



Impact of certain endophytic fungi as biocontrol agents against sesame wilt disease

Hegazy M. G. A. ^{*a}, El Shazly A. M. ^b, Mohamed A. A. ^a, Hassan M. H. A. ^c

^aAgricultural Botany Department, Faculty of Agriculture, Al-Azhar University, 71524 Assiut, Egypt

^bAgricultural Botany Department, Faculty of Agriculture, Al-Azhar University, Cairo, Egypt

^cPlant Pathology Department, Faculty of Agriculture, Al-Azhar University, 71526 Assiut, Egypt

Abstract

This study was conducted to evaluate the ability of some endophytic fungi, as biocontrol agents against sesame wilt caused by *Fusarium oxysporum* f. sp. *sesame*. Eleven fungal species belong to genus *Fusarium* was isolated from different localities in Sohag and Assiut governorates. All isolated species proved to be pathogenic on Giza-32 sesame cultivar. Twelve endophytic fungi isolates were isolated from some parts of various plants. The endophytic fungi isolates were tested for antagonistic activity by the dual culture and culture filtrate techniques. All endophytic fungal isolates were showed antagonistic activity against the causal pathogen in *vitro*. The endophytic *Aspergillus niger* was more antagonistic than other tested isolates toward the causal pathogen followed by *A. clavatus*, *Penicillium chrysogenum* and *P. crustosum*. All the tested fungal culture filtrates (FCF) were significantly affected the growth of the causal pathogen. FCF of *Aspergillus terreus* isolate at 20 % concentration displayed the greatest inhibitory action to the pathogen mycelia growth. Single application with endophytic fungi or combined with *Trichoderma* sp. were significantly decreased the disease severity (%) and increased the plant growth parameters such as shoot length, shoot weight, root weight and number of pods.

Keywords: endophytic fungi, *Fusarium oxysporum* f. sp. *sesame*, antifungal activity, *Aspergillus* spp., *Trichoderma* spp.

*Corresponding author: Hegazy M. G. A.,
E-mail address: mohamedhegazy.5419@azhar.edu.eg

1. Introduction

Sesame (*Sesamum indicum* L.) is one of the most important crops cultivated in tropical and subtropical areas of Asia, Africa, South and Central America. It has been adapted to grow in semi-arid regions (Al-Yemeni *et al.*, 2000). Sesame oil is used as edible oil, in paints and soap industry and has great medicinal value. This crop suffers from many pest and diseases like other crops. *Fusarium* wilt (*Fusarium oxysporum*), charcoal rot (*Macrophomina phaseolina*), leaf spot (*Alternaria sesami*), powdery mildew and *Phytophthora* blight are the most common diseases (Kotle, 1985). Sesame wilt caused by *Fusarium oxysporum* f. sp. *seseame* (Zap.) Cast was seriously damaging disease on sesame crop in Egypt and other countries (Abdou *et al.*, 2001; Ahmed Hoda, 2008). Bacon *et al.* (1977) defines endophytes as microbes that colonize living internal tissues of plants without causing any immediate or negative effects. Endophytes can be transmitted vertically as well as horizontally: vertical transmission occurs through seeds and vegetative propagation of the host and horizontal transmission occurs through spores, external to host tissues (Carroll, 1988). In the 1970's, endophytes considered to be neutral: that is, they were believed to neither cause any harm nor benefit the plant. However, in the course of time, many studies revealed that endophytes play an important role in host protection against predators and pathogens (Azevedo *et al.*, 2000). Many reports have provided evidence that endophytes synthesize myriads of secondary metabolites, which are not

directly involved in the metabolism of the microorganisms but play a role in the fitness and survival of the endophytes and their hosts (Schulz *et al.*, 2002). Thus, endophytes can be an important source of bio-compounds for combating fungal pathogens which reducing crop losses, and improving agricultural productivity with reduced agricultural chemical pesticide inputs. The present work aims to evaluate the ability of some endophytic fungi as biocontrol agents against *Fusarium oxysporum* f. sp. *seseame* the causal pathogen of sesame wilt.

2. Materials and methods

2.1 Isolation and identification of the causal pathogens

Isolation of the causal pathogenic fungi was carried out on PDA medium by agar plate method. *Fusarium* spp. were isolated from the infected sesame plants and brought into pure culture following standard procedure of hyphal tip technique. 250 mg/L. of Chloramphenicol (L-Chloramphenicol) added to the medium before sterilizing to prevent the bacterial growth. The developed fungi were purified using the hyphal tip and/or the single spore techniques. Stock cultures were maintained on PDA slants at 5°C in a refrigerator for subsequent studies. Fungal isolates were identified by using the morphological features of mycelia and spores as described by (John and Brett, 2006; Ismail *et al.*, 2015) and confirmed by Botany and Microbiology Department, Faculty of Science, Al-Azhar University, Assiut, Egypt.

2.2 Isolation and identification of endophytic fungi

Isolation of endophytic fungi was carried out by the modified method of Hallmann *et al.* (2007). The collected root and branch samples were washed with mild detergent and thoroughly with running tap water to remove the soil particles and adhered debris and then finally washed with sterile distilled water. The samples were subjected to surface sterilization with 70 % ethanol for 1 min. For further surface sterilization and to remove adhering microorganisms, the samples were immersed in 4% sodium hypochlorite for 3 min. Finally, the samples were rinsed with sterile distilled water and blot dried on sterile filter paper. The samples were cut into 5-10 mm using a sterile scalpel and cultured in Petri dishes containing PDA medium supplemented with chloramphenicol, (250 mg/L) to suppress bacterial growth. Petri dishes were sealed with parafilm and incubated at $27 \pm 2^\circ\text{C}$ for 15 days under dark condition and monitored every day. The purified endophytic fungal isolates were transferred separately to PDA slants. Finally, all the purified endophytic fungi were maintained at 4°C till further used. Endophytic fungi isolates were identified at Assiut University Moubasher Mycological Center (AUMMC), Assiut, Egypt based on their macro- and microscopic characteristics according to Raper and Fennell (1965), Ellis (1971), Pitt (1979) and Domsch *et al.* (1980, 2007). Identified isolates of *Aspergillus clavatus*, *Aspergillus fumigatus*, *A. niger*, *A. terreus* and *Trichoderma* sp. obtained friendly from the stock culture of Botany

and Microbiology Department, Faculty of Science Al-Azhar University, Assiut, Egypt.

2.3 Pathogenicity tests

The experiments were carried out at the experimental greenhouse of Plant Pathology Department, Faculty of Agriculture, Assiut University, Assiut, Egypt in 2017 growing season. Sesame seeds from Giza-32 cultivar were sterilized by 1% sodium hypochlorite for 3 minutes, then washed gently by sterilized distilled water then dried well. Pathogenicity of *F. oxysporum* isolates were tested under greenhouse conditions. Pottery pots (25 cm in diameter) were filled with formalin disinfested soil. Inocula were prepared by growing the desired fungal isolates at 25°C for two weeks on autoclaved sand barley medium. The prepared inoculum was added to the potted soil at the rate of 3.0% weight, mixed thoroughly with the soil, watered and let for a week. Pathogen-free sterilized sand barley was mixed at the same rate with the potted soil to serve as control treatment. Pots were planted with apparently healthy surface sterilized sesame seeds of Giza-32 cultivar at the rate of four seeds per pot. Four replicated pots were used for each treatment. The disease severity index (DSI) was recorded after 60 days from planting date. The disease index scale (0-5) according to Abdel-Razik Sahar *et al.* (2012) was used to measure the disease severity (%) of Fusarium wilt, in which (0 indicated no visible symptoms; 1, slight vein-clearing and chlorosis of the leaves; 2, yellowing and wilting of lower leaves and extend to

upper leaves; 3, brown (discoloration) of the vascular systems of tap rot and stem; 4, necrotic streaks on the base spread toward the stem apex and 5, premature plant death. The following equation was used to calculate the percentage of disease severity index (DSI) for each tested isolate:

$$\text{DSI \%} = (\sum d / d_{\text{max}} \times n) \times 100$$

Where, (d) is disease rating of each plant, (d_{max}) is the maximum disease rating and n is the total number of plants examined in each replicate.

2.4 Antagonistic activity of the isolated endophytic fungi in vitro

2.4.1 Using the dual-culture technique

In dual culture technique, the antagonistic effect of endophytic fungi was examined against *Fusarium oxysporum* f. sp. *sesame*. Discs of each isolate (6-mm discs in diameter) of endophytic fungi (7-days old) and the same size of another agar disc containing tested fungi were placed opposite to each other and close to the periphery of 90 mm petri plates containing PDA medium. For control treatment, *Fusarium oxysporum* f. sp. *sesame* was placed in a similar manner on PDA Petri plates. All pairing was carried out in three replicates and incubated at $25^{\circ}\text{C} \pm 2$ for 7 days. After incubation, the clear zone between the two colonies at the interaction point was measured. After the desired incubation time, the colonies growth of both the tested endophytic fungi and pathogen were examined and photographed.

2.4.2 Effect of endophytic fungi culture filtrate on pathogen radial growth

The efficacy of culture filtrates of endophytic fungi against *F. oxysporum* f. sp. *sesame* was assessed. Culture filtrates of *Aspergillus niger*, *A. terreus* isolate No. (1), *Aspergillus terreus* isolate No. (2), *Penicillium chrysogenum* isolate No. (1) with five concentrations (1, 2, 5, 10, 20 %) were poured in sterilized Petri dishes. After solidification of PDA, mycelia discs of the *F. oxysporum* f. sp. *sesame* (6 mm in diameter) obtained from actively growing colonies were placed in the center of the solidified agar plates. Sterilized distilled water was added to PDA instead of culture filtrate served as control. The Petri dishes were incubated at 25 ± 2 °C for four days and later the percent of inhibition in the radial colony growth compared to the control and calculated. The percent inhibition of mycelia growth of the pathogens was calculated using following formula (Hassan, 1992):

$$\text{Inhibition (\%)} = (D_1 - D_2) / D_1 \times 100$$

Where, D_1 = colony diameter in control plate and D_2 = colony diameter in treated plate.

2.5 Effect of endophytic fungi only or combined with *Trichoderma* sp. on controlling sesame wilt under greenhouse conditions

This experiment was carried out in 2018 growing season. Sterilized pots (25 cm in diameter) containing sterilized soil were

infested by adding the inoculums of *F. oxysporum* f. sp. *sesame* grown on sand barley medium at the rate of (2.0 % weight), mixed thoroughly with the soil. After one-week, equal amounts of inocula of the antagonistic fungi only and/ or with *Trichoderma* sp. were separately added to each pot and thoroughly watered. Pots containing inoculum of *F. oxysporum* f. sp. *sesame* only were used as control. Pots containing *Trichoderma* sp. only was used as positive control. Three replicates were used for each treatment and 5 sterilized sesame seeds Giza 32 cultivar were sown in each pot. Plants were irrigated when it needs and examined periodically. Percentages of disease severity index of sesame wilt were recorded as above mentioned. The plant growth parameter such as shoot length, shoot weight, root weight and number of pods were recorded.

2.6 Statistical analysis

Analyses of variance were carried out using COSTAT program and employed to Duncan's Multiple Range Test to compare the treatments means at $p \leq 0.05$ (Gomez and Gomez, 1984).

3. Results

3.1 Isolation, identification and pathogenic capability of the causal pathogens isolates

Fifty-five samples were randomly collected from certain areas related to Sohag and Assiut governorates. These localities were, El Maragha, Al- Tolihat, Nazlet Ali (Sohag governorate), Bacor,

Sedfa (Assiut governorate), Egypt. Fungal species recovered from these previous areas are listed in Table (1). In general, data indicate that, eleven fungal species belong to genus *Fusarium* were: *Fusarium camptoceras* (two isolates), *F. proliferatum* (two isolates), *Fusarium tricinctum*, *Fusarium oxysporum* f. sp. *sesame* (two isolates), *Fusarium dimerum* (two isolates), *Fusarium solani* (two isolates), and *Fusarium verticillioides*. Data in Table (1) reveal that, *Fusarium oxysporum* f. sp. *sesame* caused the highest percentage of disease severity (85 %). While *Fusarium dimerum* isolate No. (2) and *Fusarium solani* isolate No. (1) gave the same percent of disease severity (72.5 %). The percentage of disease severity for isolates *Fusarium tricinctum* isolate No. (1) and *Fusarium camptoceras* isolate No. (1) were (66 % and 65 %) respectively. On the other hand, *Fusarium camptoceras* isolate No. (2), *Fusarium dimerum* isolate No. (1) and *Fusarium verticillioides* gave the lowest percentage of disease severity (42.5 %, 39 % and 39 %, respectively).

3.2 Isolation and Identification of endophytic fungi

Eight endophytic fungi isolates were isolated from some parts of various plants, i.e. *Aspergillus sydowii* isolated from Olive (*Olea europaea*). Two species of *Penicillium crustosum* isolate No. (1), *P. crustosum* isolate No. (2) and *Alternaria alternata* were isolated from Alhagi plant (*Alhagi maurorum*) (Sohag – Al Tolihat). *Aspergillus terreus* isolate No. (2), *Penicillium crustosum* isolate No. (3) and *Penicillium chrysogenum* isolate

No. (1) were isolated from Alhagi plant (*Alhagi maurorum*) collected from Sohag – Nazlet Ali. Also, *Penicillium chrysogenum* isolate No. (2) was isolated from Pomegranate (*Punicam granatum*). Finally, *Aspergillus clavatus*, *Aspergillus*

fumigatus, *Aspergillus niger*, *Aspergillus terreus* isolate No. (1) obtained from stock culture of Department of Botany and Microbiology, Faculty of Science, Al-Azhar University, Assiut, Egypt (Table 2, 3).

Table (1): Isolation, identification localities and pathogenic capability of the causal pathogens.

Fungal isolate	Locality	Disease severity (%)
<i>Fusarium camptoceras</i> (1)	El Maragha	65 ^{bc}
<i>Fusarium camptoceras</i> (2)	Sedfa	42.5 ^e
<i>Fusarium proliferatum</i> (1)	El Maragha	51 ^{cde}
<i>Fusarium proliferatum</i> (2)	El Maragha	46 ^{de}
<i>Fusarium tricinctum</i> (1)	El Maragha	66 ^{bc}
<i>Fusarium oxysporum</i> f. sp. <i>sesame</i>	Nazlet Ali	85 ^a
<i>Fusarium dimerum</i> (1)	Bacor	39 ^e
<i>Fusarium dimerum</i> (2)	Nazlet Ali	72.5 ^{ab}
<i>Fusarium solani</i> (1)	Bacor	72.5 ^{ab}
<i>Fusarium solani</i> (2)	Nazlet Ali	64 ^{bcd}
<i>Fusarium verticillioides</i>	Sedfa	39 ^e
Control (uninfected plants)		00 ^f

Means followed by the same letters (s) in a column are not significantly different at (p≤0.05) according to Duncan's multiple-range test.

3.3 Antagonistic activity of the isolated endophytic fungi in vitro

3.3.1 Using the dual-culture method

The isolates of endophytic fungi were tested for antagonistic activity by the dual culture technique. A total of 12 endophytic fungal isolates were showed antagonistic activity against *F. oxysporum* f. sp. *sesame*. These endophytic fungi were: *Alternaria alternate*, *Aspergillus clavatus*, *Aspergillus fumigatus*, *Aspergillus niger*, *Aspergillus sydowii*, *Aspergillus terreus* isolate No. (1), *Aspergillus terreus* isolate No. (2), *Penicillium chrysogenum* isolate No. (1), *Penicillium chrysogenum* isolate No. (2),

Penicillium crustosum isolate No. (1), *Penicillium crustosum* isolate No. (2) and *Penicillium crustosum* isolate No. (3). Data in Table (4) indicated that the endophytic fungus *Aspergillus niger* was more active against *Fusarium oxysporum* f. sp. *sesame* with antagonistic activity (11.6 mm) followed by *Aspergillus clavatus* and *Penicillium chrysogenum* (1) (9.2 mm) and *Penicillium crustosum* (2). While, *Aspergillus terreus* (2) gave moderate antagonistic activity (5.9 mm) against the causal pathogen. On the other hand, *Alternaria alternate*, *Aspergillus sydowii* and *Aspergillus fumigatus* exhibited the lowest antagonistic activity against causal pathogen (1.5 mm, 1.3 mm and 0.8 mm), respectively.

Table (2): List of endophytic fungi isolates which isolated from some parts of various plants.

Isolates	Localities	Host	Source
<i>Aspergillus sydowii</i>	Assiut	Olive (<i>Oleae uropaea</i>)	Branch
<i>Penicillium crustosum</i> (1)	Sohag Al- Tolihat	Alhagi (<i>Alhagi maurorum</i>)	Root
<i>Penicillium crustosum</i> (2)			Root
<i>Alternaria alternata</i>			Root
<i>Aspergillus terreus</i> (2)	Sohag Nazlet Ali	Alhagi (<i>Alhagi maurorum</i>)	Root
<i>Penicillium crustosum</i> (3)			Root
<i>Penicillium chrysogenum</i> (1)			Root
<i>Penicillium chrysogenum</i> (2)			Branch
		Pomegranate (<i>Punicam granatum</i>)	Branch

Table (3): Identification of endophytic fungi isolated from some parts of various plants.

Isolate code	AUMMC No.	Identification
AL ₁	13894	<i>Penicillium crustosum</i> Thom
AL ₂	13895	<i>Penicillium crustosum</i> Thom
AL ₃	13896	<i>Alternaria alternata</i> (Fries) Keissler
AL ₆	13897	<i>Aspergillus terreus</i> Thom
AL ₇	13898	<i>Penicillium crustosum</i> Thom
AL ₈	13899	<i>Penicillium chrysogenum</i> Thom
P ₃	13900	<i>Penicillium chrysogenum</i> Thom
O ₃	13904	<i>Aspergillus sydowii</i> (Bainier & Sartory) Thom & Church

(AL: Alhagi; P: Pomegranate; O: Olive).

3.3.2 Effect of endophytic fungi culture filtrate on the growth of the causal pathogens in vitro

A laboratory experiment was carried out

to determine the effect of fungal culture filtrates (FCF) of the endophytic fungi and evaluating their role as bioagent against the causal pathogen. The obtained

results are listed in Table (5).

Table (4): Antagonistic activity of the isolated endophytic fungi against *Fusarium oxysporum* f. sp. *sesame* in dual culture.

Isolate No.	Endophytic fungi isolate	Average of inhibition zone diameter (mm)
1	<i>Alternaria alternata</i>	1.5 ^{et}
2	<i>Aspergillus clavatus</i>	9.2 ^b
3	<i>Aspergillus fumigatus</i>	0.8 ^{et}
4	<i>Aspergillus niger</i>	11.6 ^a
5	<i>Aspergillus sydowii</i>	1.3 ^{et}
6	<i>Aspergillus terreus</i> (1)	2.3 ^e
7	<i>Aspergillus terreus</i> (2)	5.9 ^{cd}
8	<i>Penicillium chrysogenum</i> (1)	9.2 ^b
9	<i>Penicillium chrysogenum</i> (2)	2.3 ^e
10	<i>Penicillium crustosum</i> (1)	7.1 ^{bc}
11	<i>Penicillium crustosum</i> (2)	8.2 ^b
12	<i>Penicillium crustosum</i> (3)	2.8 ^e
13	Control	0.0 ^f

Means followed by the same letters (s) in a column are not significantly different at (p≤0.05) according to Duncan's multiple-range test.

Table (5): Effect of endophytic fungi culture filtrates on radial growth of *F. oxysporum* f. sp. *sesame* the causal pathogen of sesame wilt.

Endophytic fungi	Concentration (%)	Average of colony diameter (mm)	Inhibition (%)
<i>Aspergillus niger</i>	1	73.30	18.55 ⁱ
	2	69.30	23.00 ^{ghi}
	5	63.30	29.66 ^{fgh}
	10	57.00	36.66 ^{ef}
	20	45.00	50.00 ^d
<i>Aspergillus terreus</i> (1)	1	68.30	24.11 ^{ghi}
	2	55.00	38.88 ^e
	5	45.00	50.00 ^d
	10	36.60	59.33 ^{bc}
	20	26.60	70.44 ^a
<i>Aspergillus terreus</i> (2)	1	73.30	18.55 ⁱ
	2	65.30	27.44 ^{gh}
	5	56.60	37.11 ^{ef}
	10	43.30	51.88 ^{cd}
	20	33.30	63.00 ^{ab}
<i>Penicillium chrysogenum</i> (1)	1	75.00	16.66 ⁱ
	2	70.00	22.22 ^{hi}
	5	62.00	31.11 ^{efg}
	10	55.00	38.88 ^e
	20	44.00	51.11 ^{cd}
Control		90.00	00.00 ^j

Means followed by the same letters (s) in a column are not significantly different at ($p \leq 0.05$) according to Duncan's multiple-range test.

Data in Table (5) showed that all the tested fungal culture filtrates were significantly affected the growth of the causal pathogen. FCF of *Aspergillus terreus* isolate No. (1) at 20 % concentration and *Aspergillus terreus* isolate No. (2) at the same concentration displayed the greatest inhibitory action to the pathogen. While, *Penicillium chrysogenum* isolate No. (1), *Aspergillus niger* and *Aspergillus terreus* isolate No. (1) at 1 % concentration exhibited the lowest inhibitory effect. Other tested concentration showed moderate inhibitory effect.

3.4 Effect of endophytic fungi in controlling sesame wilt and plant growth parameters under greenhouse conditions

Data in Table (6) indicated that application of endophytic fungi affected both disease severity and plant growth parameters. Endophytic fungi have significantly decreased the disease severity (%) and increased the plant growth parameter such as shoot length, shoot weight, root weight and number of pods. Endophytic fungi can decrease the disease severity percentage. *Aspergillus niger* gave the lowest percentage of disease severity (36 %). Both *A. terreus* (1) and *Penicillium chrysogenum* (1) gave the same percentage of disease severity (41 % and 38.6 % respectively) without significant differences. While, *Aspergillus terreus* (2) gave moderate disease severity percentage (52 %). Concerning the plant growth parameter, *Aspergillus terreus* (2)

gave the highest value. This treatment increased shoot length (96 cm), fresh shoot weight (10.3 g) and fresh root weight (7.6 g).

3.5 Effect of *Trichoderma* sp. alone or combined with endophytic fungi in controlling sesame wilt and plant growth parameters under greenhouse conditions

Data in Table (7) indicated that, *Trichoderma* sp. alone or combined with endophytic fungi isolates significantly affected both wilt severity percentage and increased the plant growth parameters. *Trichoderma* sp. as single treatment or combined treatment as *A. niger* +

Trichoderma sp. gave the lowest percentage of disease severity (13.3 % and 16 % respectively). Moderate of disease severity obtained by *A. terreus* (1) + *Trichoderma* sp. (25.3 %) On the other side, *A. terreus* (2) + *Trichoderma* sp. as combined treatment gave the highest percentage of disease severity (46.6 %). In general, single treatment was better than combined treatments. *Trichoderma* sp. was the best treatment and it increased shoot height (98 cm), fresh shoot weight (42.3 g) and fresh root weight (25.6 g). While, combined treatment *Trichoderma* sp. + *Penicillium chrysogenum* (1) was the lowest treatment shoot height (80 cm), fresh shoot weight (17.5 g) and fresh root weight (9.3 g).

Table (6): Effect of endophytic fungi on sesame wilt severity and plant growth parameters under greenhouse conditions.

Treatments	Disease Severity (%)	Plant growth parameters					
		Shoot			Root		
		Plant height (cm)	Fresh weight (g)	Dry weight (g)	Fresh weight (g)	Dry weight (g)	Number of bods
<i>Aspergillus niger</i>	36 ^c	73 ^{ab}	11.8 ^b	3.66 ^b	3.33 ^c	1.3 ^c	10 ^c
<i>Aspergillus terreus</i> (1)	41 ^{bc}	83 ^b	19 ^a	4.66 ^a	7.83 ^a	3.43 ^a	16 ^a
<i>Aspergillus terreus</i> (2)	52 ^b	96 ^a	10.3 ^{bc}	3 ^c	7.6 ^a	2.66 ^b	11 ^b
<i>Penicillium chrysogenum</i> (1)	38.6 ^{bc}	85 ^b	10.6 ^{bc}	2.2 ^d	6.66 ^{ab}	1.46 ^c	10 ^c
Control (untreated)	94.3 ^a	56 ^c	9.6 ^c	2 ^c	4 ^b	1.16 ^c	9 ^d

Means followed by the same letters (s) in a column are not significantly different at (p<0.05) according to Duncan’s multiple-range test.

Table (7): Effect of endophytic fungi and *Trichoderma* sp. in controlling sesame wilt and plant growth parameters in greenhouse.

Treatments	Disease Severity (%)	Growth parameters					
		Shoot			Root		
		Shoot height (cm)	Fresh weight (g)	Dry weight (g)	Fresh weight (g)	Dry weight (g)	Number of bods
<i>A. niger</i> + <i>Trichoderma</i> sp.	16 ^c	91.3 ^b	24.6 ^c	9.6 ^b	14.6 ^b	7.1 ^b	8 ^c
<i>A. terreus</i> (1) + <i>Trichoderma</i> sp.	25.3 ^{bc}	84 ^c	27 ^b	8.5 ^c	15.3 ^b	5.6 ^c	12 ^b
<i>A. terreus</i> (2) + <i>Trichoderma</i> sp.	46.6 ^a	84 ^c	27.5 ^b	7.6 ^d	13 ^c	5.4 ^c	6 ^d
<i>P. chrysogenum</i> (1) + <i>Trichoderma</i> sp.	36 ^{ab}	80 ^d	17.5 ^d	6 ^e	9.3 ^d	3.5 ^d	13 ^a
Control (<i>Trichoderma</i> sp. only)	13.3 ^c	98 ^a	42.3 ^a	12.6 ^a	25.6 ^a	14.3 ^a	12 ^b

Means followed by the same letters (s) in a column are not significantly different at (p<0.05) according to Duncan’s multiple-range test.

4. Discussion

In Egypt, sesame (*Sesamum indicum* L.) is subject to be attacked by many soil borne pathogens. The most destructive diseases are Fusarium wilt caused by *F. oxysporum* f. sp. *sesame* at any growth stages and causing considerable losses of seed yield (Sahab *et al.*, 2008; Elewa *et al.* (2011) and Ziedan *et al.* (2011). Similar results obtained by this study which revealed that, *Fusarium oxysporum* f. sp. *sesame* caused the highest percentage of disease severity. Eight endophytic fungi such as *Aspergillus sydowii*, *Penicillium crustosum*, *Alternaria alternata*, *Aspergillus terreus* and *P. chrysogenum*, were isolated from some parts of various plants. This result agreement with those obtained by Garoe *et al.* (2012). *Aspergillus niger* gave the highest activity against *Fusarium oxysporum* f. sp. *sesame* followed by *Aspergillus clavatus* and *Penicillium chrysogenum*. These results in agreement with those obtained by Kim *et al.* (2007), Rukachaisirikul *et al.*, (2007) and Ge *et al.* (2008) who reported that, endophytic fungi isolated from vegetable plants showed *in vitro* antagonism against *Pythium ultimum*, *P. infestans* and *P. capsici*. All the tested fungal culture filtrates (FCF) were significantly affected the growth of the causal pathogen. FCF of *Aspergillus terreus* isolate at 20 % concentration displayed the greatest inhibitory action to the pathogen mycelial growth. These results in a harmony with those obtained by several investigators (Nair and Padmavathy, 2014; Vidal and Jaber, 2015). They reported that some endophytic fungi can produce compounds

that have growth-inhibitory activities toward herbivores and phytopathogens and thus can be used as biological control agents. Single application with endophytic fungi or combined with *Trichoderma* sp. were significantly decreased the disease severity (%) and increased the plant growth parameters such as shoot length, shoot weight, root weight and number of pods. These results in accordance with those obtained by many workers (Elewa *et al.*, 2011; Harman *et al.*, 2004; Ribeiro *et al.*, 2018; Terhonen *et al.*, 2016). According to the available literature the mode of action of endophytic fungi may be due to or more from the following action: endophytic fungi are promising agents for biological control and may inhibit or reduce phytopathogen growth in several ways, including mycoparasitism, antibiosis, the production of metabolites, competition for nutrients, or resistance induction in plants. Several roles have been described to fungal endophytes, including providing protection against herbivorous insects, plant parasitic nematodes and plant pathogens (Breen, 1994). Fungal endophytes can reduce damage caused by pathogens in plants. Endophytes-induced defense responses against Fusarium wilt diseases have been reported for tomato, barley *Asparagus* sp. and bananas (Duijff *et al.*, 1998; Fuchs *et al.*, 1997). Mutualistic fungi may confer several benefits to plants such as tolerance to drought, metals disease, temperature and growth enhancement and nutrient acquisition. The endophytic fungus is having rich source of secondary metabolites which act as biological active agent in the higher plants. The natural

compounds from the endophytes acts as growth inhibitor of plant pathogenic organism (Read 1999; Redman *et al.*, 2002; Varma *et al.*, 1999).

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