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# Biocontrol of Sweet Melon Fruit Rot Caused by *Fusarium solani* using an Endophytic Fungus Isolated from the Medicinal Plant *Solenostemma arghel*

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**Abstract**

The fruit rot disease of sweet melon is responsible for serious of economic crop losses that have occurred sporadically in Aswan, Egypt recently. The symptoms appeared as water-soaking lesion which advanced to the rotting of the fruit surface. White mycelial mats with brown color inside appear on the lesion at the surface of the fruit. Disease symptoms, morphological and mycological characteristics, pathogenicity and molecular identification, indicated as *Fusarium solani*, are the disease causative. When healthy fruits of sweet melon sprayed with spore suspension of the isolated *F. solani*, the disease symptoms appeared as white spot, which enlarged and turned brown. Dual culture techniques showed that the endophytic fungi, *Aspergillus terreus*, *Fusarium solani* and *Penicillium verrucosum* isolated from the medicinal plant *Solenostemma arghel* inhibited the pathogen growth in variable levels. The extract of ethyl acetate of the endophytic fungal was found to be active against *Fusarium solani*. The ethyl acetate fractions of *Penicillium verrucosum* inhibited the pathogen growth by 47% while *A. terreus*, and *F. solani* showed inhibition percentage of 45% and 40%, respectively.

**Keywords:** Sweet Melon, *Fusarium solani*, fruit wilt, *Solenostemma arghel*

## 1. Introduction

Sweet melon, (*Cucumis melo* ) is a very important fruit crop cultivated worlwide. The crop is

cultivated in Egypt for local market consumption and exportation. It is known as a very healthful fruit containing vitamins, sugars, folic acid, ascorbic acid and other health-bioactive compounds (Nuangmek *et al.,* 2019). The sweet melon is sensitive to the disease of *fusarium* rot when cultivated in the same soil without regular rotation (Soriano-Martin *et al.,* 2006). According to Nuangmek *et al.,* (2019), the disease caused by *Fusarium* species is considered the high prevalent postharvest and preharvest diseases of cucurbit fruits (melon and cucumbers). The melon disease caused by *Fusarium* species leads to serious economic losses. In addition, the plant pathogen can stay dormant in the soil for many years via the production of chlamydospores, making it very tough to be controlled (Garret, 1970). Many strategies have been used to control this severe pathogen like crop rotation strategy, but completely failed when a disease outbreak occured (Zhao *et al.,* 2011). The alternaive emerging strategy to control *Fusarium* rot disease is the biological control. Many antagonistic microorganisms and microbial endophytes have been confirmed to be effective as biocontrol agents against many plant pathogens (Bhakthavatchalu and Shivakumar, 2018; Marrez *et al.,* 2019). Many of these endophytes are capable of synthesizing antimicrobial secondary metabolites which act as biocontrol agents (Kamel *et al.,* 2020). Many studies reported that the soil incorporation with fungal antagonists results in reducing the incidence of diseases in different crops (Alwathnani and Perveen, 2012).

The purpose of our study was to isolate and identify the causal agent of the sweet melon fruit rot disease using morphological and molecular teqniques and to evaluate the efficacy of extracts of fungal endophytes isolated from the medicinal plant *Solenostemma arghel* as biocontrol to the pathogen.

## 2. Materials and Methods

### *2.1. Samples collection*

In summer 2019, brown color appeared inside the fruits of the native Sweet Melon (shamam) for the first time in Upper Egypt at Aswan University farm (Aswan city, Egypt). The fruits were collected and kept in bags and then transferred immediately for pathogen isolation.

### *2.2. Isolation of the Pathogen from infected fruits of Sweet Melon*

The skin of the sympotomatic part of infected sweet melon was firstly surface sterilized by ethanol (70%) for 1 min and NaClO (1%) for 1 min then washed four times

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with sterilized distilled water. Small parts were aseptically cut and plated on potato dextrose agar (PDA) plates for 4 days at 28 °C. Within two days, fungal mycelia were visibly grown from the fruit pieces. Using hyphal tip method for purification, a single hypha was transmitted and inoculated on fresh PDA plates then examined by microscope (Ghuffar *et al.,* 2018).

*2.3. Morphological and Molecular identification*

Isolated fungal species were identified morphologically based on their colonial and hyphal characteristics (Booth, 1977; Christensen and Raber, 1978; Moubasher, 1993; Raper and Fennell, 1965). The pathogen molecular identification was performed by rRNA gene sequencing. CTAB method (Gontia-Mishra *et al.,* 2014) was used to extract DNA from five days incubated cultured fungus. The partial fragment of rDNA gene was amplified by using two fungal primers ITS1 and ITS4 (Suarez *et al*., 2005). The PCR amplifications were detected by electrophoreses on 1% agarose gel. These bands were eluted and sequenced in Korea Solgent Company. NCBI Blast website was used to analyze sequences. MEGA6 software programme was used for construction of phylogenetic tree (Tamura *et al.,* 2013).

*2.4. Pathogenicity test*

The re-inoculation test of pathogenicity was done by the method according to Berner *et al.* (2007), the pathogen was injected on PDA media and incubated at 28 ℃ for 11 days. The harvested conidia were suspended in sterilized water at 1 x $10^6$ Conidia/ ml, the sweet melon fruits were sprayed by the conidial suspension then covered with bags and incubated at 28℃ for two weeks. In the same conditions, the control fruits were sprayed with sterile distilled water.

*2.5. Selection of antagonistic isolates from Solenostemma arghel*

From fungal lab collection from Faculty of Science, Aswan University, endophytic fungi isolated from the medicinal plant, *S. arghel* (*Aspergillus terreus*, *Fusarium solani* and *Penicillium verrucosum*) (unpublished result) were obtained. Each isolate was sub-cultured on PDA media for their cultivation and allowed to flourish at 28 °C.

*2.6. In vitro screening of antagonistic fungi against sweet melon pathogen*

Antagonistic effects of endophytic fungi isolated from *S. arghel* were tested *in vitro* against sweet melon pathogen by dual culture assay (Albert *et al.,* 2011). The control plates were made by culturing the pathogenic fungus against agar plug. The tests were done in four replicates and incubated at 28±2 °C and the growth diameter of tested pathogen was measured. The inhibition percentage was calculated after 7 days based on the inhibition of colony diameter:

$$\text{Inhibition (\%)} = D1\text{-}D2/D1 \times 100$$

D1: the colony diameter of the pathogen co-inoculated with agar plug (control), and D2: the pathogen colony diameter co-inoculated with fungal endophytes.

*2.7. Extraction of secondary metabolites from endophytic fungi*

Endophytic fungi isolated from *S. arhgel* were incubated as 6 mm disc in 1000 ml flask containing 400 ml PDA media under shaking condition (210 rpm) along 10 days. Then ethyl acetate (EtOAc) was combined with culture and left 24h under continuous shaking, and then the extract of EtOAc was separated by separating funnel and vacuum dried (Abdel-Motaal *et al.,* 2010).

*2.8. Antifungal activity of endophytic fungi against sweet melon pathogen*

Fungal ethyl acetate extracts were added into PDA at various concentrations (0.5, 1.0 and 2.0 mg/ml), shaken well to homogenize. The disc of mycelia (0.8 cm diameter) was transferred in the center of the plate (6.0 cm in diameter) according to the 'poisoned food method' that used to check the antimicrobial effect against the pathogen (Balouiri *et al.,* 2016). According to Singh and Tripathi, (1999), the fungal growth diameters of the treated and control plates were measured after 3 days, and the inhibition percentage was calculated. The minimum inhibitory concentration (MIC) values of each extract for fungal growth were determined in comparison with the control according to Balouiri *et al.,* (2016).

*2.9. Statistical analysis*

All experiments were done in triplicate. One-way analysis of variance (ANOVA) was used to analyze the obtained results with the help of Minitab 18 software (www.minitab.com). Tukey test was run to verify the significant differences between the control and treatments ($P \leq 0.05$). Values shown in the figures are the means ± standard errors (SEs).

## 3. Results

*3.1. Examined symptoms*

Sweet melon fruits showed the typical rot symptoms on the base of the fruits in which the fissures are found as white mycelia present in the epidermal tissue (Fig. 1A). A cross section through a mature lesion showed brown and spongy internal rot with a light brown halo (Fig. 1B).

*3.2. Morphological characterisation of the pathogen*

Colonies of the examined fungi were cottony white color (Fig 1C) with hyalline hyphae, and the mycelium became yellowish white and its reverse is yellow-brown. *Fusarium solani* has aerial hyphae that grow to form conidiophores which branch into monophialides which produce conidia. Phialids were more or less erect. The macroconidia were slightly curved; hyaline and have three septa but may have as many as 4–5. Microconidia were oval or cylindrical, hyaline, smooth and absecnce of septa, but sometimes they may have one or two. Chlamydospores also forms by *F. solani* that most usually emerged under suboptimal growth conditions (Fig. 1D, 1E, 1F, 1G). This fungus was identified as *F. solani* according to morphological characteristics, (Summerbell, 2003; Chehri *et al.,* 2015).

### *3.3. Molecular characterisation of sweet melon pathogen*

To confirm the morphological identification, *F. solani* was exposed to a genomic DNA extraction and gene amplification. The partial 28S nrDNA gene and internal transcribed spacer region (ITS) were amplified by using primers ITS1 and ITS4 (Suarez *et al*., 2005; White *et al*., 1990). According to the NCBI-BLAST analysis, the ITS sequence showed 97–100% similarity with all *F. solani* (LC70340.1, MF359555.1, JN786598.1) in the Phylogenetic tree (Fig. 2). The ITS sequences of *F. solani* were placed in the Gen Bank database with accession number LC510255.

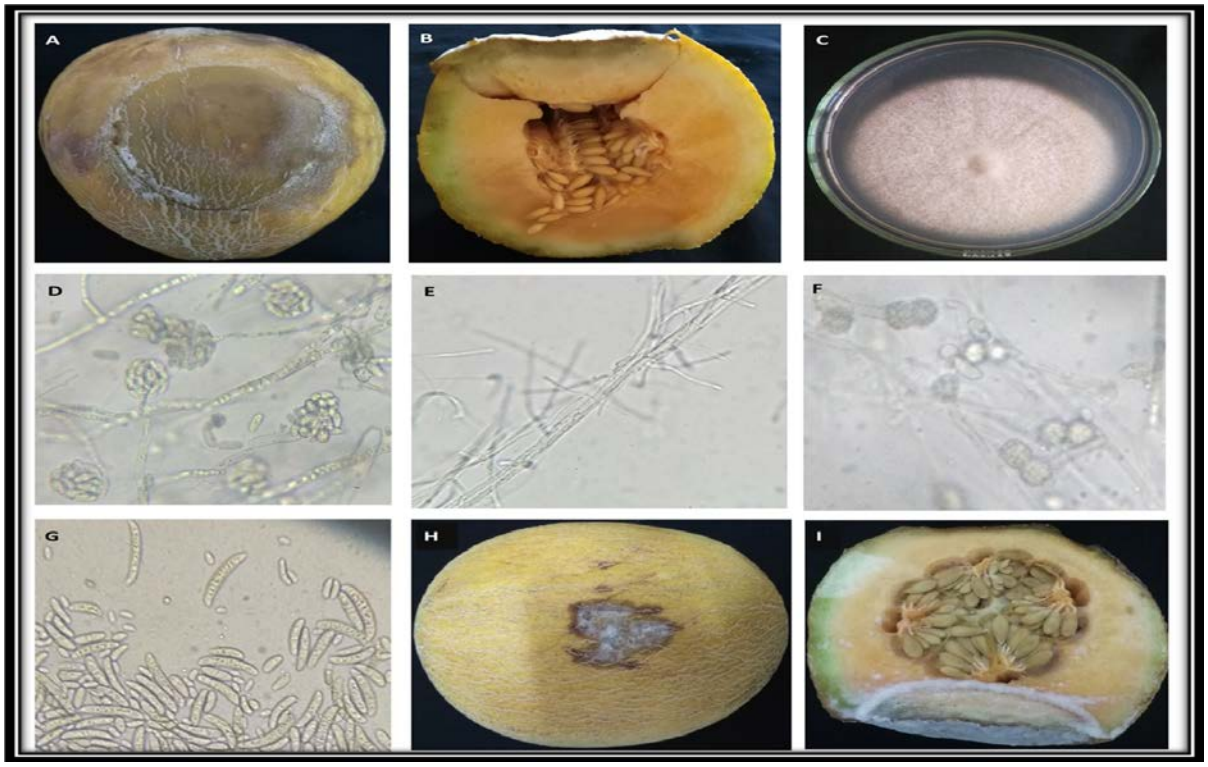


**Figure 1.** Fruit rot symptoms on Sweet Melon after natural infection by *Fusarium solani*. (A,B) Natural disease symptoms on Sweet Melon fruit. (C) Colony shape. (D) False head. (E) Phialids. (F) chlamydospores. (G) Macro and Micro-conidia. (H, I) Pathogenicity re-test using isolated *Fusarium solani* on Sweet Melon. (*Cucumis melo*) fruits one week after inoculation.

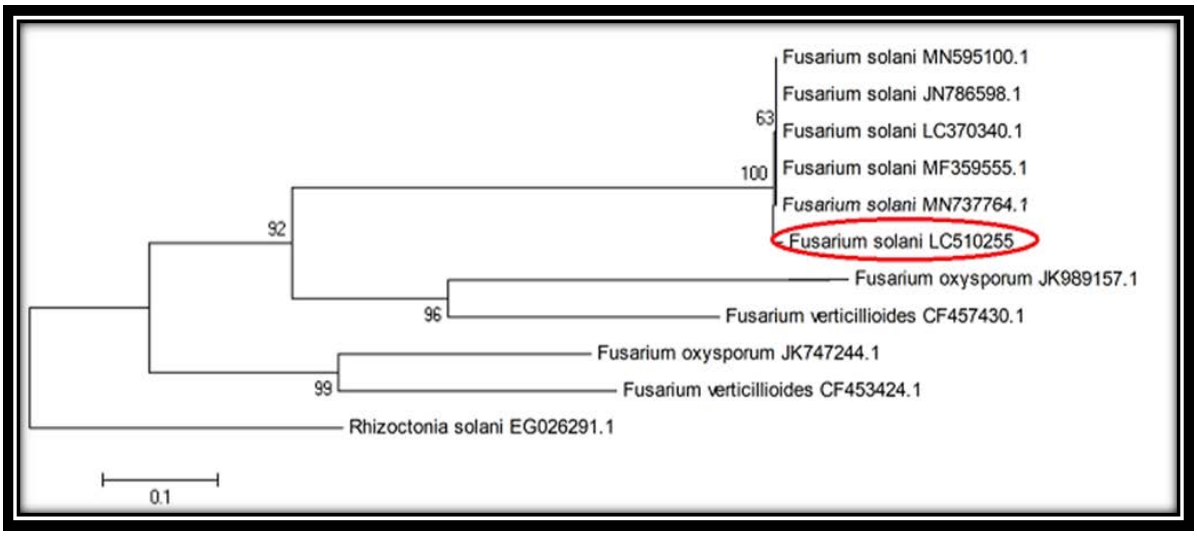


**Figure 2.** Phylogenetic relationships of partial sequences of rRNA gen from *Fusarium solani* fungal pathogenic and selected fungi derived from NCBI Genbank. The construction of phylogenetic tree represents Neighbor-joining method using MEGA 6 software.

### *3.4. Pathogenicity test*

When the healthy fruits of sweet melon were sprayed with *F. solani*, the symptoms of disesae appeared after one week as a white spots, that turned to brown after two weeks (Fig. 1H, 1I) while control fruits did not show any morphological symptoms. *Fusarium solani* was reisolated from the parts of diseased fruits but not from the control fruits.

### *3.5. Antagonist activity of Solenostemma arghel endophytes against sweet melon pathogen*

The dual culture assay demonstrated that the tested endophytic fungi obtained from *S. arghel* (*Aspergillus terreus*, *Fusarium solani* and *Penicillium verrucosum*) inhibited the growth of *F. solani* (the sweet melon pathogen). Figure (3) showed that the antagonistic isolates change the shape of phytopathogen colonies from circular as in control to elongated ellipse. In comparison with the control, the above mentioned isolates had a significant inhibition effect ($p<0.05$) on the growth of pathogen colonies. The antagonistic activity of the selected endophytic fungi showed varying degrees of inhibition against the phytopathogenic *F. solani*, the highest inhibition percentage demonstrated by *F. solani* followed by *A. terreus* and *P. verrucosum* with inhibition percentage (35%, 26.1% and 22.7%), respectively (Fig. 3).

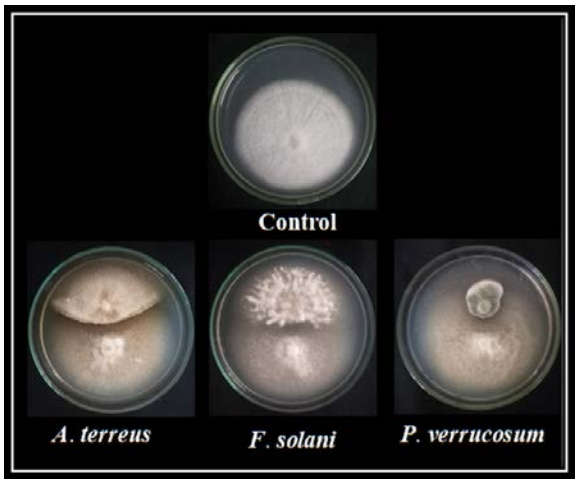


**Figure 3.** Antagonistic activity of *S. arghel* fungi against the pathogen *F. solani*.

### *3.6. Antifungal activity of endophytic fungi against sweet melon pathogen*

The antifungal activities of selected endophyte had a significant efficacy against sweet melon pathogen (Fig. 4).

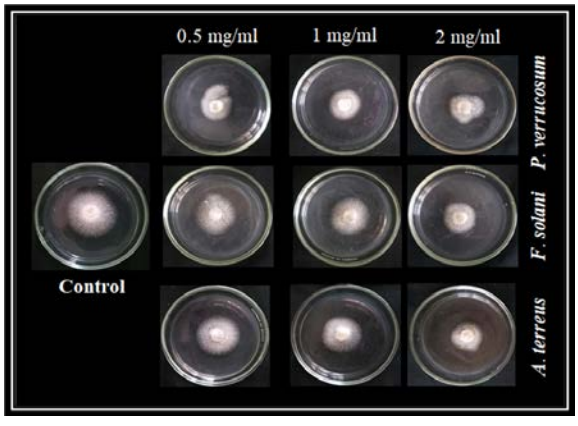


**Figure 4.** Antifungal activity of *S. arghel* endophytic fungal extracts against *F. solani* (the sweet melon pathogen).

The growth inhibition percentage of *F. solani* by various extract concentrations (0.5, 1.0 and 2.0 mg/ml) of *S. arghel* endophytes was determined by measuring the inhibition zones diameter. The concentrations of the selected fungal endophytes were active significantly ($p<0.05$) against the sweet melon pathogen with variable inhibitory effects. In Figure (5), *Penicillium verrucosum* produced the most active substances in the EtOAc fraction which showed the best antifungal activity at concentration 2.0 mg/ ml ($MIC_{47}$) and at 0.5 mg/ ml, the inhibition percentage was 41.6%. *Aspergillus terreus* and *F. solani* exhibited antifungal activity against the pathogenic fungi *F. solani* at concentration 2 mg/ml ($MIC_{45}$, $MIC_{40}$), respectively (Fig. 5).

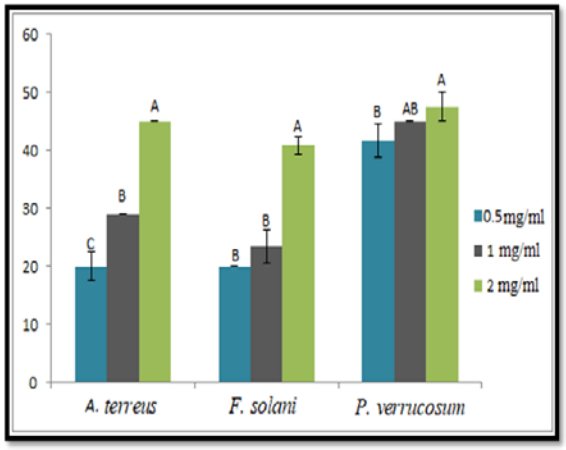


**Figure 5.** Histogram showing the antifungal activity of *S. arghel* endophytic fungi against *Fusarium solani*, values are mean ± stander errors (SEs) of three independent replicates (n=3). Letters a, b and c indicate significant differences $p<0.05$ (ANOVA after Tukey's test analysis).

## 4. Discussion

Rot disease caused by *Fusarium* species is severe for many crops. It is a very common disease of cucurbit fruits during preharvest and postharvest time according to Nuangmek *et al.*, (2019). Our results reported that *F. solani* is the causal agent of fruit rot disease to sweet melon in this study. *Fusarium solani* was reported as a predominant factor in rotting mature melon fruits under wet weather conditions in Texas (Toussoun and Snyder1961). Vast isolates of *F. solani* obtained from roots of muskmelon plants showed root-rot symptoms (Champaco, 1990). Also, other *Fusarium* species like *F. equiseti* have been recorded as a causal agent of fruit rot of cantaloupes by Kim and Kim, (2004) and *F. incaratum* caused fruit rot to muskmelon (Wonglom and Sunpapao, 2020). To reduce losses caused by *Fusarium* to sweet melon fruits, control actions are currently subjected to research. In the present study, the above-mentioned endophytic fungi considerably inhibited the growth of the phytopathogenic *Fusarium in vitro*. They were effective as biological control agents against the sweet melon pathogen. Previous researches reported that several antagonistic species have been confirmed to be effective as bio-control agents in controlled laboratory conditions (Zhao *et al.*, 2011), like *Peniciliium* species (Sabuquillo *et al.*, 2006), *Aspergillus* species (Kandhari *et al.*, 2000) and *F. solani* which isolated from cotton plants as endophytic fungi (Wei *et al.*, 2019).

Our results clearly show that *S. arghel* endophytes affected the growth of the phytopathogenic fungus *F. solani*. This inhibition is propably attributed to the secretion of phytoanticipins or other inhibitory substances produced by antagonists such as aspergillic acid and

dermadin. This inhibition differs according to the nature, quantity and quality of antibiotics/inhibitory substances (Alwathnani and Perveen, 2012). Purification of active molecules may enhance the biocontrol process through the increase in the inhibition percentage compared to the crude extract.

## 5. Conclusion

*Fusarium solani* is the causal agent of fruit rot disease of Sweet Melon. Biological control of *F. solani* by friendly endophytic fungi, *Aspergillus terreus*, *Fusarium solani* and *Penicillium verrucosum* isolated from the medicinal plant *Solenostemma arghel* inhibited the growth of the pathogen at a variable manner. These fungal endophytes can be used as biocontrol agents to supress the Fusarium pathogenicity.

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