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Endophytic fungi associated with *Allium* Plants and their antagonistic activity against *Fusarium oxysporum f.sp. Cepae*

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Abstract

Fungal endophytes isolated from healthy tissues allium species: onion (*allium cepa*), garlic (*allium sativum* L.) and leek (*alium porrum*) were screened in vitro for antagonism against *Fusarium oxysporum f.sp. cepae* the causal pathogen of basal rot disease in onion., among the twenty nine isolates tested , three fungal isolates namely *Chaetomium globosum* , *Stachybotrys chartarum* and *Trichothecium roseum* were selected to study the antagonistic effect against *Fusarium oxysporum*. *T. roseum* was found to have the highest percentage of inhibition of mycelial growth of *Fusarium oxysporum* in vitro. (58.11%) followed by *C. globosum* (44 %) and *S. chartarum* (41.33%). The application of the tested powder formulation of fungi to infested soil two weeks before transplanting significantly reduced the incidence of basal rot on Giza 6 onion cultivars. The use of these bioagents in the form of powder formulations as soil treatment could be recommended for the control of basal rot in onion.

Keywords:

Fungi – *Allium* - *Fusarium*.

INTRODUCTION

Onion is one of the main crops grown for bulb production (Campbell *et al.*, 1986). Onion plants are infected with a wide spectrum of diseases; bacterial, viral, fungal and nematodes (Fritsch and Friesen, 2002). Basal rot is a highly destructive disease of onion (*Allium cepa* L.) that is caused by the fungus *Fusarium oxysporum f. sp. cepae* (Hans.) causes severe losses in the productivity both in the field and storage (Lager, 2011). Endophytic fungi exist widely inside the healthy tissues of living plants, and are important components of plant micro-ecosystems. Endophytic fungi were reported to produce novel antibacterial, antifungal, antiviral, anti-inflammatory, antitumor, antimalarial, and other bioactive compounds (Suman *et al.* 2016). Fungal endophytes occur ubiquitously in plants and are being increasingly studied for their ability to support plant health and protect the host from diseases. Using endophytes in disease control provides potential advantages compared to other biocontrol agents since they colonise the plant internally and thereby stay protected from environmental stresses and fluctuations. Looking at the great role of endophytic fungi to control plant diseases, the current study aims to: firstly identification of endophytic fungi associated with allium species; secondly, to evaluate their antagonism against *Fusarium oxysporum in vitro* and use of these bioagents in the form of powder formulations for control basal rot in onion.

MATERIALS AND METHODS

Isolation and Identification of the causal pathogen of onion basal rot

Thirty infected onion samples showing typical symptoms of basal rot were collected from onion fields in Assiut, Sohag (Sohag, AL-Maragha, Tahta, Gerga, Al-Mensha) Qena (Qous and Qena) and Monofya governorates. Infected plants were cut into small pieces, thoroughly washed with tap water, surface sterilized by immersing for 2 minutes in 2 % sodium hypochloride solution then rinsed several times in sterilized distill water and dried with sterilized filter papers. The surface sterilized plant pieces were plated on sterilized potato dextrose agar medium PDA in Petri plates and incubated at

25±2°C. After 4-5 days incubation period, the developed fungal colonies were purified by hyphal tip isolation technique on PDA medium. The pure fungal isolates were then grown on PDA slants at 25±2°C and kept in refrigerator at 4°C for further studies. All isolates were identified as *Fusarium spp* by Assiut University Mycological Center (AUMC).

Pathogenicity tests

Pathogenicity tests of *Fusarium spp* isolates on Giza 6 onion cultivar were carried out under greenhouse conditions at Shandaweel Agriculture Research Station, Sohag in 2017/2018 onion growing season. Soil was sterilized by autoclaving at 121°C for 3 hrs. The inoculum was prepared by using five agar disks (5 mm) of each isolate were grown on barley grains. Five replicates were used for each tested isolate (5 seedling /pot). The percentage of infection of each isolate was recorded after 4 months from planting date.

Isolation and identification of endophytes from allium plants

Samples

Healthy plants of onion (*Allium cepa*), Garlic (*Allium sativum* L.) and leek (*Allium porrum*), were collected from Sohag ((Sohag, AL-Maragha, Tahta, Gerga, Al-Mensha)) and stored in cold storage at 5°C.

Isolation of endophytic fungi

Endophytic fungi were isolated by the method as described previously by (Hazalin *et al.*, 2009). Roots, seeds, stems and leaves were respectively washed in tap water, sterilized in 70% ethanol for 1 min followed by 0.1% HgCl₂ for 4 min, rinsed three times in de-ionized water, cut into segments approximately 5 mm in diameter and placed in 90 mm Petri dishes containing PDA medium with plate supplemented with antibiotic (Tetracycline) and incubated at 28 ±2 C for 5 to 7 days. Pure colonies were transferred on PDA slant. The fungal strains in the pure culture were preserved on potato dextrose agar (PDA) slant at 4 to 5°C with proper labeling and were sub-cultured from time to time.

Preliminary test for antagonistic capability of certain isolated endophytic fungi against *F. oxysporum in vitro*

The tested isolates of fungi were plated on sterilized potato dextrose agar medium PDA in Petri plates and incubated at 20°C. After 4-5 days

incubation period on each plate (9 cm in diameter) containing 10 ml of tested medium, one disk (0.5 cm in diameter) of *F. oxysporum* was placed at one side of the Petri dish near the periphery and on the other side of the plate, a disk of isolated fungi was also placed at the same distance from the periphery of the plate.

Preparation of talc-based powder formulations of fungi

Fungi isolates were produced in Erlenmeyer flasks (250 ml) containing 100 ml of PDA, incubated at 25°C for 15 days under static conditions. The conidia suspension was prepared by adding 40 ml of sterilized distilled water containing Tween 80 (0.1%) and stirred for 30 min on a magnetic stirrer. The spores were counted in Neubauer's chamber. The inoculum was prepared by washing fungal propagules from 10-21 days old cultures of fungi grown on PDA medium at 27 °C., number of propagules was adjusted to 10⁶ CFU /ml by using hemocytometer. Sterilized talc (1 kg) was prepared for the talc powder formulation. The pH was adjusted to 7 by adding calcium carbonate. Fungal suspension (2x10⁸ /cfu/ml) was added to the mixture and mixed well under sterile conditions. Carboxyl methyl cellulose (1%) was added. The materials (35% moisture content) were packed in polyethylene bags sealed and stored at 4°C until used as described by Jayaraj et al. (2006).

Effect of fungal formulations on incidence of onion basal rot caused by *F. oxysporum* under greenhouse conditions

Experiments were carried out under greenhouse conditions at Shandaweel Agriculture Research Station, Sohag in 2018 onion growing season. Inocula of *F. oxysporum* were growing on Barley Medium. Sterilized pots (30 cm in diameter) were filled with sterilized clay soil and infested by *F. oxysporum* isolate at a rate of 3% of clay soil (w/w), two weeks before planting. The formulated antagonistic fungi were added to the infested soil at a rate of 1.5% of soil (w/w) in pots, two weeks before or at the time of planting. Three pots were used for each treatment as replicates (6 seedlings /pot). Untreated pots with antagonists were used as the control.

RESULTS

Results presented in Table (1) and Fig. (1) Significant differences were observed in disease

levels Amongst the 30 *Fusarium spp.* isolates, there were 18 pathogenic isolates resulting in 13.33–100 % percentage of infection compared with the control (0%), and 12 non-pathogenic isolates having no significant effect .Isolate No. 17 caused the highest percentage of infection (100%) followed by isolate 9(93.33%). Isolate 26 exhibited the lowest percentage of infection (13.33%). The other tested isolates were moderately virulent.

Table (1): Pathogenicity tests of *Fusarium Spp* isolates on Giza 6 onion cultivar under greenhouse conditions in 2017 / 2018 onion growing season.

Isolates	Fungi	Infection (%)
1	<i>Fusarium subglutinans</i>	0 ^{h*}
2	<i>Fusarium subglutinans</i>	0 ^h
3	<i>Fusarium proliferatum</i>	46.66 ^{bc}
4	<i>Fusarium oxysporum</i>	53.33 ^b
5	<i>Fusarium proliferatum</i>	26.66 ^{def}
6	<i>Fusarium verticillioides</i>	0 ^h
7	<i>Fusarium nygamai</i>	0 ^h
8	<i>Fusarium semitectum</i>	0 ^h
9	<i>Fusarium oxysporum</i>	93.33 ^a
10	<i>Fusarium oxysporum</i>	33.33 ^{cde}
11	<i>Fusarium oxysporum</i>	40.00 ^{bcd}
12	<i>Fusarium semitectum</i>	13.33 ^{gh}
13	<i>Fusarium semitectum</i>	0 ^h
14	<i>Fusarium oxysporum</i>	26.66 ^{def}
15	<i>Fusarium oxysporum</i>	0 ^h
16	<i>Fusarium oxysporum</i>	26.66 ^{def}
17	<i>Fusarium oxysporum</i>	100 ^a
18	<i>Fusarium oxysporum</i>	26.66 ^{def}
19	<i>Fusarium oxysporum</i>	33.33 ^{cde}
20	<i>Fusarium chlamyosporum</i>	0 ^h
21	<i>Fusarium verticillioides</i>	0 ^h
22	<i>Fusarium oxysporum</i>	53.33 ^b
23	<i>Fusarium oxysporum</i>	26.66 ^{def}
24	<i>Fusarium oxysporum</i>	26.66 ^{def}
25	<i>Fusarium incarnatum</i>	0 ^h
26	<i>Fusarium verticillioides</i>	13.33 ^{gh}
27	<i>Fusarium oxysporum</i>	40.00 ^{bcd}
28	<i>Fusarium verticillioides</i>	26.66 ^{def}
29	<i>Fusarium verticillioides</i>	20.00 ^{efg}
30	<i>Fusarium chlamyosporum</i>	0 ^h
31	Control	0 ^h

*Means followed by the same letter are not significantly different according to Duncan's multiple range tests at 5%.

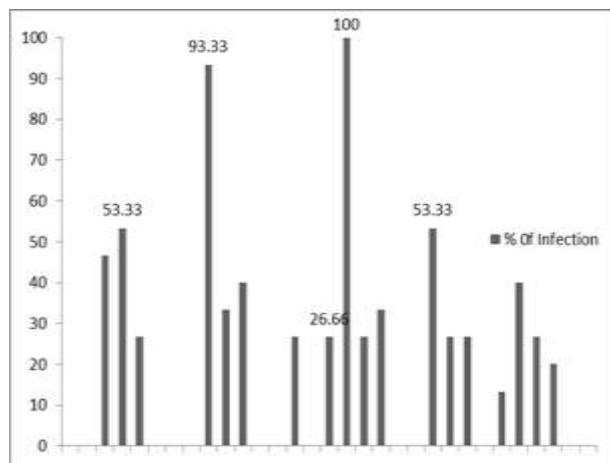


Fig. (1) Pathogenicity tests of *Fusarium Spp* on Giza 6 onion cultivar under greenhouse conditions in 2017 / 2018 onion owing season.

Isolation of endophytic fungi

Results presented in Table (2) showed that twenty nine endophytic fungi isolated from healthy allium species: onion (*allium cepa*), garlic (*allium sativum* L.) and leek (*allium porrum*) plants, fungal species belonging to 9 genera and 17 species.

Antagonistic capability of endophytic fungi against growth of *Fusarium oxysporum* in vitro

Twenty nine isolates of endophytic fungi were firstly tested for the antagonistic capability against *Fusarium oxysporum* (isolate No. 17) in vitro. Results presented in Table (3) and Fig.(2) showed that three fungal isolates exhibited inhibition percentages that were *T. roseum* caused the highest percentage of inhibition (58.11%) followed by *C. globosum* (44%) then *S. chartarum* (41.33%) while other tested isolates having no effect.

Effect of fungal formulations on incidence of onion basal rot caused by *F. oxysporum* under greenhouse conditions

Results presented in Table (4) and Fig. (3) Indicate that, in general, application of all tested fungal formulations, individually, decreased significantly percentage of onion plants infection. The least percentage of infection was observed with *T. roseum* from 100 % to 36%. The lowest effect in was observed with *S. chartarum* formulation treatment. Data also indicate that there is no significant effect between the *C. globosum* formulation and *S. chartarum* formulation.

Table (2): Entophytic fungi isolated from allium species

Allium Species	Isolates	Plant tissue
<i>Allium sativum</i>		
1	<i>Aspergillus terreus</i> -	clove
2	<i>Alternaria tenuissima</i>	
3	<i>Penicillium sp</i>	
4	<i>Aspergillus terreus</i> -	
5	<i>Penicillium sp.</i>	
6	<i>Cladosporium tenuissimum</i>	
7	<i>Chaetomium globosum</i>	
8	<i>Stachybotrys chartarum</i>	
9	<i>Trichothecium roseum</i>	
10	<i>Alternaria alternata</i>	
11	<i>Fusarium verticillioides</i>	
<i>Allium cepa</i>		
12	<i>Fusarium nygamai</i>	leaf
13	<i>Alternaria alternata</i>	
14	<i>Aspergillus terreus</i>	
15	<i>Aspergillus flavus</i>	
16	<i>Fusarium semitectum</i>	
17	<i>Fusarium chlamydosporum</i>	root
18	<i>Fusarium verticillioides</i>	
19	<i>Fusarium subglutinans</i>	
20	<i>Fusarium incarnatum</i>	
21	<i>Aspergillus flavus</i>	bulb
22	<i>Fusarium nygamai</i>	
23	<i>Trichothecium roseum</i>	seed
24	<i>Nigrospora sphaerica</i>	
25	<i>Alternaria alternata</i>	
26	<i>Aspergillus flvus</i>	
27	<i>Aspergillus terreus</i>	
<i>Allium porrum</i>		
28	<i>Aspergillus terreus</i>	leaf
29	<i>Penicillium sp</i>	

Table (3): Antagonistic capability of endophytic fungi against growth of *Fusarium oxysporum* in vitro.

Isolates of endophytic fungi	Inhibition (%)
Control	00.00
<i>Trichothecium roseum</i>	58.11
<i>Chaetomium globosum</i>	44.00
<i>Stachybotrys chartarum</i>	41.33

Table (4): Effect of endophytic fungi formulations on incidence of onion basal rot disease under greenhouse conditions.

Endophytic fungi formulations	Infection (%)
<i>Chaetomium globosum</i>	75.33 ^b
<i>Trichothecium roseum</i>	36 ^c
<i>Stachybotrys chartarum</i>	74.66 ^b
Control	100 ^a

*Bulb infection%; *Means followed by the same letter are not significantly different according to Duncan's multiple range test at 5%.

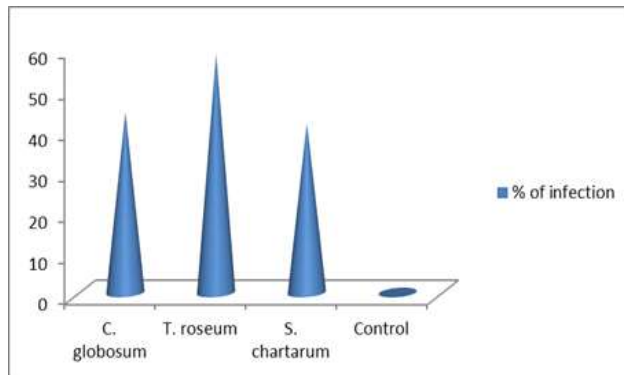


Fig. (2): Antagonistic capability of endophytic fungi against growth of *Fusarium oxysporum* in vitro.

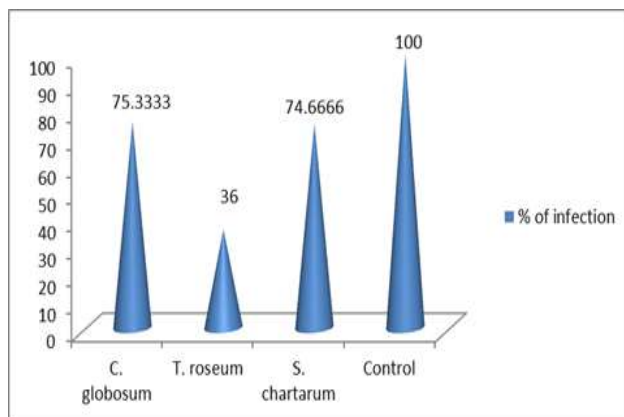


Fig (3): Effect of endophytic fungi formulations on incidence of onion basal rot disease under greenhouse conditions.

DISCUSSION

Basal rot is a highly destructive disease of onion (*Allium cepa* L.) that is caused by the fungus *Fusarium oxysporum* f. sp. *cepae* (Hans.) Snyder and Hans. Basal rot occurs both in the nursery and in the field Cramer and Christopher (2000). The present study also confirms that it was the

aggressive pathogen of onion caused characteristic symptoms of basal rot disease. Our obtained results revealed that significant differences were observed in disease levels amongst the 30 *Fusarium spp.* isolates, there were 18 pathogenic isolates resulting in 13.33–100 % percentage of infection compared with the control (0%) Galván *et al.*, (2009), Bayraktar and Dolar (2010), Chandel and Deepika (2010) also reported the pathogenic nature of this pathogen.

In vitro, the antagonistic effect of twenty nine endophytic fungi isolated from healthy allium species: onion (*allium cepa*), garlic (*allium sativum* L.) and leek (*allium porrum*) plants on the growth of *F. oxysporum* were investigated.

Obtained Data revealed that amongst all the fungi isolated and identified, only three of them were found antagonistic activity against *Fusarium oxysporum* f. sp. *cepae* the causal pathogen of basal rot disease in onion, Similar effects were reported by (Wang *et al.*, 2016; Wu *et al.*, 2016) who demonstrated that several endophytic fungal species are now successfully tested as biocontrol agents against plant pathogenic fungi as well as for plant growth promotion. Numerous attempts were made to control wilt disease through biocontrol agents using endophytic fungi and bacteria (Aydi Ben Abdallah *et al.*, 2016; Hong *et al.*, 2007; Raza *et al.*, 2017; Saravanakumar *et al.*, 2016). However, our present study, along with a previous report supports this notion that endophytic fungi have the ability to control plant pathogens in *in vitro* conditions with diverse mechanisms. (Worapong and Strobel, 2009). From *in vitro* results the three best isolates, such as, *Trichothecium roseum*, *Chaetomium globosum* and *Stachybotrys chartarum* were selected for greenhouse studies. Such results are similar to those reported by (Poulina Moya *et al.* 2016) who found that *Chaetomium globosum* is a potential biocontrol agent against various seed and soil borne pathogens. There are many studies with promising results on using endophyte *Chaetomium spp.* as a biocontrol agent. Endophytes are capable to reduce the host effect of fungi diseases, through secondary metabolites production as alkaloids. Soyong (1991) showed that *C. globosum* have been screened and found to control other economically important plant pathogens like *Phytophthora palmivora*, *Phytophthora parasitica*, and *Colletotrichum gloeosporioides*.

Park *et al.* (2005) stated that liquid culture of *C. globosum* F0142 could suppress the development of disease more than 80% and can exhibit antifungal activity against *Phytophthora infestans* in tomato, several strains of *Chaetomium* spp. showed different *in vitro* and *in vivo* antifungal potencies against many phytopathogens (Vitale *et al.* 2012) While Fayyadh and Yousif (2019) showed that *C. globosum* in reducing tomato leaf spot disease caused by *A. alternata*. In this context, Abo-Elyousr *et al.* (2017) pointed that *T. harzianum* isolate 3013 and *Stachybotrys chartarum* isolate 2031 could be used to control onion Stemphylium blight disease.

Our results showed that formulation of *Trichothecium roseum* successfully suppressed basal rot disease in onion under greenhouse conditions effectively reduced the basal rot disease up to 64 % compared to other formulations, these results are in agreement with those obtained by Zhang *et al.* (2010) suggest that the antifungal compound trichothecin contributed the principal antagonistic action. *T. roseum* can obviously inhibit the growth of pathogenic fungi, in this context; Jayaprakashvel *et al.* (2009) showed that the crude metabolites of *T. roseum* MML003 effectively reduced the sheath blight disease of rice.

CONCLUSION

The evaluation of the endophytic fungi associated with healthy allium species: onion, garlic and leek plants with antifungal potential against *F. oxysporum* causing basal rot of onion under *in vitro* conditions led to the selection of three the most promising biocontrol agents like *Trichothecium roseum*, *Chaetomium globosum* and *Stachybotrys chartarum* were found very effective in reducing basal rot of onion severity. The application of the tested powder formulation of fungi significantly reduced the incidence of basal rot on Giza 6 onion cultivars. This strategy is very promising as an alternative to chemical fungicides.

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الملخص العربي

Endophytic fungi associated with *Allium* Plants and their antagonistic activity against *Fusarium oxysporum* f.sp. *Cepae*

أماني أحمد سيد، آمال محمد إبراهيم عراقى، ثروت محمد عبدالرحمن، عبد الرازق عبدالعليم عبدالرازق

تم عزل 29 عذلة من الفطريات الداخلية المرتبطة بانواع من العائلة البصلية (البصل والكراث والثوم) ووجد ان ثلاثة انواع من الفطريات لها تأثير مضاد على نمو فطر الفيوزاريوم مسبب عفن القاعدة في البصل *Chaetomium Trichothecium roseum*, *globosum*, *Stachybotrys chartarum* وهى كما ادى معالجة التربة الملوتة بالمسبب المرضي بالفطريات الثلاثة المختبرة فى صورة مسحوق او بودر قبل الزراعة باسبوعين الى انخفاض معنوي في حدوث مرض عفن القاعدة في صنف البصل جيزة 6. يمكن التوصية باستخدام العوامل الحيوية للحد من مرض العفن القاعدى في البصل وقد تكون هذه الاستراتيجيات واعدة للعناية كاحد البدائل لمبيدات الفطريات الكيميائية.