the studied isolates, molecular analyses were performed. β-tubulin was successfully amplified from 88 isolates of *Fusarium solani* species complex recovered from potato tubers samples. A single band of 350 bp was obtained by Bt2a and Bt2b primer pairs (Figure 2). Translation elongation factor-1α (TEF-1α) was also successfully amplified from 83 *Fusarium solani* isolates and five *Fusarium solani* isolates (SVUFf1, SVUFf2, SVUFs44, SVUFs86 and SVUFs90) have short sequence by TEF-1α gene, so didn't show in Phylogenetic analysis. A single band of 700 bp was obtained using EF1 and EF2 primer pairs (Figure 3). All the sequences of β-tubulin and TEF-1α were deposited in the GenBank and their accession numbers were indicated in Figure (4) and (5). The β-tubulin and TEF-1α sequences of *Fusarium solani* isolates were subjected to GenBank database using BLAST search and the results were recorded as the most closely related sequences with high percentage of homology. TEF-1α showed the highly resolution comparable to β- tubulin in identification of *Fusarium solani* species complex

Mega Blast analyses of the 2X consensus nucleotide sequence of β-tubulin and TEF-1α gene showed 99-100% similarity with several *Fusarium* species sequences of the same region deposited in GenBank. Neighbor joining tree using Mega 6 was used to study genetic relatedness of *Fusarium* strains (Figures 4 and 5).

β- tubulin dataset of *F. solani* species complex (88 *F. solani*) from the current study and 7 GenBank sequences (Figure 4). The phylogenic tree (Figure 4) revealed that, the strains of *F. solani* species complex were categorized into 3 clades. Phylogenetic analysis of this dataset resulted in the *F. solani* species complex clustered together in one cluster to the exclusion of out-group taxa.

SVUFs87 (*F. solani*) was found to be at a separate branch. The first and second clade isolates were closer to each other than the remaining clades with strong support 92% bootstrap value. SVUFf1 and SVUFf2 (*F. falciforme*), SVUFs 24, 28, 34, 53, 57, 59, 71, 74, 75, 76, 77, 81, 85, 88 and SVUFs104 (*F. solani*) clustered together with *Fusarium falciforme* (KY776685 and KY776684) in the first clade. Strains in this clade have the same morphological characters except isolates (SVUs53 and SVUs59), but all strains in this clade have morphological characters different from other *Fusarium solani* species complex in macroconidia, which was shorter than *Fusarium solani* species complex and these isolates gave the same results in the pathogenicity test (lesion sizes 10:16mm) and α-amylase production (level of this isolates less than ≤10 mm). The second clade comprised SVUFs102 of *F. solani* species complex. The third clade was divided into sub clades A, B, C and D. In the sub-clade A, SVUFs52 *Fusarium solani* was a base for all other *Fusarium solani* species complex presented in the third clade. Sub-clade B consisted from 2 strains (SVUFs62 and SVUFs72) of *Fusarium solani* species complex, which clustered together in one clade. Sub-clade C included strains of SVUFs91, 92, 93, 94, 95, 97, 98 and 101, these eight isolates considered as *F. solani* species complex. Sub-clade D included SVUs19, 20, 21, 22, 23, 25, 26, 27, 29, 30, 31, 32, 33, 35, 36,

37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 54, 55, 56, 58, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 73, 78, 79, 80, 82, 83, 84, 86, 89, 90, 96, 99 and 103 strains forming distinct clade with different *F. solani* species complex sequences (MF662654, KU938965, KT374270, KU938962 and KU938955), which were obtained from NCBI.

Phylogenetic analysis was done by TEF-1α sequences for the 83 strains of *F. solani* species complex under study along with 5 GenBank sequences (Figure 5). In addition to the out group sequence *Acremonium sclerotigenum* (KT878381). The phylogenetic tree revealed that, the tested strains could be categorized into six clades (Figure 5).

First clade comprised 2 isolates (SVUFs37and 84) of *F. solani*, which grouped together with *F. solani* MG252286. Second clade includes strains (SVUFs82, 91 and 93); these three isolates were *F. solani*. First clade and second clade were found to be at the base of the tree. Third clade SVUFs 39 and SVUFs72 (*F. solani*) clustered together in one clade with 64% bootstrap value. Fourth clade consisted from 14 strains (SVUFs24, 28, 34, 57, 63, 71, 74, 75, 76, 77, 81, 85, 88 and 104) closely related to *Fusarium falciforme* MH463544. Isolates in this clade have the same morphological characters and also showed the same results in the pathogenicity test (lesion sizes 10:16mm) and α-amylase production (levels of these isolates less than ≤10 mm). Isolates in this clade were previously explained that they had morphological characters different from other *Fusarium solani* species complex in macroconidia, which was the shortest. The tree showed a well-supported relationship (99% bootstrap) between *F. keratoplasticum* (KC808192), which was achieved from GenBank and isolate (SVUFs87) in fifth clade. Isolate in this clade was the same isolate (Figure 4), which was represented as a separate branch in β- tubulin phylogenetic tree (Figure 4), so it was presented in a separate group at the base of the tree (Figure 4); isolate SVUFs87 was morphologically similar with other *F. solani* isolates. However, the TEF-1α gene sequence of SVUFs87 was different from another *F. solani* isolates and was classified as *F. keratoplasticum*. The latter was considered partially different genetically from most *F. solani* species, but basically similar in terms of biological characters. The sixth clade *F. solani* (SVUFs19, 20, 21, 22, 23, 25, 26, 27, 29, 30, 31, 32, 33, 35, 36, 38, 40, 41, 42, 43,, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 58, 59, 60, 61, 62, 64, 65, 66, 67, 68, 69, 70, 73, 78, 79, 80, 83, 89, 92, 94, 95, 96, 97, 98, 99, 100, 101, 102 and 103) grouped with the other *Fusarium solani* species complex (MH996883) that was obtained from GenBank.

Screening of *Fusarium solani* for α-amylase production

88 *Fusarium solani* isolates which were collected from potato tubers during this study were screened for production of α-amylase qualitative assay depending on color change of Iodine indicator from blue to colorless in culture of *Fusarium solani*. All results recorded in Figure 6 and 7, each sample was tested in three replicates.

It was observed that all *Fusarium* strains produced α-amylase and production was more than ≥ 9 mm.

High levels of amylase (≥ 15 mm) were produced by isolates of *Fusarium solani* SVUFs73, SVUFs93, SVUFs96 and SVUFs97.

Others isolates of *Fusarium solani* produced α-amylase at levels ranging from 11–14 mm. These isolates were belonging to *F. solani* species complex SVUFs20, 21, 23, 25, 26, 32, 35, 39, 42, 45, 47, 49, 50, 54, 56, 58, 59, 61, 62, 65, 66, 69, 89, 90 and 91 (Figure 6).

Low levels of α-amylase (≤10 mm) were produced by others isolates of *F. solani* species complex (SVUFf1, SVUFf2, SVUFs19, 22, 24, 27, 28, 29, 30, 31, 33,34, 36, 37, 38, 40, 41, 43, 44, 46, 48, 51, 52, 53, 55, 57, 60, 63, 64, 67, 68, 70, 71, 72, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 92, 94, 98, 99, 100, 101 and 103) (Figure 6).

Pathogenicity of *Fusarium solani*

Fusarium solani were evaluated for their pathogenicity on healthy potato tubers. Measurements consisted into measuring the lesion's size in the pathogenicity test. Lesion sizes were completely variable and ranged from 0.0 mm to 35 mm (Figure 6). Lesion size less than 10 mm wasn't pathogenic to potato tubers. The results of the pathogenicity test revealed that 88 isolates caused discoloration, necrosis and lesion of the tubers. Of the 88 isolates with putative dry rot symptom.

Tubers inoculated with *F. solani* SVUFs73 showed a mean lesion size of 32.7 mm, the highest average lesion size among all tested isolates Figure 6 and 8. *Fusarium solani* SVUFs73 showed higher pathogenicity effect than other isolates, based on average lesion sizes (Figure 8).

On the other hand, no differences were observed between the four isolates of *F. solani* species complex (SVUFs93, 96, 97 and 102), which showed a similar lesion size (22.7) mm (Figure 8).

Tubers inoculated with isolates *F. solani* species complex isolates (SVUFf1, SVUFf2, SVUFs19, 22, 23, 24, 27, 28, 29, 30, 31, 33,34, 36, 37, 38, 40, 41, 43, 44, 46, 48, 51, 52, 53, 55, 57, 59, 60, 63, 64, 67, 68, 70, 71, 72, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 92, 94, 98, 99, 101, 103 and 104) were pathogenic to inoculated potato tubers and caused lesions of 10:16 mm (Figure 8).

Discussion

This study is the comprehensive research for identification and genetic diversity of *Fusarium solani* , affecting the potato tubers in Upper Egypt. In this study, *Fusarium solani* were associated with potato tubers collected from the markets in Upper Egypt. This result in agreement with [3, 14] showed that *F. solani* was the most frequently species associated with potato dry-rot in Egypt. According to other studies, *Fusarium solani* was the most frequent and aggressive among all *Fusarium* species isolated from potato tubers in different parts of the world [9]. This concurred with Chehri et al.[35] who assessed that *F. solani* was considered as in the virulent group. However, the genus of *Fusarium solani* is complex and morphological differences may be difficult to observe. Therefore, the DNA analysis is necessary for accurate identification and characterization of the species [36].

β-tubulin sequences are often used to identify fungal species, and vast numbers of these sequences are already available in databases [37]. The results of Donaldson et al. [38] demonstrated the utility of β-tubulin sequences for phylogenetic studies in the genus *Fusarium* and a wide array of Ascomycetes. Sequence of translation elongation factor-1α (TEF-1α) gene always offered a finer resolution and separated strains of most *Fusarium* complex species at species rank [16]. For precise identification of FSSC in this study, a molecular systematic study of β-tubulin and TEF-1α genes was used. In this study, PCR assays with primers Bt2a/Bt2b and Ef1/Ef2 that amplify β-tubulin and TEF sequences enabled us to acquire the product with numerous species of *F. solani*. It was found that β-tubulin separated *F. solani* species complex under study to *F. falciforme* and *F. solani*. While, TEF-1α separated FSSC to *F. falciforme*, *F. keratoplasticum* and *F. solani*. *Fusarium solani* isolates were additionally clustered in the same main clade with several groupes which demonstrated intraspecific variations. *Fusarium solani* also represent a species complex of 45 phylogenetic species which formed *Fusarium solani* species complex [39]. Inside *F. solani* species complex (FSSC), the isolates from soil and plant debris isolated worldwide are typically gathered in one or two phylogenetic species, known as FSSC3 and FSSC4 [40,41]. Intraspecific variations of *F. solani* have likewise been reported by Balmas et al. [42] in which two phylogenetic species, FSSC5 and FSSC9 were identified among 23 *Fusarium solani* species complex. These results came in agreement with Taha et al. [18] who performed β- tubulin gene sequencing of *Fusarium* isolates and phylogenetic analysis showed that the clade of *F. solani* divided into sub-groups. Mehl and Epstein [43] and Short et al. [44] reported that three of the most common species in *F. solani* species complex (*F. keratoplasticum* , *F. falciforme* and *F. solani*), showed a good deal of intraspecific variation, and overlapping morphological traits. This result was in concurrence with Chehri et al. [45] who examined and phylogenetically analyzed 55 strains of the FSSC based on internal transcribed spacer (ITS) regions and partial translation elongation factor-1 (TEF-1α) sequences. They showed that the strains were characterized into four portrayed *Fusarium* species, to be specific *Fusarium keratoplasticum*, *F. falciforme*, FSSC , and *Fusarium* cf. *ensiforme*. Also, the phylogenetic trees unmistakably distinguished firmly related species and particularly separated all morphological taxa. As indicated by Short et al.[44], members from *F. falciforme*, *F. keratoplasticum*, and FSSC ordinarily were related with human infectious diseases while these strains were related with plants. These results were in agreement with Chehri et al.[35] who reported that based on the sequence data from translation elongation factor (TEF-1α) gene, all of the selected FSSC isolates were divided into two major groups. the first group on molecular identification of FSSC strains isolated from potato tubers in Iran and the second group were *Fusarium falciforme,* which were reported for the first time in Iran.

In this study, all the *Fusarium solani* were active in producing amylase especially *F. F. solani* (SVUFs73, 93, 96, 97, 102) (mean value ≥15 mm), whereas, low enzyme activity was exhibited by other isolates of *F. solani* (mean value ≤14mm). These results are in agreement with Kumar et al. [46] who reported that the primary and secondary screening results showed that a fungus isolated from degrading potato tuber, *Fusarium solani*, expressed maximum amylase production over other isolates. Subsequently, it was

selected for amylase production and optimization studies indicated that as independent variable, pH-4 and incubation temperature of 30°C were found ideal for amylase production by *F. solani*.

All *Fusarium solani* isolates were pathogenic for potato tuber with different degrees. Isolate SVUFs73 of *F. solani* was highly pathogenic to potato tubers, whereas, other isolates which belong to the same species were weakly pathogenic to potato tubers. The mechanisms underlying this behaviour still vague. Some studies proposed that mycotoxins produced by *Fusarium* spp. play a key role in this regard [47, 48, 49]. These results were in agreement with the results published by [35] who confirmed that some isolates of *F.solani* were highly virulent and other isolates were virulent or nonvirulent. In addition, Ashour et al.[3] confirmed that, of the 10 isolates of *F. solani* with dry rot symptoms, one isolate was the most pathogenic while others were either less pathogenic (5 isolates) or non pathogenic, 4 isolates to potato tubers. According to Saber et al.[2] , 8 isolates of *Fusarium solani* were obtained from infected potato tubers of which 3 isolates were highly pathogenic to potato tubers, 2 isolates were avirulent, and other isolates were non pathogenic to potato tubers.

Conclusions

Based on morphological, microscopic characteristic and molecular identification by sequencing of β-tubulin and TEF-1α genes, we proved the presence of *Fusarium keratoplasticum*, *F. falciforme* and *F. solani* on potato tubers in Upper Egypt. Based on our knowledge and research, this is the first comprehensive report on identity (morphological and molecular), pathogenicity and distribution of members of *F. solani* species complex from potato tubers in Upper Egypt. TEF-1α gene in molecular identification of FSSC was better than β- tubulin. FSSC demonstrated the most aggressive properties and amylase production, with present strong relationships between pathogenicity and α-amylase enzyme production.

Declarations

Acknowledgements

The authors extend their appreciation to Taif University for funding the current work by Taif University Researchers Supporting Project number (TURSP-2020/111), Taif University, Taif, Saudi Arabia.

Conflict of Interest: The authors declare that they have no conflict of interest.

Authors' contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by [**Youssuf A. Gherbawy**], [**Mohamed A. Hussein**], [**Eman G. A. El-Dawy**] and [**Nabila A. Hassany]**. The first draft of the manuscript was written by [**Yassmin M. Shebany**] and [**Mohamed F. Awad**] and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Data availability

Sequence data have been deposited in GenBank, (https://www.ncbi.nlm.nih.gov/nuccore/MN310668.1?report=GenBank).

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Tables

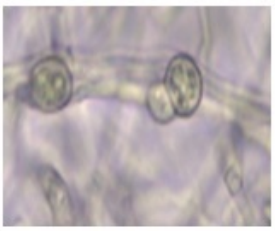
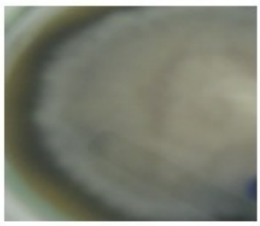
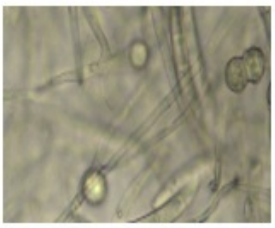
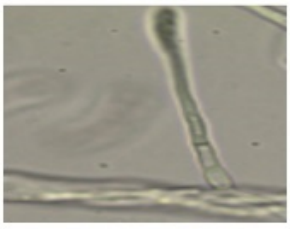
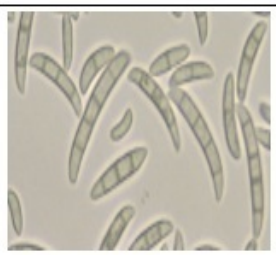
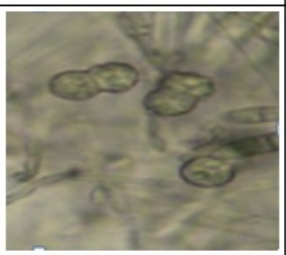
Table (1): Total counts (TC, calculated per 480 segments, frequency (F, calculated per 40 samples) and percentage frequency (%F) of *Fusarium solani* recovered from potato tubers.

Samples / Species	Infected potato tubers			Healthy potato tubers		
	TC	F	%F	TC	F	%F
Fusarium solani	128	32	80	59	20	50

Table (2): Morphological characteristics of *Fusarium solani* isolated from potato tubers

Code of isolates	Colony color	Microconidia			Macroconidia			Phialides	Chlamydospores
		shape	Size (µm)	Form	Shape	Size (µm)	Septate		
SVUFf1, SVUFf2, SVUFs24, 28, 34, 57, 63, 71, 74, 75, 76, 77, 81, 85, 88, 104	Off-white to pale cream	Ellipsoidal to kidney shaped	9.7- 17.7 × 2.9- 4.5	False head	Falcate shape with pointed apexes	28.8- 39.6 × 3.1- 5.6	3	monophialides	Singly and in pairs
SVUFs87	Deep gray colony with a white leading at center	Oval to kidney-shape and have thicker walls	9.8- 15.7 × 3.0- 4.5	False head	Fusiform and thick wall (The apical cell was blunt while the basal cell was foot-shaped)	28.5- 31.7 ×3.2- 5.1	3	Long monophialides	Singly and in pairs
SVUFs19, 20, 21, 22, 23, 25, 26, 27, 29, 30, 31, 32, 33, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 58, 59, 60, 61, 62, 64, 65, 66, 67, 68, 69, 70, 72, 73, 78, 79, 80, 82, 83, 84, 86, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103	off-white to gray and white to violet	Oval to kidney-shape, larger and have thicker walls	14- 23.4 × 2.9- 5.6	False head	Fusiform and thick wall (The apical cell was blunt while the basal cell was foot-shaped)	35.3- 50.6 × 3.5- 6.2	3-5	Long monophialides	Singly and in pairs

Table (3): Microscopic characteristics of *Fusarium solani* isolated from potato tubers

Code of isolates	Colony color	Macro& Microconidia	Chlamydospores	Phialides
SVUFf1, SVUFf2, SVUFs24, 28, 34, 57, 63, 71, 74, 75, 76, 77, 81, 85, 88, 104				
SVUFs87				
SVUFs19, 20, 21, 22, 23, 25, 26, 27, 29, 30, 31, 32, 33, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 58, 59, 60, 61, 62, 64, 65, 66, 67, 68, 69, 70, 72, 73, 78, 79, 80, 82, 83, 84, 86, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103				

Figures

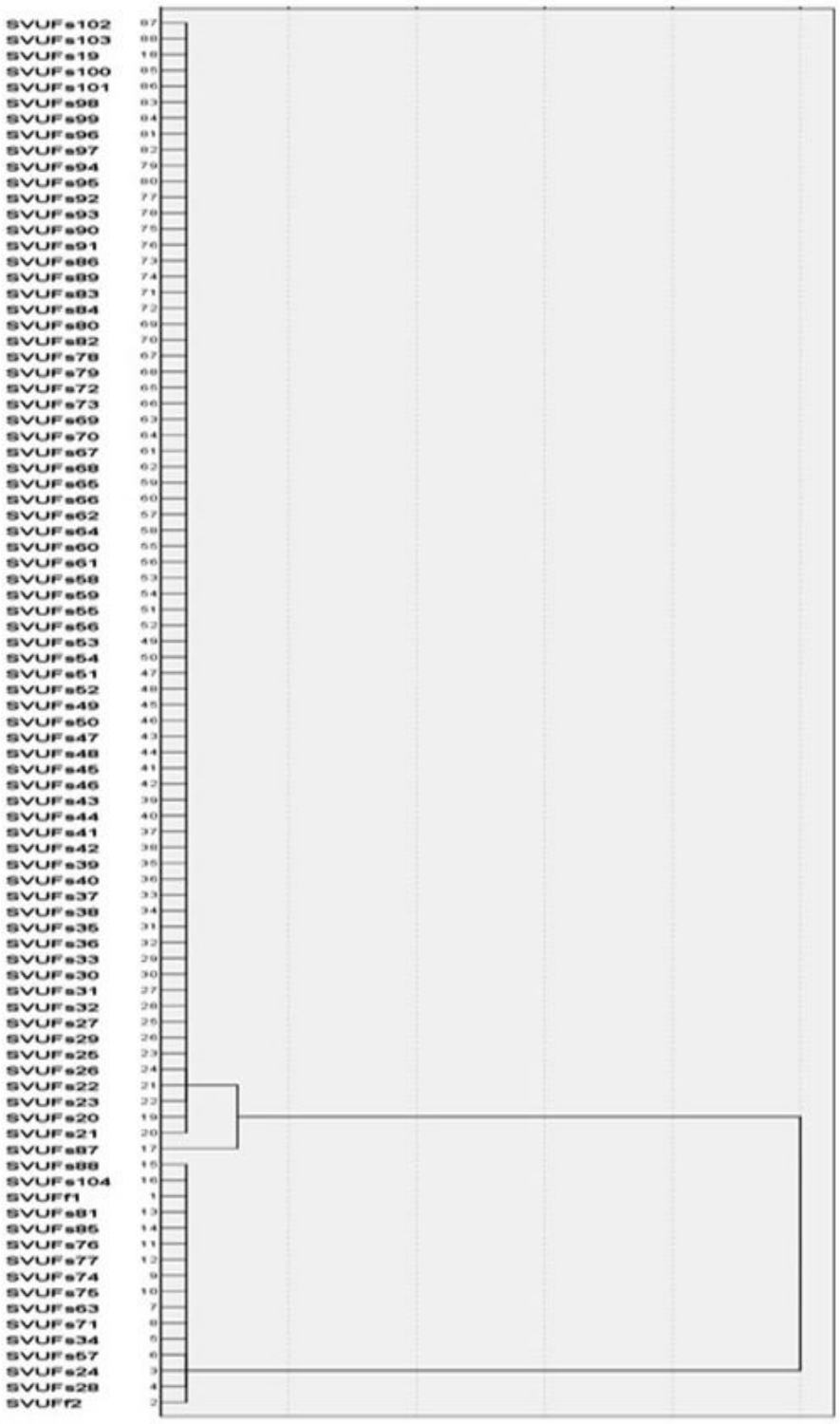


Figure 1

Dendrogram (based on morphological characteristics) showing relationships among 88 isolates of Fusarium solani isolated from potato tubers.

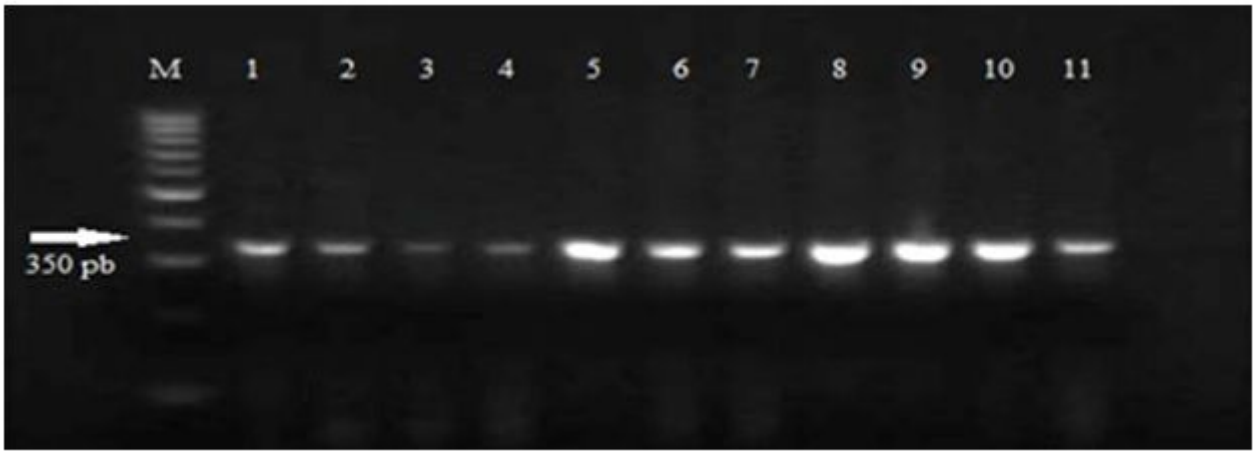


Figure 2

PCR products obtained from different Fusarium solani by using primer pair Bt2a / Bt2b. M: 100 bp molecular size marker.

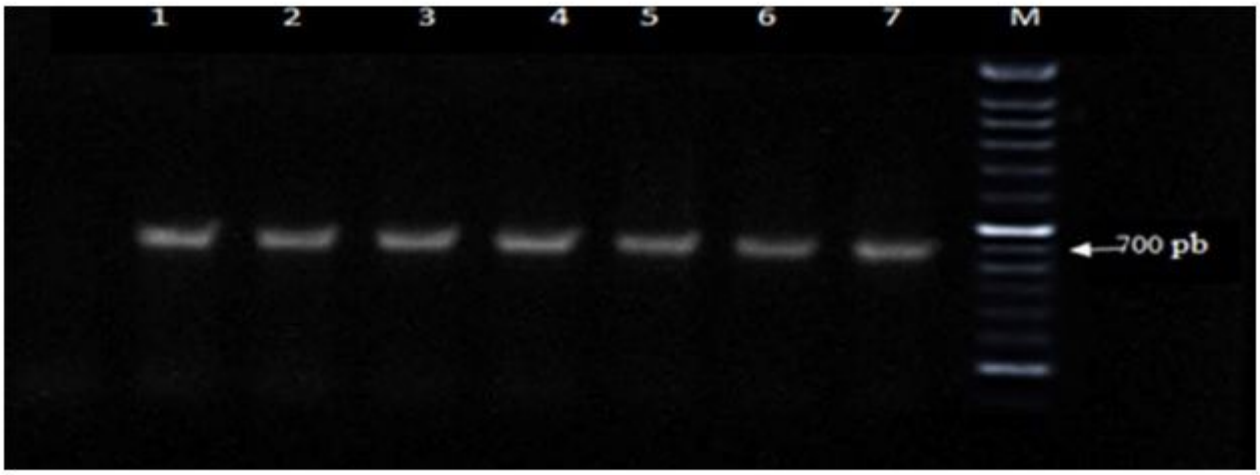


Figure 3

PCR products obtained from different Fusarium solani by using primer pair EF1/ EF2. M: 100 bp molecular size marker.

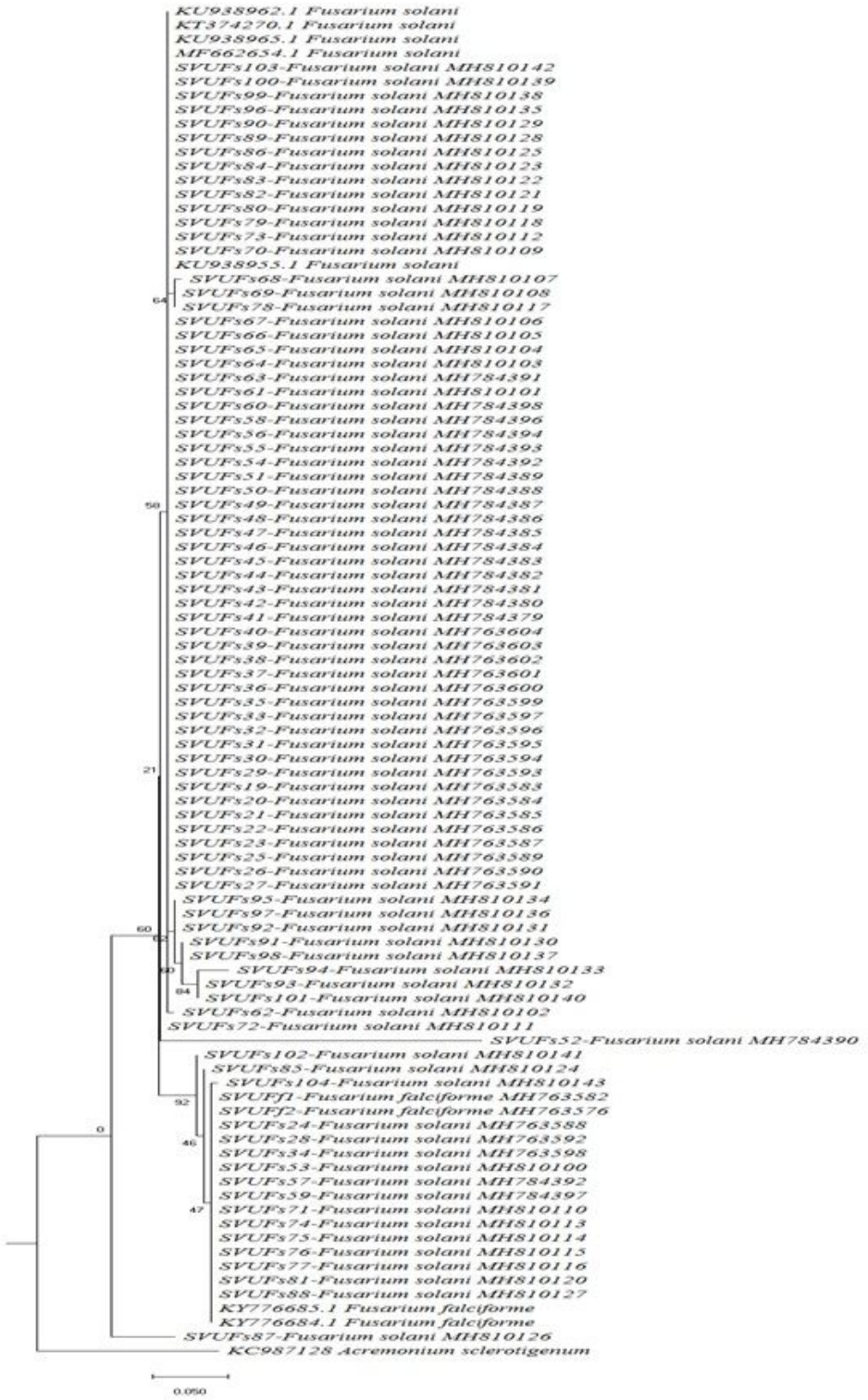


Figure 4

Neighbor- joining phylogenic tree of 88 isolates of Fusarium solani species complex resulting from the sequence results of β-tubulin gene using Acremonium sclerotigenum as out group.

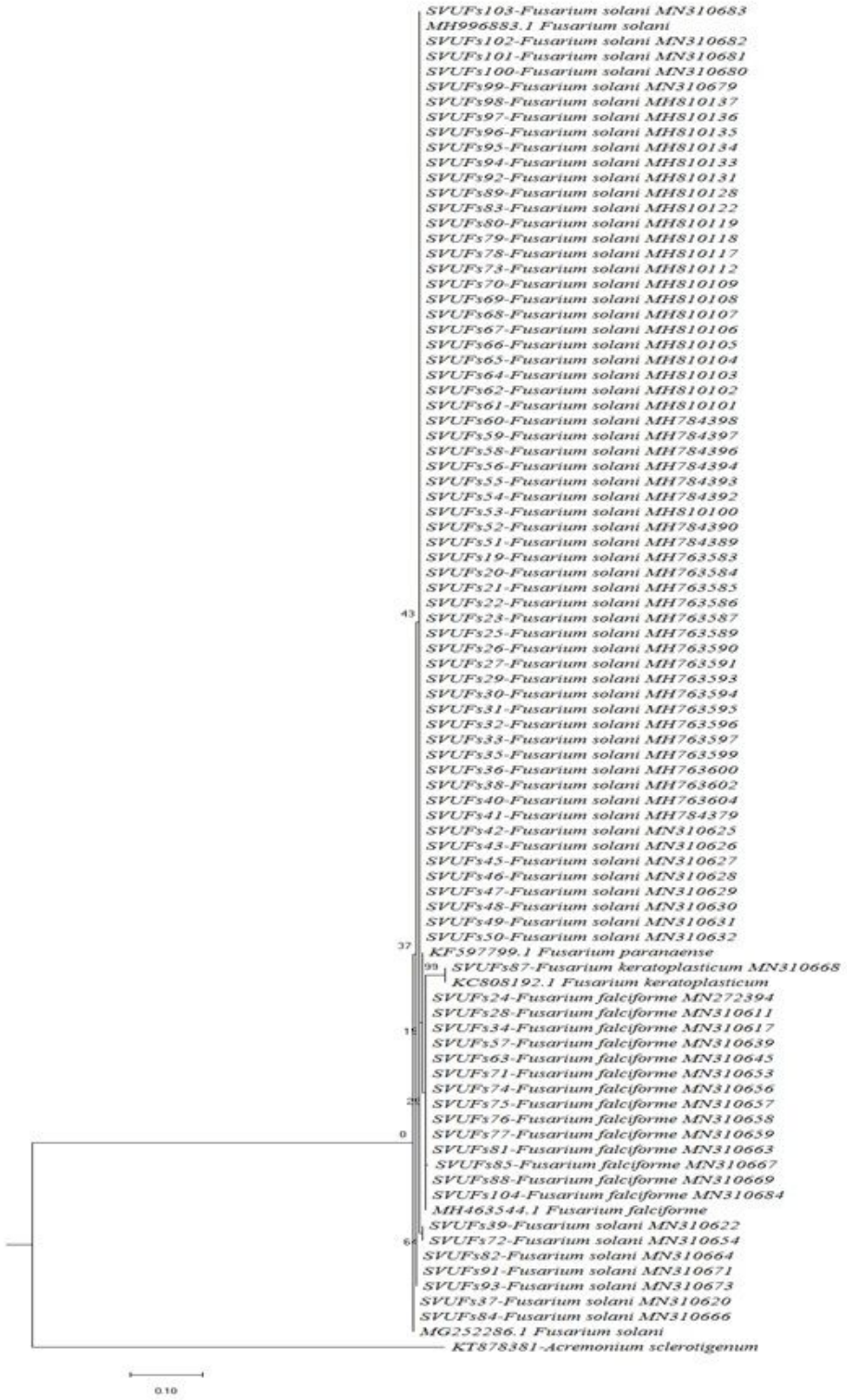


Figure 5

Neighbor- joining phylogenic tree of 83 isolates of Fusarium solani species complex resulting from the sequence results of TEF-1α gene using Acremonium sclerotigenum as out group.

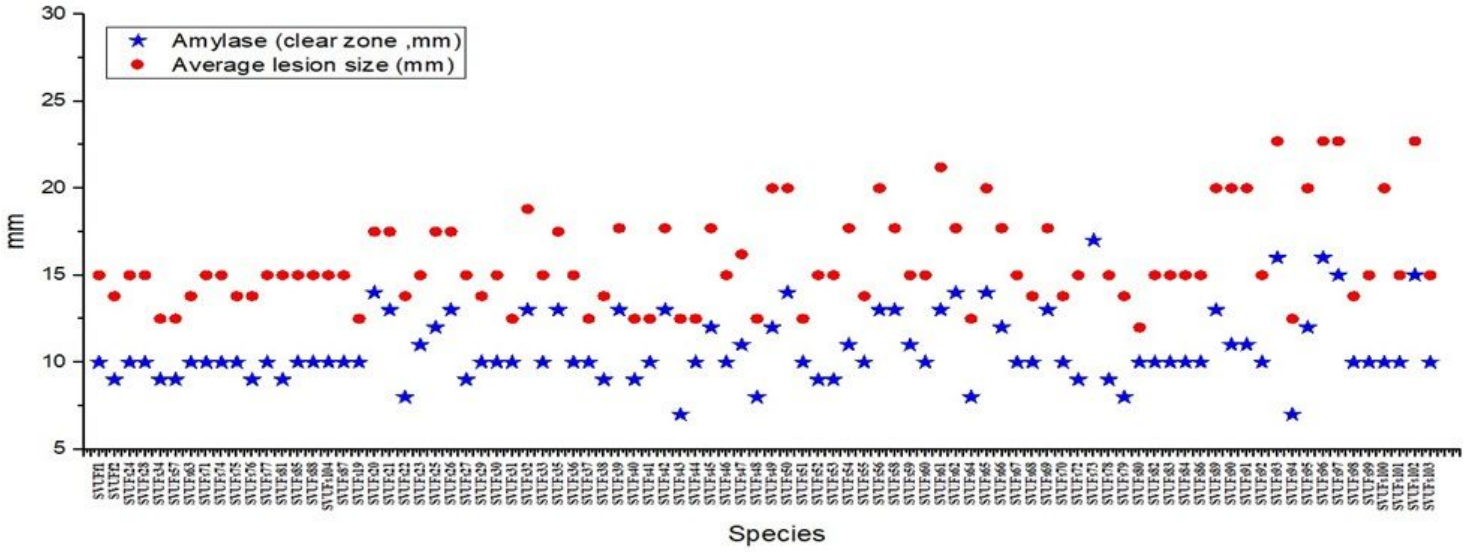


Figure 6

Amylolytic activities and measurement of lesion expansions in the potato tubers of each sample by different strains of Fusarium solani species complex.

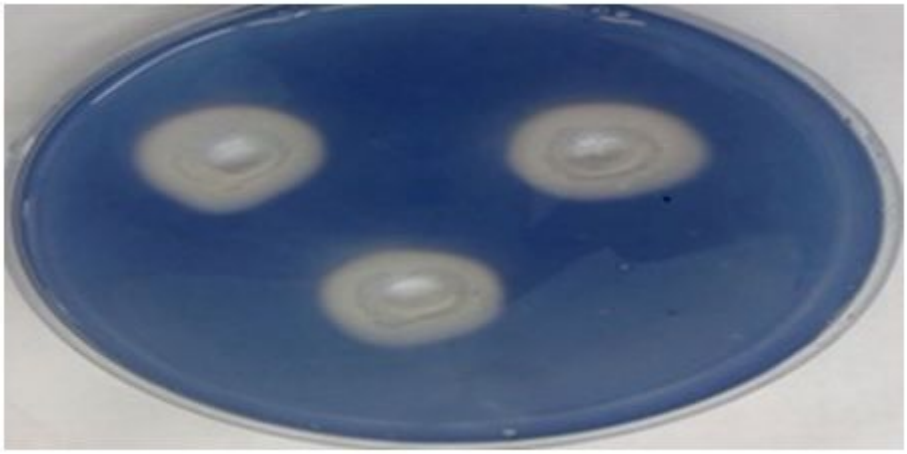


Figure 7

Clear zone representing amylase activity produced by Fusarium solani (SVUFs73).

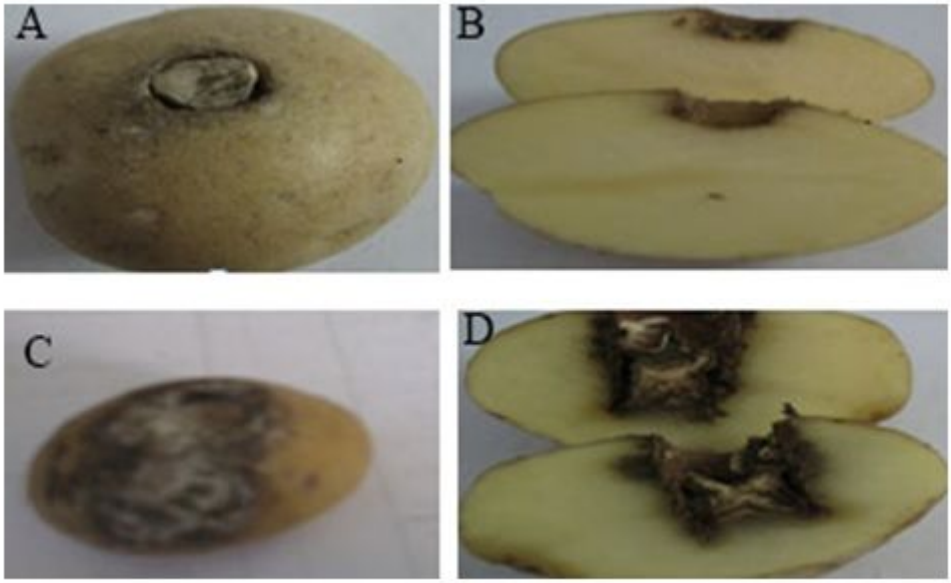


Figure 8

External and internal symptoms of dry rot caused by Fusarium solani on potato tubers, after 21 days of incubation; A and B: control sample; C and D: inoculated sample by SVUFs73.