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Biological Control of Onion White Rot Disease Caused by *Sclerotium cepivorum*

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ONION (*Allium cepa* L.) is a very important vegetable crop in Egypt and all over the world. White rot is a serious disease of *Allium* spp. caused by the soil-borne fungus *Sclerotium cepivorum*. In this study, three *Trichoderma* species and one vegan compost, alone or in combinations with the fungicide folicur were tested for their ability to inhibit mycelial growth of *S. cepivorum*. The results of the laboratory experiment showed that *T. hamatum* is the most effective as it recorded the highest inhibition (100% over growth) of the mycelial growth of the fungus followed by *T. viride* (64.6%) and *T. harzianum* (63.5%). The results indicated also that the compost tea caused inhibition of the fungus by 57.1%. In the pots experiment, folicur showed the highest efficiency (75.0%) to reduce the disease incidence, followed by *T. harzianum* combined with *T. hamatum* and folicur combined with *T. viride* and *T. hamatum*that had 70.8% efficiency.

Keywords: *Sclerotium cepivorum*, White rot, Onion, Biological control, *trichoderma*.

Introduction

Onion (*Allium cepa* L.) has important position among all vegetable crops in Egypt. The dry-land area cultivated with onion in Egypt reached 68,487 ha and producing 2,505,189 tons annually (FAO, 2014). Onion plants are subjected to various phytopathogens that cause considerable loss in quantity and quality of onion yield. White-rot disease caused by *Sclerotium cepivorum* is one of the most essential diseases around the world that causes yield loss up to 100% (Yesuf, 2013).

The fungus produces long-lived survival structures named sclerotia (Ahmed and Ahmed, 2015) that can stay in the soil without having a host plant for over 20 years. They start to develop and germinate within the sight of *Allium* plants particularly root exudates. By this way, the sclerotia cause the infection exceptionally particular to *Allium* species as they penetrate onion plants causing white rot disease (Maude, 2006; Davis *et al.,* 2007). Control of this disease has been accomplished primarily by chemical fungicides such as folicur (Pung *et al*., 2008). Due to concerns of health and environmental hazards and many pathogens could develop resistance against most recommended fungicides, there is a great demand for safer, alternative and effective control agents. The objective of this study was to control the onion white rot disease caused by *S. cepivorum* by using some fungal isolates as bio-agents.

Materials and Methods

Isolation and identification of the pathogen

Onion plants showing white rot symptoms were collected from different localities of EL-Gharbia, El-Dakahlia and Giza Governorates. Diseased bulbs were washed with tap water, cut into small pieces and surface sterilized by 0.5% sodium hypochloride solution for two minutes and then, washed three times with sterilized distilled water. Samples were dried between two layers of sterilized filter papers to remove the excess water and placed on potato dextrose agar (PDA) medium in Petri dishes. Inoculated dishes were incubated at 18-20 °C for 4-5 days, and the developed fungal cultures were purified by using hyphal tip isolation techniques (Brown, 1924). Identification of the fungal isolates was carried out according to Ciements and Shear (1957). The obtained pure cultures were kept at 5 °C on PDA slants for further studies.

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Pathogenicity test

Five isolated of *S. cepivorum* were tested for their pathogenicity against onion cultivar (Giza 20) under greenhouse conditions during 2013/2014 season. Plastic pots (25 cm in diameter) were filled with sterilized soil. The experiment was carried out in a randomized complete block design with three replicates for each particular treatment. According to the methods of Abd El-Moity (1976), inoculate of *S. cepivorum* isolates were separately prepared. Glass bottles of 500 ml capacity containing 100g barley grains and 50 ml water were autoclaved and then, they were inculcated with 5 mm diameter discs of pathogenic fungal isolates using 10 days-old cultures and incubated at 18-20°C for 25 days. At the greenhouse of Gemiza Research station, four healthy onion seedlings (60 days old) were transplanted in each pot containing 3kg soil. The soil was infested with the tested fungus at a rate of 2% fungus to soil (w/w). The soil was then moistened with water for two weeks before transplanting.

Laboratory experiment

Three fungal antagonists' *viz.*, *Trichoderma viride, T. harzianum and T. hamatum* were evaluated *in vitro* against the most aggressive isolate of *S. cepivorum* applying dual culture technique (Cherif and Benhamou, 1990). Each treatment replicated 3 times and incubated at 27±1°C until the growth of control treatment (with only plant pathogen disk) covered the Petri dish. The effect of *Trichoderma* strains on plant pathogens was determined by the percentage of mycelia growth inhibition calculated with the following formula according to Castillo *et al*. (2011) .

Inhibition (%) = [(D1-D2) / D1] ×100, where D1 is the growth of the phytopathogen in the absence of antagonist, while D2 is the growth of the phytopathogen in the presence of antagonist. The days of contact between plant pathogen-antagonistic and antagonistic ability of *Trichoderma* isolates according to the methodology proposed by Bell *et al*. (1982) were also determined using the following scale:

Class 1 = The antagonist completely overgrown the pathogen (100 % overgrowth)\

Class 2 = The antagonist overgrown at least 3/4th of pathogen surface (75% overgrowth).

Class 3 = The antagonist colonized on half of the growth of the pathogen (50% overgrowth).

Class 4 = The pathogen and the antagonist locked at the point of contact.

Class 5 = The pathogen overgrown the mycoparasite.

To study the effect of vegan compost on *S. cepivorum* isolate, one kg of a vegan compost provided from Gemmeiza research station was immersed in 10 L water and filtered to obtain tea compost. Some chemical properties of compost were described in Table 1. Four concentrations of compost tea (25, 50, 75 and 100 mg/ml) were evaluated *in vitro* against the aggressive isolate of *S. cepivorum*. The requisite quantity of each concentration was calculated and mixed thoroughly with autoclaved and cooled (45°C) PDA medium, while sterilized distilled water was used as a negative control. Compost amended PDA medium was then poured aseptically in Petri plates (7cm) and allowed to solidify. After solidification of medium, all the plates were inculcated aseptically with 5mm culture disk of 7-day old culture of *S. cepivorum* and incubated at 18-20°C until mycelial growth of pathogenic fungi covered the surface of medium in control treatment (after 7 days). The percent of growth inhibition of the test pathogen was calculated using the formula according to Otadoh *et al.* (2011) as follows:

I= (C-T /C) ×100, where I= Percent inhibition (Reduction), C= Growth diameter of the pathogen in control and T= Growth diameter of the pathogen in treatment.

TABLE 1. Some chemical and physical properties of the vegan compost.

Property	Value
Acidity (pH)	5.85 (1:5)
EC (dS m^{-1})	8.35 (1:5)
Organic carbon (%)	11.85
Total nitrogen (%)	1.18
Potassium (%)	1.8
Phosphorus (%)	0.44
C/N ratio	1:10.04
Calcium (%)	0.91
Manganese (ppm)	388.0
Zinc (ppm)	12.55
Copper (ppm)	7.18
Ferric (%)	1.05

To study the effect of fungicide (folicur) against mycelial growth of S. cepivorum, six concentrations (0.02, 0.05, 0.2, 0.4, 0.8 and 1 ppm) of folicur were evaluated against *S. cepivorum*. Based on active ingredient, the requisite quantity of each

fungicide was calculated and mixed thoroughly with autoclaved and cooled (45°C) PDA medium, while sterilized distilled water was used as negative control. Fungicide amended PDA medium was then poured aseptically in Petri plates (7cm) and allowed to solidify. After solidification of medium, all the plates were inculcated aseptically with 5mm culture disc of 7-day old culture of *S. cepivorum* and incubated at 18-20°C until mycelial growth of pathogenic fungi covered the surface of medium in control treatment (after 7 days). The percent of growth inhibition of the test pathogen was calculated using the formula according to Otadoh *et al*., 2011.

Greenhouse experiment

Greenhouse experiment was conducted to evaluate the effect of bioagents and folicur, alone or in combinations, on the incidence of onion white rot disease in the greenhouse. The experiment was carried out in pots against onion plants (Giza- 20) under outdoor conditions during 2016-2017 season. Pots (30 cm in diameter) were filed with sterilized soil. The experiment was carried out in a randomized complete block design with three replicates for each particular treatment. Pots infested with the most aggressive isolates (I_5). Glass bottles of 500 ml capacity, containing 100g barley grains and 50 ml water, were autoclaved and then, they were inculcated with 5 mm diameter discs of pathogenic isolate using 10 days-old cultures and incubated at 18-20°C for 25 days. Fungal inoculum was mixed with the soil surface of each pot at rate of 20g/kg soil. The soil was then moistened with water for two weeks before transplanting.

Preparation of the treatments

Three fungal antagonists' *viz.*, *T. viride, T. harzianum and T. hamatum* were grown on PDA plates for 10 days at 27±1°C then its growth was flooded with sterile-distilled water and scraped with a brush. A spore suspension of each fungal isolate was prepared approx. 1×10^7 spore/ml.

Folicur (Tebuconazole, alpha-[2-(4chlorophenyl) ethyl]-alpha-(1,1-dimethylethyl)-1H-1,2,4-triazole-1- ethanol) was used at the recommended dose (25ml/l).

Disease assessment

After three months from sowing, the disease assessment was made based on the percentages of disease incidence (DI) and it was calculated using the following formula:

Disease incidence = (No. of dead plants /Total sowing seedlings) ×100

The percentage of efficiency was calculated as follows:

% Efficiency = C - T/C × 100, where*:* C = % disease incidence in control treatment, T = % disease incidence in different treatments.

Effect of treatments on growth traits and yield of onion plants

Growth characters of onion plants including plant height (cm), fresh and dry weight (g) were recorded in different treatments and control after 60 days of transplanting. At harvesting time, total onion yield per pots (g) and total soluble solids (TSS) were calculated in each treatment.

Statistical analysis

Data were analyzed through one way analysis of variance (ANOVA) with the least significant difference (LSD) at the 0.05 probability level and Duncan's Multiple range test using CoStat Version 6.4 (CoHort Software, Monterey, CA).

Results and Discussion

Pathogenicity test of S. cepivorum

Five isolates of *S. cepivorum* were collected and isolated from different localities of EL-Gharbia, El-Dakahlia and Giza Governorates. All obtained fungal isolates proved to be able to infect onion plants causing white rot symptoms. According to the results presented in Fig. 1, isolate No. 5 (I_5) recorded the highest percentage of infection (97.9%), while isolate I_3 showed the lowest (37.5%). Isolate I_5 was selected for further experiments. Differences in the pathogenicity of isolates tested might be due to the presence of genetic differences among the fungal isolates (Nattrass, 1932; Stewart and McLean, 2007).

In vitro efficacy of bioagents

Three fungal bioagents (*T. viride, T. harzianum* and *T. hamatum*) were evaluated *in vitro* against (I_5) the aggressive isolate of *S. cepivorum*. The results presented in Table (2) indicated that all the bioagents assessed showed fungistatic action and significantly inhibited mycelial growth of *S. cepivorum*. *T. hamatum* was the most effective that recorded the highest inhibition of mycelia growth by 100% (over growth) followed by *T. viride* (64.58%) and *T. harzianum* (63.54%). *Trichoderma* species were efficient in inhibiting the development of mycelial growth of *S. cepivorum* by direct competition, stimulation, and antibiosis by *Trichoderma* isolates (Benítez *et al*., 2004).

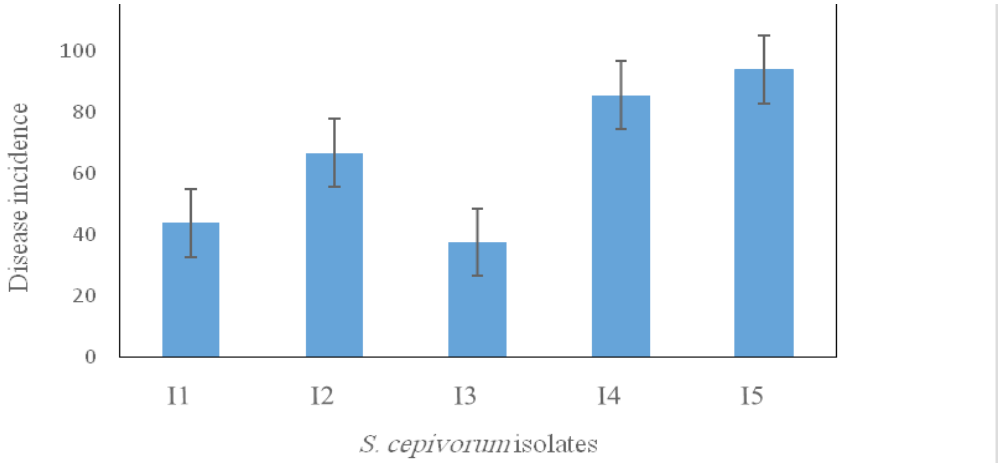

Fig. 1. Pathogenicity test of S. cepivorum isolates on onion cv. Giza 20 under greenhouse conditions.

TABLE 2. Effect of three bioagents against *S. cepivorum*.

Bioagents	Growth inhibition (%)	Bell scale
TV	64.58±0.47	2
TH	63.54±0.47	2
THM	100.0±0.0	1

- Values are means of three replicates±SE in each treatment.
- TV: Trichoderma vride, TH: T. harzianum, THM: T. hamatu

In vitro efficacy of compost tea

Results in Table 3 revealed that compost tea showed low inhibitory effect against radial growth of the fungus and gave the maximum inhibition (57.1%) when applied at high concentration (100 mg/ml). Mechanisms of action underlying the efficacy of compost tea in controlling plant pathogens have been reported as single or multiple mechanisms involving microbial antagonism (through antibiosis, parasitism, competition for nutrients /space or induced plant resistance) (Al-Mughrabi *et al.,* 2008)or suppressive physicochemical properties (improved nutrient status of the plant, toxic compounds or induced resistance) (Siddiqui *et al.,* 2008). This was supported by Noble and Coventry (2005) who demonstrated that disease suppressive effect of compost tea against *Pythium ultimum*, *Rhizoctonia Solani*, *Phytophthora Spp*, *Fusarium Oxysporium* and *Verticillium dahliae* was increased due to increase rate of application to at least 20%.

In vitro efficacy of fungicide folicur

Results in Table 4 indicated that fungicide (folicur) has significant inhibited mycelial growth of *S. cepivorum*. In addition it was found that percent inhibition was increased with the increase in concentration of the fungicides. Foilcur was found complete inhibition (100%) at 0.2 ppm concentration and with IC_{50} values ppm 0.036. Pung, (2008) assessed *in vitro* the efficacy of Folicur to reduce the mycelial growth of the onion white rot disease. Complete fungal inhibition was recorded at 1 ppm. At lower fungicide levels, it only causes partial inhibition of the pathogen.

Efficacy of bioagents, folicur and their combinations in greenhouse

The ability of three bioagents and fungicide Folicur, alone or in combinations, to control *S. cepivorum* were evaluated under greenhouse conditions. The results in Table 5 indicated that disease incidence was significantly reduced in all treatments in comparison with the control. No significant differences were found between the efficiency values exhibited by folicur alone (75.0%), *T. harzianum* combined with *T. hamatum* (70.8%), folicur combined with *T. viride* and *T. harzianum* (70.8%), folicur combined with *T. viride* (62.5%), folicur combined with *T. harzianum* (62.5%), Folicur combined with *T. hamatum* (62.5%) and folicur combined with *T. viride* and *T. hamatum*(62.5%).

Furthermore, results in Table 6 revealed that vegetative growth parameters of onion plants were significantly increased as a response to treatment with bioagents individually or in combination with fungicide folicur. Meanwhile, a significant increase in onion bulb yield was recorded in the bioagents treatments alone or in combination with folicur and the highest increase was found in the folicur alone (216.4 g/pot) followed by *T. harzianum* combined with *T. hamatum* (192.6g/pot). Additionally, some treatments significantly increased TSS in onion bulbs and the highest increase was recorded in folicur (16.0%).

TABLE 3. Effect of compost against the aggressive isolate of S. cepivorum.

Concentration (mg/ml)	Growth inhibition (%)
25	42.85±0.001
50	50.0±0.001
75	54.28±0.001
100	57.14±0.001
IC_{50}	61.96

- Values are means of three replicates ± SE.
- IC_{50}: Inhibitory concentration (the concentration in mg/ml that inhibits 50% of the fungus).

TABLE 4. Effect of fungicide (Folicur) against the aggressive isolate of *S. cepivorum* .

Concentrations (ppm)	Growth inhibition (%)
0.02	30.00±0.00
0.05	59.04±0.47
0.2	100.0±0.00
0.4	100.0±0.00
0.8	100.0±0.00
1	100.0±0.00
IC_{50}	0.036

TABLE 5. Effect of the bioagents and folicur alone or in combinations on disease incidence of white rot disease.

Treatments	Disease incidence (%)	Efficiency
Control	87.5±0.0 a	-
TV	50.0±0.0 b	37.5
THM	54.2±4.2 b	33.3
TH	54.2±4.2 b	33.3
F	12.5±0.0 e	75.0
TV + THM	29.2±4.2 cd	58.3
TV + TH	33.3±4.2 c	54.2
TH + THM	16.7±4.2 de	70.8
TV + THM + TH	37.5±0.0 c	50.0
F + TV	25.0±0.0 cde	62.5
F + TH	25.0±0.0 cde	62.5
F + THM	25.0±0.0 cde	62.5
F + TV + THM	25.0±0.0 cde	62.5
F + TV + TH	16.6±4.2 de	70.8
F + THA+ THM	33.3±4.2 c	54.2
F + TV + THM + TH	33.3±4.2 c	54.2
LSD 0.05	8.48	-

- Values are means of three replicates in each treatment ± SE.
- Means values in each column followed by the same letter are not significantly different ($P \leq 0.05$).
- TV: *Trichoderma vride*, TH: *T. harzianum*, THM: *T. hamatum*, F: Folicur.

Trichoderma spp. have a lot of mechanisms to reduce disease severity and increase vegetative growth for plant such as, as production of growth hormones, solubilization of insoluble minor nutrients in soil and increased uptake and translocation of less-available minerals (Kleifeld and Chet, 1992; Near *et al.,* 1994). *Trichoderma* strains grow rapidly when inoculated in the soil, because they are naturally resistant to many toxic compounds, including herbicides, fungicides and pesticides such as DDT and phenolic compounds (Chet *et al.,* 1997). *Trichoderma* strains are very effective in controlling many phytopathogens such as *R. solani*, *P. ultimum* and *Sclerotium rolfsii*, when alternated with methyl

TABLE 6. Effect of the bioagents alone or in combinations with folicur against onion growth and yield.

Treatments	Vegetative growth			Yield and quality	
	Plant height (cm)	Plant fresh weight (g)	Plant dry weight (g)	Bulb weight/ pot (g)	TSS (%)
Control (infected)	38.0±1.2 g	13.6±0.3 g	1.9±0.04 e	33.4±1.2 h	11.7±0.3 d
Control (healthy)	56.0±1.6 a	32.6±0.2 a	6.9±0.1 a	248.1±1.5 a	16.0±0.33 a
TV	50.3±0.3 bc	19.2±0.6 de	4.9±0.6 bc	66.6±3.6 g	12.2±0.2 d
THM	50.0±0.6 bc	21.4±1.7 d	5.9±0.2 ab	66.59±4.6 g	12.0±0.5 d
TH	52.0±1.1 abc	19.4±0.4 de	4.6±0.4 bc	71.98±1.0 g	14.2±0.1 bc
F	52.3±1.2 abc	29.5±0.3 b	4.8±0.3 bc	216.4±1.7 b	16.0±0.3 a
TV + THM	53.0±0.6 abc	16.9±0.4 ef	3.4±0.4 d	103.4±5.8 f	14.8±0.4 b
TV + TH	52.3±1.2 abc	16.7±0.8 f	3.6±0.2 cd	105.9±5.2 f	14.0±0.0 bc
TH + THM	51.3±0.7 abc	19.3±0.2 de	5.0±0.4 bc	192.6±2.1 c	14.0±0.0 bc
TV + THM + TH	48.7±0.7 cd	14.9±0.2 fg	2.7±0.5 de	155.1±2.6 e	14.3±0.4 bc
F + TV	52.3±1.2 abc	24.0±0.5 c	5.8±0.3 ab	108.2±4.9 f	14.0±0.3 bc
F + TH	51.3±0.7 abc	15.2±0.3 fg	2.7±0.28 de	174.0±2.2 d	13.7±0.4 bc
F + THM	53.7±0.9 ab	17.1±0.2 ef	5.1±0.19 b	169.1±5.0 d	13.5±0.2 c
F + TV + THM	51.7±0.9 abc	20.8±0.9 d	5.7±0.4 ab	175.1±1.0 d	12.7±0.5 d
F + TV + TH	46.3±0.9 de	13.9±0.4 g	4.8±0.4 bc	174.2±2.7 d	12.5±0.0 d
F + THA+ THM	44.0 ±1.0 ef	16.4±0.3 f	5.2±0.1 bc	170.3±2.4 d	12.7±0.3 d
F + TV + THM + TH	41.3±1.3 f	13.8±0.2 g	2.7±0.23 de	174.2±5.5 d	14.5±0.0 bc
LSD 0.05	2.85	1.72	0.95	10.13	0.79

- Values are means of three replicates in each treatment ± SE.
- Means values in each column followed by the same letter are not significantly different ($P \leq 0.05$).
- TV: *Trichoderma vride*, TH: *T. harzianum*, THM: *T. hamatum*, F: Folicur, TSS: Total soluble solids.

bromide, benomyl, captan or other chemicals (Vyas and Vyas, 1995).Some researchers also reported that combinations of tebuconazole and a biocontrol agent enhanced the control of onion white rot (Clarkson *et al.,* 2006).

Conclusions

Mixing the soil surface with *Trichoderma vride*, *T. harzianum* or *T. hamatum* alone or in a combination with the fungicide folicur could be recommended for controlling the white-rot disease in onion plants caused by *S. cepivorum* and enhancing the vegetative growth and bulb yield of onion plants.

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