

COMPARATIVE STUDY BETWEEN CHEMICAL AND NON-CHEMICAL CONTROL AGAINST *Sclerotium cepivorum*, THE CASUAL WHITE ROT OF ONION UNDER EGYPTIAN CONDITIONS.

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ABSTRACT

Sclerotium cepivorum one of the most destructive soilborne pathogens affecting onion and other *Allium* species and causing considerable damage to the host under congenial environments. In this work, it was isolated from North Egypt; many attempts were done to control onion white rot disease caused by the pathogen. Three fungicides; procymidone (sumisclex®25), vinclozolin (Ronilan) and tolclofos-methyl (Rizolex) were used against *S. cepivorum* and compared with some biological non-chemical treatments. These treatments include a five antagonistic fungi, two antagonistic bacteria, three essential oils and three plant extracts. Among all fungicides tested, it was found that procymidone application was the best chemical treatment giving 100% inhibition in mycelial growth and reduction of sclerotia germination at all concentrates tested, *in-vitro*. While, *T. harzianum* was the best antagonistic fungi against the pathogen giving 82.8% reduction. On the other hand, *Pseudomonas fluorescens* and *Bacillus subtilis* have achieved complete inhibition of mycelial growth giving 100 and 94.00%, respectively and suppressed completely sclerotial germination when exposed to bacterial culture in half-strength Nutrient Broth (NB). Among all tested essential oils, it was noticed that, cinnamon (*Cinnamomum zeylanicum*) was the best essential oil for reducing mycelial growth giving 100% at 0.50 and 0.75%. While the cinnamon extract was the best against *S. cepivorum* with 94.40% inhibition in mycelial growth at 50% concentrate and completely suppressed sclerotial germination. Conversely, onion white peels extract gave the lowest rate of mycelium growth reduction giving (11.11%) and stimulated sclerotial germination than other extracts.

Keywords: chemical control, non-chemical control, *S. cepivorum*, onion

INTRODUCTION

Onion (*Allium Cepa* L.) is the most widely cultivated *Allium* species in Egypt. Onion production has been significantly reduced mainly in Upper Egypt due to white rot caused by *Sclerotium cepivorum* Berk. It has become a recurrent problem in major onion production areas all over the world (Mengistu and Seid, 1993; Mengistu, 1994). The disease is prevalent in many *Allium* growing regions worldwide and causes serious economic losses in onion and garlic crops (Perez *et al.*, 1994; Crowe, *et al.*, 1980; Andrea *et al.*, 1996 and Pinto *et al.*, 1998). *S. cepivorum* has a global importance causing serious white rot disease on *Allium* species like onion, garlic, shallot and leek (Coley-Smith *et al.*, 1987 and Entwistle, 1986, 1990a). The pathogen

produces numerous small size sclerotia which aid in survival, and consider as a primary source of inoculum. On germination, the sclerotia produce mycelium which penetrates the root epidermis and invades the cortical parenchyma both intra and intercellularly causing extensive tissue degradation (Abd-El-Razik et al., 1973). Infected plants suffer water stress and usually die prematurely (Entwistle, 1990b). Management of soilborne diseases especially those that produce sclerotia is very difficult and need an integrated strategy. Crop rotation with non host crops (Banks and Edgington, 1989), soil solarization (Porter and Merriman, 1983 and Melero-Vara *et al.*, 2000), biological control agents (Harrison and Stewart, 1988; Kay and Stewart, 1994b and Gerlagh *et al.*, 1996), sclerotia germination stimulants (Coley-Smith and Parfitt, 1986), and composted onion waste (Coventry *et al.*, 2002) have been tried with varying levels of success. However, no single method gave the desired level of white rot control. Fungicides are among the most effective options for white rot management. Avila De Moreno, 1991 found that vinclozolin and carbendazim applied 45 and 75 days after sowing gave the best control of the disease. Previously it has been also reported that vinclozolin and iprodione (Utkhede and Rahe, 1979), procymidone (Stewart and Fullerton, 1991; Fullerton and Stewart, 1991) gave reduction of disease incidence up to 75–95% applied as seed and soil treatment. (Melero-Vara *et al.*, 2000) found that tebuconazole was effective in reducing the incidence and progress of the disease and in increasing the yield when applied as a clove treatment. According to (Duff *et al.*, 2001) procymidone and tebuconazole applied as seed treatment resulted in better yields. Application of fungicides could also be integrated with other disease management components for effective control of white rot on garlic. In view of the extensive recurrent incidence of white rot in different areas of Egypt, the present studies were conducted with the objectives to (I) determine the effects of fungicides on epidemics of white rot of onion (II) determine the effects of antagonistic fungi (III) determine the effects of antagonistic bacteria (IV) determine the effects of some essential oils (V) determine the effects of some plant extracts. The efficiency of these biocontrol agents and other non-chemical control means were tested for different points of view i.e. on mycelial growth and germination of sclerotia.

MATERIALS AND METHODS

Isolation, purification and identification of the causal of onion white rot disease (*S. cepivorum*)

Samples of infected onion bulbs showed the typical symptoms of white rot were collected from different locations of Dakahlia and Gharbia governorates. Two methods were used to isolate the pathogen from the collected samples, i.e., by means of mycelium or sclerotia. Isolation from infected bulbs was conducted by picking off mycelial growth from diseased onion bulbs and roots according to Clarkson, *et al.* (2002). Where isolation from sclerotia was according to Harper and Stewart (2000) and Clarkson, *et al.* (2002)

Effect of certain fungal antagonists on growth of *S. cepivorum in-vitro*:

Five antagonistic fungi, (*T. viride*, *T. harzianum*, *Coniothyrium minitans*, *G. virens* and *Penicillium janthinallum*.) were isolated from rhizosphere of healthy onion plants grown in Dakahlia governorate. Roots of plants were washed carefully with tap water to remove the adhering soil particles. The washed roots were cut into small pieces and divided into two groups. The first group was surface sterilized by immersing the root pieces in 1% Na-hypochlorite solution for 5 minutes and then washed several times in sterilized distilled water to remove any residual effect of Na-hypochlorite, while the second group was left without sterilization in order to isolate the surface organisms. The washed root pieces were dried between two sterilized filter papers, then transferred to the surface of Potato dextrose agar (PDA) amended with rose Bengal (0.003 %) and streptomycin sulfate (0.01 %) in Petri dishes and incubated at $25\pm 2^{\circ}\text{C}$ for 4-7 days. The growing fungi were individually transferred to PDA medium. Purification of fungi was carried out using single spore or hyphal tip technique. The purified fungal isolates were identified by Dept. of Plant Pathology, Faculty of Agriculture, Mansoura University. *C. minitans* was obtained as a kind gift from Prof. Dr. Laszlo Vajna, Dept. of Plant Pathology, Institute of Plant Protection Research, Budapest, Hungary. The effect of these antagonistic fungi on radial growth of *S. cepivorum* was studied. Each of obtained antagonistic fungi was grown on PDA for 5-7 days at ($25\pm 2^{\circ}\text{C}$), the fungal pathogen was grown on PDA for 5-7 days at ($20\pm 2^{\circ}\text{C}$) under dark condition. The antagonistic was done through using one disc (5 mm. in diameter) of the antagonist facing one disc of the pathogen on the PDA surface and relatively closed to the periphery of the plates. The untreated control treatment was done on the same medium in Petri dishes by growing one disc of the pathogenic fungus in the same place but without antagonistic disc plate. Three replicates were used. All plates were incubated at ($20\pm 2^{\circ}\text{C}$) in dark for 7 days after inoculation; average of radial growth (mm.) was recorded and compared with the untreated control to calculate the inhibition %

Effect of antagonistic bacterial action on radial growth of pathogen:

The inhibitory effects of *Bacillus subtilis* and *Pseudomonas fluorescens* on radial growth of *S. cepivorum* were studied. *B. subtilis* and *P. fluorescens* were obtained from laboratory of organic agriculture, Agriculture Research Center (ARC) in Cairo. All pure cultures of *B. subtilis* and *P. fluorescens* were grown on Nutrient Agar medium (NA) for 48h. ($30\pm 2^{\circ}\text{C}$). The fungal pathogen was grown on PDA for 5-7 days ($20\pm 2^{\circ}\text{C}$). The antagonistic effects of the used bacteria on the fungal pathogen was done through streaking the antagonistic bacteria facing one disc of the pathogen on the PDA surface and relatively closed to the periphery of the plates. The untreated control treatment was done on the same medium in Petri dishes by growing one disc of the pathogenic fungus in the same place of treatments without antagonistic bacteria. Incubation was done for 7 days at ($20\pm 2^{\circ}\text{C}$) in dark; average of the pathogenic fungus radial growth (mm.) was recorded and compared with the untreated control %.

Antifungal activity of some essential oils on growth of *S. cepivorum*:

Three commercial essential oils of cinnamon (*Cinnamomum zeylanicum*), garlic (*Allium sativum*) and onion (*Allium cepa*) were tested for their antifungal activity at three concentrations 0.25, 0.50 and 0.75% (v/v) against the fungal pathogen. The three essential oils concentrates prepared by mixing with PDA medium, after autoclaving with 0.5% of Tween-80 (v/v) to enhance oil solubility, then the essential oil concentrates were added before solidifying, and then poured in sterile Petri dishes(9cm). Three replicates for each concentrate were used. Control treatment was done by mixing PDA with tween-80 only with no essential oils added. All plates were left for 30 min to be solidified before inoculation with 5 mm discs diameter taken from 7 days old culture of pathogen. After 7 days, average of radial growth (mm.) was recorded and compared with the untreated control %.

Antifungal activity of plant extracts on growth of *S. cepivorum*:

Three extracts from onion red peels, onion white peels and cinnamon bark were tested against the fungal pathogen. Their extracts were prepared from dry part ; Onion dry peels (100g) were ground into fine powder in a high-speed micro mill. The powder were soaked in distilled water at the rate of 1:4 (w/v) then, the mixture was heated at 100°C for 30min.,and filtrated through cheese cloth under a strong hand pressure. The extract was centrifuged at 12000 rpm for 30 min. and sterilized by filtering through a 0.22 µm membrane filter at 25±3°C to avoid any bacterial or fungal contamination. The extract was considered as 100% concentration. Then was mixed with PDA at 48°C to obtain concentrations of 10, 25 and 50 %, with 0.5% of Tween-80 (v/v). Cinnamon soaked in distilled water at the rate of 1:2 (w/v) then was mixed with PDA at 48°C to obtain concentrations of 0,10, 25 and 50 % with 0.5% of Tween-80 (v/v) to enhance oil solubility, The amended media were poured into 9 cm Petri dishes (12 ml per plate).Three replicates for each concentrate were used. Control treatment (0%) was done by mixing PDA with tween-80 only with no extracts added. All plates were left for 30 min to be solidified before inoculation with 5mm disks of the pathogen, taken from 7day old culture in the centre of each plate and then incubated at 20°C.Average of radial growth was recorded after 7 days compared with the untreated control percentage when mycelial growth covered the surface of all cultures in the control treatment. Inhibition of growth was calculated in relation to the growth in the control, according to the equation proposed by Pinto, *et al.* (1998).

Effect of some fungicides on the radial growth of *S. cepivorum* causing onion white rot:

The fungicidal effects of three fungicides were determined on the radial growth of *S. cepivorum*. The tested fungicides were procimidone (Sumiscler®25), vinclozolin (Ronilan®) and tolclofos-methyl (Rizolex®). Fungicides were added to molten autoclaving PDA medium to produce the concentrations 0.5, 1, 1.5, 2, 2.5 and 3mg /100ml. Unamended PDA considered as a control, then poured in sterile Petri dishes. Three replicates for each concentrate were used, all plates were left for 3min to be solidified

before inoculating with 5mm discs taken from the edge of 7day old culture of pathogen and placed in the center of plates, (Kay, A.J. and A. Stewart, 1994). Average of radial growth was recorded after 7 days compared with the untreated control percentage.

RESULTS AND DISCUSSION

1- Effect of antagonistic fungal action on radial growth of pathogen.

Table (1) show that, after 5days of incubation *P. janthinallum* was the best antagonistic fungus for inhibition radial growth of the pathogen giving 79.18% when compared with the untreated control. This was followed by *G. virens* and *C. minitans* giving 57.50% and 50.00% inhibition, respectively. Whereas, *T. harzianum* and *T. viride* were not effective on *S. cepivorum* giving 18.75% and 0% reduction when compared with untreated control.

After 10 days of incubation, it was noticed that *P. janthinallum* was the best antagonistic fungus reducing radial growth of *S. cepivorum* giving 86.09% inhibition, followed by *T. harzianum*, *C. minitans*, *G. virens* and *T. viride* giving 76.39%, 75.65%, 73.91% and 71.74%, respectively.

After 15days, it was observed that *T. harzianum*, *T. viride*, *G. virens* and *P. janthinallum* achieved a best inhibition among the tested antagonistic fungi giving 82.69%, 80.00%, 78.08% and 76.19% ,respectively in radial growth of *S. cepivorum* ,but *C. minitans* gave moderate inhibition rates of 58.85% when compared with the untreated control. These results are in agreement with Djonovic, *et al* ,2006; Karthikeyan *et al.*,2006; Embaby, *et al.* ,2007 and Yang, 2007.

This high antagonistic action of *T. harzianum* due to mycoparasitism, produce several fungitoxic cell-wall-degrading enzymes (Chet *et al.*1998), and probably also peptaibol antibiotics. These lytic enzymes, which act as fungal cell-wall degrading agents such as *N*-acetyl- β -*D*-glucosedeaminidase, chitinase, β -1,3glucose, chitobiosidase and protease (Elad *et al.*,1995; Elad *et al.*,1998; Antal *et al.*,2000 and Harman *et al.*,2004). Moreover, *Trichoderma* spp. could manufacture cyanamide hydratase, rhodanese and β -cyanoalnine synthases, which play an important function in reducing the growth of pathogenic fungi (Ezzi-Mufaddal and James, 2002).

P. janthinallum gave high reduction of mycelial growth and sclerotia germination (Table 2). This result agree with (Gudrun, *et al.*, 2005) who reported that *P. janthinallum* limited the growth of *S. cepivorum* through secreting some enzymes such as peptidas and endoglucanase.

On the other hand, *C. minitans* gave moderate inhibition rates giving 58.85% of mycelial growth of pathogen and inhibition sclerotial germination of 100%. This result showed that *C. minitans* specialized antifungal agent that targets sclerotia of Ascomycotina and Deuteromycotina and required a host to be in vegetative stage (Lebertz, 1995). Degradation of mycelial growth and sclerotia of *S. cepivorum* are due to *C. minitans* secreting β -1, 3- glucanase and chitinase (Li Ren, *et al.* (2007).

Table (1): Effect of antagonistic fungal action on radial growth of *S. cepivorum*:-

Treatment	After 5day		After 10day		After 15day		Ger. S.	
	*R.G	Inh. %	R.G	Inh. %	R.G	Inh. %	No.S.	Inh. %
Control	4.00a	0.00	7.67a	0.00	8.67a	0.00	25.00a	83.33
<i>T. viride</i>	4.00a	0.00	2.17bc	71.74	1.73c	80.00	0.00d	100
<i>T. harzianum</i>	3.25b	18.75	2.50b	76.39	1.50c	82.69	0.00c	100
<i>G. virens</i>	1.70c	57.50	2.0bc	73.91	1.90c	78.08	3.00b	10
<i>C. minitans.</i>	2.00c	50.00	1.87c	75.65	3.57b	58.85	0.00e	100
<i>P. janthinallum</i>	0.83d	79.18	1.07d	86.09	2.06c	76.19	0.00f	100
L.S.D.	0.7138		0.5641		0.6967		0	

*R.G=Radial growth (cm.) Inh. %= inhibition % Ger.S. = germination of sclerotia.

2-Effect of antagonistic bacterial action on radial growth of *S. cepivorum*.

Results in Table (2) indicated that *P. fluorescens* followed by *B. subtilis* achieve the highest inhibition of radial growth during the incubation period, without any significant differences between them. After 15 days, they gave maximum reduction in radial growth by 100% and 93.46%. These results have the same opinion with (Chen and Dickman, 2005; Basha, et al. 2006; Ahmadzadeh, et al. 2007 and Antelmann, et al. 2008). They reported that *P. fluorescens* and *B. subtilis* reduced the mycelial growth of *S. cepivorum*, *R. solani* and *F. oxysporum*. This high antifungal effect of *P. fluorescens* probably related to the degradation of chitin in hyphal and sclerotial cells by several hydrolyzing enzymes (Gooday, 1990), such as endochitinase (1,4-L-poly-N-acetyl-galactosaminidase) or, exochitinase, chitobiosidase and/or N-acetyl-galactosaminidase or NA Gase are exuded from this antagonistic bacteria (Tronsmo and Harman, 1993). In addition these include simple metabolites such as 2,4-diacetylphloroglucinol, phenazine-1-carboxylic acid and pyrrolnitrin [3-chloro-4-(2-nitro-3-chlorophenyl)-pyrrole], as well as the complex macrocyclic lactone, 2,3-deoxy-2,3-didehydro-rhizoxin. Study of the biochemistry and mechanism of formation of these metabolites has proved useful in several ways. Pyrrolnitrin is active against *Rhizoctonia spp*, *Fusarium spp.*, and other plant pathogenic fungi, and it has been used as a lead structure in the development of a new phenylpyrrole agricultural fungicide. (Leonardo et al., 2004).

Table (2): Effect of antagonistic bacterial action on radial growth of pathogen:

Treatment	Radial growth of pathogen						Ger. S.	
	After 5day		After 10day		After 15day		No.S.	Inh. %
	R.G	Inh. %	R.G	Inh. %	R.G	Inh. %		
Control	4.00a	0.00	7.67a	0.00	8.67a	0.00	25.00a	83.33
<i>B. subtilis</i>	2.13b	46.6675	2.13b	72.17	0.57b	93.46	1.00b	3.33
<i>P. fluorescens</i>	1.77b	55.8325	1.77b	76.96	0.00b	100	0.00c	100
L.S.D.	0.6693		0.7475		0.6792		0	

*R.G=Radial growth (cm.) Inh. %= inhibition % Ger.S. = germination of sclerotia.

Whereas, the high antifungal activity of *B. subtilis* depends on the production of antibiotic compounds, including peptides (Banerjee and Hansen 1988 and Paik *et al.*, 1998), lipopeptides (Arima *et al.*, 1968), phenylpropanol derivatives (Pinchuk *et al.*, 2002), and a novel phospholipid compound (Tamechiro *et al.*, 2002).

3-Effect of Antifungal activity of some essential oils on radial growth and germination sclerotia of *S. Cepivorum*.

Results in Table (3) showed that Cinnamon oil was the most effective on radial growth of *S. cepivorum* giving 94%, 100% and 100% reduction in mycelium growth at all concentrates 0.25%, 0.5% and 0.75%, and suppressed sclerotia formation, except 0.25% concentrate gave 100 sclerotia/plat, 3.33% germinated sclerotia when exposed in 0.5% concentrate. This is in agreement with the results of many authors who reported the antifungal activity of cinnamon oil against plant pathogenic fungi (Atta-Ur-Rahman *et al.*, 1999 and Ranasinghe *et al.*, 2002). This antifungal activity of cinnamon oil due to presence of some active compounds such as cinnamaldehyde, eugenol, cinnamic acid and weitherhin. Also, the antifungal activity of oil can be attributed to the presence of an aromatic nucleus and phenolics of OH group, which is Known to be reactive and form hydrogen bounds with active sites target enzymes (Velluti *et al.*, 2003)

While, onion oil and garlic oil increased the mycelial growth gradually in all concentrations, giving 9 cm growth over than the untreated control with 8%. And forming a large number of sclerotia in all concentrations giving 3.9×10^5 and 3.7×10^5 sclerotia/plat. and onion oil germinated 93.33% of sclerotia followed by garlic oil giving 90% when compared with untreated control.

Table (3): Effect of Essential oil on radial growth and on germination of sclerotia of pathogen:

Concentration Essential oils	0.25%			0.5%			0.75%			Ger. S.	
	R.G.	Inh. %	No.S	R.G.	Inh. %	No.S	R.G.	Inh. %	No.S	No.S.	Inh. %
Control	8.33b	0.00	830	8.33b	0.00	830	8.33b	0.00	830	25.00b	83.33
Onion oil	9.00a	-8.00	5400	9.00a	-8.00	46500	9.00a	-8.00	3.9×10^5	28.33a	93.33
Garlic Oil	9.00a	-8.00	2753	9.00a	-8.00	26600	9.00a	-8.00	3.7×10^5	27.00a	90
Cinnamon Oil	0.50c	94.00	100	0.00c	100	0	0.00c	100	0	1.00c	3.33
L.S.D.	0.2718			0.2718			0.2718			1.438	

*R.G=Radial growth (cm.) No.S=number of sclerotia Ger.S. = germination of sclerotia. Inh. %= inhibition %. (-)= increase mycelial growth.

4--Effect of Antifungal activity of plant extracts on radial growth and sclerotial germination of *S. Cepivorum*:

Data in Table (4) illustrates the highest inhibition rates of radial growth, sclerotia forming and germination which came from Cinnamon extract, which reduced the mycelial growth by 67.22%, 89.00% and 94.44% at the all tested concentrates 10%, 25% and 50%, respectively, when compared with untreated control. It suppressed forming sclerotia by 100% at

25% and 50% concentrate, and reduced germination sclerotia giving 17.77% germination. These results agree with those obtained by Wilson, *et al.* (1997), they reported that cinnamon extract inhibited the radial growth of *Botrytis cinerea*.

Onion Red peels extract gave reduction in mycelial growth at the higher concentrate of 50 % giving 51.85% and formed 1×10^3 sclerotia /plate, but it was deformed, while, Onion White peels gave the lowest reduction of radial growth, forming sclerotia and germination at all tested concentrates respectively. These result in agreement with Coventry, *et al.* (2002) and Coventry, *et al.* (2006) . They proved that application of the leaf extract of onion alone or combined with *T. viride* could eliminate *S. cepivorum*. Alternatively, Dini, *et al.* (2008) stated that the extracts of onion Red peel discrete antioxidant capacity which increased after boiling, although cooking methods caused significant losses of the cysteine derivatives in water.

Table (4): Effect of water plant extracts on radial growth and on germination of sclerotia of pathogen:

Concentration	10%			25%			50%			Ger. S.	
	R.G.	Inh. %	No.S	R.G.	Inh. %	No.S	R.G.	Inh. %	No.S	No.S.	Inh. %
Treatment											
Control	9.00a	0.00	72 X10 ³ a	9.00a	0.00	72X10 ³ a	9.00a	0.00	72X10 ³ a	25.00a	83.33
Onion White Scale.	9.00a	0.00	72 X10 ³ a	9.00a	0.00	72X10 ³ a	8.00a	11.11	70X10 ³ b	19.33b	64.43
Onion Red Scale.	9.00a	0.00	14.67X10 ³ b	7.83b	12.96	4.33X10 ³ b	4.33b	51.85	1 X10 ³ c	12.33c	41.10
Cinnamon.	2.95b	67.22	3.33 X10 ³ c	1.00c	89.00	0.00c	0.50c	94.44	0.00d	5.33d	17.77
L.S.D.	0.1697		1.5373	0.2718		1.7188	1.0871		0.9414	2.4908	

*R.G=Radial growth (cm.) No.S=number of sclerotia Ger.S. = germination of sclerotia. Inh. %= inhibition %.

5-Effect of some fungicides on the radial growth and sclerotial germination of *S. cepivorum* causing onion White rot:

Data in Table (5) show that the highest inhibition of fungal growth came from Sumisclex®25, which reduced the mycelial growth by 100% at the maximum concentrate(3mg/100ml) and giving 93.3% to 94.4% at concentrates of 0.5,1,1.5,2 and 2.5 mg/100ml ,when compared with untreated control, while suppressed sclerotial germination at100%at all tested concentrates, respectively. Rizolex suppressed the fungal growth significantly by 93.7. % and 94.4%inhibition, at the higher concentrate (2.5, 3%) respectively, while suppressed sclerotial germination at100% at all tested concentrates, except in case of 0.5mg/100ml concentrate. While, Ronilan did not show any effect on radial growth but suppressed sclerotial germination by 100% at all tested concentrates. These results are in agreement with those obtained by Macleod and Nielsen (1995) and Macleod and Ryan, (1997).They found that reduction in mycelial growth of *S. cepivorum* achieved by dicarboximides such as vinclozolin and iprodione, while Fullerton *et al.*, (1992). Porter, *et al.*, (1991) and Fullerton *et al.*, (1995) found good results have been obtained with another dicarboximide, procymidone, when compared with tebuconazole.

Table (5): Effect of fungicides on radial growth and germination of sclerotia of pathogen:

Concentration Treatment	Fungicidal concentration											
	0.5		1		1.5		2		2.5		3	
	R.G.	Ger.S	R.G.	Ger.S	R.G.	Ger.S	R.G.	Ger.S	R.G.	Ger.S	R.G.	Ger.S
Control	9.00a	30a	9.00a	30a	9.00a	30a	9.00a	30a	9.00a	30a	9.00a	30a
Procymidone (Sumisclex®25)	0.60b	1c	0.60b	0b	0.50b	0d	0.50d	0d	0.50c	0b	0.00d	0b
tolclofos- methyl (Rizolex)	0.97b	29b	0.77b	0c	0.57b	0c	0.50c	0c	0.57b	1.33b	0.50c	0b
Venconazole (Ronilan)	9.00a	0d	9.00a	0d	9.00a	1b	9.00b	13b	9.00a	1.33b	9.00b	0.33b
L.S.D.	0.46	0.94	0.29	0	0.11	0	0	0	0.05	1.54	0	0.54

*R.G=Radial growth (cm.)

Ger.S. = germination of sclerotia.

REFERENCES

- Abd-El-Razik, A.A., Shatla, M.N., Rushidi, M., 1973. Studies on the infection of onion plants by *Sclerotium cepivorum* Berk. Phytopathol 76, 108–116.
- Ahmadzadeh, M.; Behnam, S.; Sharifi T. A. and Hedjaroude, G.A. (2007). Effect of timing of application *Pseudomonas fluorescens* in suppression *Sclerotinia sclerotiorum*, the causal agent of white mold in canola. Communications in Agricultural & Applied Biological Sciences. 72(4):957-60, 2007.
- Andrea, T.B., Emma, Z.M., Carmen, G.C., Ronald, F.C., 1996. The use of arbuscular mycorrhizae to control onion white rot (*Sclerotium cepivorum* Berk) under field conditions. Mycorrhizae 6, 253–257.
- Antal, Z. ; L. Manczinger ; G. Szakacs ; R. P. Tengerdy and L. Ferenczy.2000.Colony growth, *in vitro* antagonism and secretion extracellular enzymes in cold-tolerant strains of *Trichoderma* spp. Mycological Reserch,5(104):545-549.
- Antelmann, H.; Hecker, M. and Zuber, P. (2008).Proteomic signatures uncover thiol-specific electrophile resistance mechanisms in *Bacillus subtilis*. Expert Review of Proteomics. 5(1):77-90, 2008 Feb.
- Arima, K; A. Kakinuma and G. Tamura.1968. Surfactin, a crystalline peptidolipid surfactant produced by *Bacillus subtilis*: isolation, characterization, and its inhibition of fibrin clot formation. Biochem Biophys Res Com., 31:488-494.
- Atta-Ur-Rahman; M. I. Choudhary; A. Farooq; A. Ahmed; M. Z. Iqbal; B. Demirci; F. Demirci and K. H. C. Baser. 1999. Antifungal activities and essential oil constituents of some spices from Pakistan. Third International Electronic Conference on Synthetic Organic Chemistry (ECSOC-3), September 1-30.
- Avila De Moreno, C., 1991. Chemical control of *Sclerotium cepivorum* Berk. causing white rot of garlic. Fitopatol. Colombiana 15, 62–69.

- Banerjee, S. and J. N. Hansen .1988. Structure and expression of a gene encoding the precursor of subtilin, a small protein antibiotic. *J. Biol Chem.*, 263:9508-9514.
- Banks, E.and Edgington, L.V., 1989. Effect of integrated control practices on the onion white rot pathogen in organic soil. *Can. J. Plant Pathol.* 11, 268–272.
- Basha, S.A.; Sarma, B.K.; Singh, D.P.; Annapurna, K. and Singh, U.P. (2006).Differential methods of inoculation of plant growth-promoting rhizobacteria induce synthesis of phenylalanine ammonia-lyase and phenolic compounds differentially in chickpea. *Folia Microbiologica.* 51(5):463-8, 2006.
- Chen, C. and Dickman, M.B. (2005). cAMP blocks MAPK activation and sclerotial development via Rap-1 in a PKA-independent manner in *Sclerotinia sclerotiorum*. *Molecular Microbiology.* 55(1):299-311, 2005 Jan.
- Chet, I., Benhamou, N. and Haran, S. 1998. In *Trichoderma and Gliocladium* Vol. 2 (eds Kubicek, C. P. & Harman, G. E.) 153–172 (Taylor and Francis, London,).
- Clarkson, J. P.; T. Payne; A. Mead and J. M. Whipps. (2002). Selection of fungal biological control agents of *Sclerotium cepivorum* for control of white rot by sclerotial degradation in a UK soil. *Plant Pathology* 51 (6), 735–745.
- Coley-Smith, J.R. and Parfitt, D., 1986. Some effects of diallyl disulphide on sclerotia of *Sclerotium cepivorum* and possible novel control method for white rot disease of onions. *Pesticide Sci.* 37, 587–594.
- Coley-Smith, J.R., Parfitt, D. and Taylor, I.M., 1987. Studies of dormancy in sclerotia of *Sclerotium cepivorum* Berk. *Plant Pathol.* 36, 594–599.
- Coventry, E., Noble, R., Mead, A. and Whipps, J.M., 2002. Control of white rot (*Sclerotium cepivorum*) with composted onion waste. *Soil Biochem.* 34, 1037–1045.
- Coventry, E.; R. Noble; A. Mead; F. R. Marin; J. A. Perez and J. M. Whipps. (2006). Allium White Rot Suppression with Composts and *Trichoderma viride* in Relation to Sclerotia Viability. *Phytopathology* 96, (9), 1009-1020.
- Crowe, F.J., Hall, D.H., Greathead, A.S., Baghott, K.G., 1980. Inoculum density of *Sclerotium cepivorum* and the incidence of white rot of onion and garlic. *Phytopathology* 70, 64–69.
- Dini, I., Tenore, G.C.and Dini, A. (2008). Chemical composition, nutritional value and antioxidant properties of *Allium cepa* L. Var. tropeana (red onion) seeds. *Food Chemistry* (2008) 107:613–621.
- Djonovic S. Pozo MJ. Kenerley C.M. (2006) Tvbg3, a beta-1, 6-glucanase from the biocontrol fungus *Trichoderma virens*, is involved in mycoparasitism and control of *Pythium ultimum*. *Applied & Environmental Microbiology.* 72(12):7661-70, 2006 Dec.
- Duff, A.A., Jackson, K.J. and O'Donnell, W.E., 2001. Tebuconazole (folicur) potential in the control of white rot (*Sclerotium cepivorum*) in garlic in subtropical Queensland, Australia, Second International Symposium on edible Alliaceae. *Acta Horticult.* 555, 247–250.

- Elad, Y; D.R.David; T.Levi; A.Kapat and E.Guvrin.1998.*Trichoderma harzianum* T39 mechanisms of biocontrol of foliar pathogens. In:Lyr,H.,Russell,D.E.,H.Dehne,H.D.Sisler,Modern fungicides and antifungal compounds II.Intercept Ltd Andover,Hampshier,UK,pp.459-467.
- Elad, Y; N.E. Malathrakis and A.J.Dik.1995.Biological control of Botrytis incited diseases and powdery mildews in greenhouse crop. Crop protection, 15:224-240.
- Embaby, El-Sayed M.; M. Eman El-Taher and E.A.M. Gado (2007). Control of Damping off and / or Sore Shin in Cotton and White Mould in Cowpea Plant Disease(s) by using a Bio-fungicide *Coniothyrium minitans* Campbell. Research Journal of Agriculture and Biological Sciences, 3(4): 267-273, 2007.
- Entwistle, A.R., 1986. Differences in the incidence of Allium white rot in direct drilled and module-grew bulb onions. In: Entwistle, A.R. (Eds.), Proceedings of the third international workshop on Allium white rot, Wellesbourne, UK, pp. 1–5.
- Entwistle, A.R., 1990a. Allium white rot and its control. Soil Use Manage. 6, 201-209.
- Entwistle, A.R., 1990b. Measuring net changes in populations of *Sclerotium cepivorum* sclerotia to evaluate the long term potential of control measures. In: Entwistle, A.R., Mattusch, P. (Eds.). Proceedings of fourth international workshop on Allium white rot. Neustadt/Weinstrasse, Federal Republic of Germany. pp: 59–68.
- Ezzi-Mufaddal, I and M. L. James. 2002. Cyanide catabolizing enzymes in *Trichoderma* spp. Enzyme Microb Technol., 31:1042-1047.
- Fullerton, R.A. and Stewart, A., 1991. Chemical control of onion white rot (*Sclerotium cepivorum* Berk) in the Pukekohe district of New Zealand. NZ J. Crop Horticul. Sci. 19, 121–127.
- Fullerton, R.A., Slade, E.A., Stewart, A. and Young, H., 1992. Degradation of the dicarboximide fungicides iprodione, vinclozolin and procymidone in Patumahoe clay loam soil in New Zealand. Pesticide Sci. 35: 95-100.
- Fullerton, R.A., Stewart, A. and Slade, E.A., 1995. Use of demethylation inhibiting fungicides (DMIs) for the control of onion white rot (*Sclerotium cepivorum* Berk.) in the New Zealand. NZ J. Crop Horticul. Sci. 23, 121–125.
- Gerlagh, M., Whipps, J.M., Budge, S.P. and Goossen-van De Geijn, H.M., 1996. Efficacy of isolates of *Coniothyrium minitans* as mycoparasites of *Sclerotium cepivorum*, and *Botrytis cinerea* on tomato stem pieces. Eur. J. Plant Pathol. 102, 787–793.
- Gooday, G.W. 1990. The ecology of chitin degradation. In: Advances in Microbial Ecology (Marshall, K.C., Ed.), pp. 387-430. Plenum Press, New York.
- Gudrun, M.; A. Koch; B. Henrissat and G. Schulz. 2005. Endoglucanase II (EGII) of *Penicillium janthinellum*: cDNA sequence, heterologous expression and promotor analysis. Current Genetics, 5 (29):490-495.

- Harman, G.E., Howell, C.R., Viterbo, A., Chet, I. and Lorito, M., 2004. *Trichoderma* species—opportunistic, avirulent plant symbionts. *Nat. Rev. Microbiol.* 2 (1), 43–56.
- Harper G. E. and A. Stewart (2000) Magnetic separation technique for the isolation of *sclerotium cepivorum* from iron-rich soil particles. *Soil Biology and Biochemistry* 32:135-137.
- Harrison, Y.A. and Stewart, A., 1988. Selection of fungal antagonists for biological control of onion white rot in New Zealand. *NZ J. Exp. Agri.* 16, 249–256.
- Karthikeyan, M.; K. Radhika; S. Mathiyazhagan; R. Bhaskaran; R. Samiyappan and R. Velazhahan. 2006. The effect of soil application of biocontrol agents (*Pseudomonas fluorescens*, *Trichoderma viride* and *T. harzianum*) against *S. rolfsii*, the causal agent of stem rot in groundnut. *Microbiology*, 11(152): 1253-1266.
- Kay, S.J. and Stewart, A., 1994b. The effect of fungicides on fungal antagonists of onion white rot and selection of dicarboximide-resistant biotypes. *Plant Pathol.* 43, 863–871.
- Lebertz, H. 1995. Investigation of the behavior of the environment-leaching behavior and side on soil microflora of spore isolate CON/M/91-08: Lab project Number: IF-95/02315-00. Unpublished study prepared by Institute Fresenius. 32pp.
- Leonardo De La Fuente; L. Thomason; D. Weller; N. Bajsa; L. quagliotto; L. Chernin and A. Arias. 2004. *Pseudomonas fluorescens* UP6 isolated from birdsfoot trefoil rhizosphere produces multiple antibiotics and exert broad spectrum of Biocontrol activity. *European J. of Plant Pathology*, 110:971-681.
- Li Ren, A.; L. Guoqing ; Y. C. Han; D. H. Jiang and Hung-Chang Huang. 2007. Degradation of oxalic acid by *Coniothyrium minitans* and its effects on production and activity of b-1, 3-glucanase of this mycoparasite. *Biological Control*, 43: 1-11..
- Macleod, I.L. and Nielsen, P. (1995). An investigation of the use of tebuconazole (Folicur) for the control of white root rot (*Sclerotium cepivorum*) in onions. *Annual Report on trials in 1995*.
- Macleod, I.L. and Ryan, D.J. (1997). The latest in fungicide-based management strategies for control of onion white rot (*Sclerotium cepivorum*). *Proceedings of the 11th Biennial Australasian Plant Pathology Conference*, Perth, Western Australia, p14.
- Melero-Vara, J.M., Parados-Ligero, A.M. and Basallote, U.M.S., 2000. Comparison of physical and chemical and biological methods of controlling garlic white rot. *Eur. J. Plant Pathol.* 166, 581–588.
- Mengistu, H., 1994. Research on vegetables disease in Ethiopia. In: Herath, E., Dessalegne, L. (Eds.), *Proceedings of the Second National Horticultural Workshop of Ethiopia*, 1–3 December. IAR, Addis Ababa, Ethiopia, pp. 209–215.
- Mengistu, H. and Seid, A., 1993. Outbreak of garlic bulb rots in Ethiopia. *Annual report*. Debre Zeit Agricultural Research Center: Alemaya University of Agriculture 124pp.

- Paik, S. H.; A. Chakicheria and J. N. Hansen. 1998. Identification and characterization of the structural and transporter genes for, and the chemical and biological properties of sublancin 168, a novel antibiotic produced by *Bacillus subtilis* 168. *J Biol Chem.*, 273: 23134-23142.
- Perez, M.L., Salinas, G.J. and Redondo, J.E., 1994. Main disease on *Allium* species in Mexico with emphasis on white rot (*Sclerotium cepivorum* Berk.). In: Entwistle A.R., Melero-Vara J.M., (Eds), Proceedings of the Fifth International Workshop on Allium white rot, pp: 6–11.
- Pinchuk, I. V.; P. Bressollier; I. B. Sorokulova; B. Verneuil and M. C. Urdaci. 2002. Amicoumacin antibiotic production and genetic diversity of *Bacillus subtilis* strains isolated from different habitats. *Res. Microbiol.* 153:269-276.
- Pinto, C.M.F., Mafia, L.A., Casali, V.W.D., Berger, R.D. and Mizubuti, E.S.G., 1998. Progress of white rot on garlic cultivars planted at different times. *Plant Dis.* 82, 1142–1146.
- Porter, I.J., Maughan, J.P. and Towers, G.B. (1991). Evaluation of seed, stem and soil applications of procimidone to control white rot (*Sclerotium cepivorum* Berk.) of onions. *Aust.J.Exp.Agric.* 31:401-406.
- Porter, I.J. and Merriman, P.R., 1983. Effects of solarization of soil on nematode and fungal pathogens at two sites in Victoria. *Soil Boil. Biochem.* 15, 39–44.
- Macleod, I.; Cross, S.; Florissen, P. and Pung, H. (2007). Investigating and developing fungicide options for onion white rot control in Australia. 22nd Biennial Australasian Plant Pathology Conference 27-30.
- Ranasingh, L.; B.Jayawardena and K. Abeywickrama. 2002. Fungicidal activity of essential oils of *Cinnamomum zeylanicum* and *Syzygium aromaticum* Merr ET L.M.Perry against crown rot and anthracnose pathogens isolated from banana. *Letters in Applied Microbiology* 35:208-211.
- Stewart, A. and Fullerton, R.A., 1991. Additional studies on the control of onion white rot (*Sclerotium cepivorum* Berk) in New Zealand. *NZ J. Crop Horticul. Sci.* 19, 129–134.
- Tamechiro, N.; Y. Okamoto-Hosoya; S. Okamoto; M. Ubukata; M. Hamada; H. Naganawa and K. Ochi. 2002. Bacilysocin, a novel phospholipids antibiotic produced by *Bacillus subtilis* 168. *Antimicrob Agents Chemother*, 46:315-320.
- Tronsmo, A. and G.E. Harman. 1993. Detection and quantification of N-acetyl-beta-D-glucosaminidase, chitobiosidase, and endochitinase in solutions and on gels. *Anal. Biochem.* , 208: 74-79.
- Utkhede, R.S. and Rahe, J.E., 1979. Evaluation of chemical fungicides for the control of onion white rot. *Pesticide Sci.* 10, 414–418.
- Velluti, A.; V. Sanchis; A.J.Ramos; J. Egido and S. Marin. 2003. Inhibitory effect of cinnamon, clove, lemongrass, oregano and palmarose essential oil on growth and fumonisin b1 production by *Fusarium proliferatum* in maize grain. *International Journal of Microbiology*, 21:649-659.

- Wilson, G. L.; M. J. Solar; A. El-Ghaouth and M. E. Winsenewski. 1997. Rapid of plant extracts and essential oils for antifungal activity against *Botrytis cinerea*. Plant Disease, 81:204-210.
- Yang L. (2007) Antifungal substances produced by *Penicillium oxalicum* strain PY-1potential antibiotics against plant pathogenic fungi. World Journal of Microbiology and Biotechnology.

دراسة مقارنة بين المقاومة الكيماوية وغير الكيماوية للفطر *S. cepivorum* المسبب لمرض العفن الأبيض في البصل تحت الظروف المصرية.
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يعتبر الفطر *S. cepivorum* من أهم وأخطر الفطريات المحمولة بالتربة والمسببة لخسائر كبيرة لنباتات البصل والعائلة البصلية تحت الظروف البيئية الملائمة. مؤخرًا ظهر المرض في محافظات الوجه البحري وتم عزله وأجريت محاولات عديدة لمقاومة هذا المرض. وذلك عن طريق استخدام ثلاث مبيدات فطرية وهي: سميسلكس، الرونيلان، الريزولكس لمقاومة المرض ومقارنتها ببعض الطرق الغير كيماوية والتي تشمل: خمسة أجناس فطرية مضادة، جنسين من البكتريا المضادة، ثلاثة زيوت طيارة، ثلاثة مستخلصات نباتية. أظهرت النتائج أنه: من بين المبيدات الفطرية المختبرة كان السميسلكس أفضل المبيدات المستخدمة في تثبيط كل من النمو الفطري وإنبات الاجسام الحجرية خلال كل التركيزات المستخدمة بمعدل ١٠٠%. أما بالنسبة لمحاولات المقاومة الغير كيماوية فقد لوحظ أنه بالنسبة للفطريات المضادة فقد أعطي الفطر *T. harzianum* افضل تثبيط لكل من النمو الفطري وإنبات الاجسام الحجرية للفطر الممرض بنسبة ٨٢,٨٠% ، ١٠٠% علي التوالي. أما علي الجانب الآخر فقد وجد أن كلا من البكتريا المضادة *B. subtilis*, *P. fluorescens* حققت تثبيطاً للنمو الفطري بنسبة ١٠٠% و ٩٤,٠٠% علي التوالي. كما حققت تثبيطاً تاماً لإنبات الاجسام الحجرية. أما بالنسبة للزيوت المختبرة فقد حقق زيت القرفة أعلى النتائج تثبيطاً لكل من النمو الفطري وإنبات الاجسام الحجرية عند استخدامه بتركيز ٠,٥% ، ٠,٧٥% معطياً ١٠٠% تثبيطاً. كما أعطي مستخلص قلف القرفة بتركيز ٥٠% تثبيطاً للنمو الفطري بمعدل ٩٤,٤٠% وتثبيطاً تاماً لإنبات الاجسام الحجرية .