CERTAIN BIOAGENTS, BOTANICAL EXTRACTS AND INTERCROPPING FOR CONTROLLING THE WHITE STEM ROT OF CHICKPEA IN EL-BEHERA GOVERNORATE

BY

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ABSTRACT

Commercially systemic fungicide, vitavax-200, significantly reduced the mycelial growth of *Sclerotinia sclerotiorum* (Lib.) de Bary at all tested concentrations of the fungicide. The *in vitro* growth of *S. sclerotiorum* was suppressed as a result of the antagonistic effect of *Trichoderma harzianum* or *Bacillus subtilis.* The results obtained indicated that the leaves extracts of absinthe, garlic or onion individualy inhibited the radial growth of the tested fungus. The inhibition effect was increased by increasing the concentration of the used extracts, and the fungal growth was completely suppressed at concentration of 15 % under different incubation periods for garlic or onion extracts. On the basis of these results, intercropping of garlic or onion plants with chickpea showed significant reduction in chickpea white stem rot disease incidence.

Key words : chickpea, stem rot, bioagents, extracts, intercropping.

INTRODUCTION

Chickpea (*Ciecer arietinum* L.) is one of the wintry legume crops. It is planted in Egypt for cooking, eating its wet seeds (Malana), its flaked seeds, preparing foods for children and old men, making kinds of sweet with sugar, and using its stems, drying leaves, and broken seeds in the animal feeding.

In Egypt, *S. sclerotiorum* is considered as a serious pathogen. The impact of the disease was increased notably during the last decade causing a remarkable yield losses in Egypt (Omar *et al.*, 1992 and Mazen, 1995) as well as in various chickpea growing areas (Letham *et al.*, 1976), (Purdy, 1979) , and (Sharma, *et al.*, 2001).

One of the most approaches to control white stem rot is the using of fungicides, so many different chemicals have been used on variety of crops *i.e,* benomyl has been reported to control *S. sclerotiorum* on bean (Natti, 1971), on tomato (Letham *et al.*, 1976), and on chickpea (Omar *et al.*, 1992). Additionally, when soybean infected seeds treated with captan, pentachloronitrobenzene, thiabendazole and fludioxonil, the fungal growth from this infected seeds was completely inhibited. Thiram and thiabendazole reduced recovery of *S. sclerotiorum* by 90 %. Also, both of the two fungicides reduced sclerotia formation from the infected seeds by more than 98 % (Mueller *et al.*, 1999).

Many trials were attempted to control the disease by means of biocontrol agents. In this respect, it was found that more than 30 species of fungi and bacteria have been reported to be parasites or antifungal of *Sclerotinia* sp (Steadman, 1979).

Trichoderma spp has more antagonistic effect on *S. sclerotiorum* on various crops (Budge and Whipps 1991, Hanging 1991, Abd El-Moity *et al.*, 1993 and Sharma *et al.*, 1999), while *Bacillus subtilis* isolates showed antagonistic effect against many pathogens *i.e, S. sclerotiorum, Fusarium* spp, *Pythium* sp , *Rhizoctonia solani, Macrophomina phaseolina, Ralstonia solanacearum* and *Phytophthora* spp (Talavera *et al.*, 1988; Sabet *et al.*, 1998; El-Sheikh, 1998 and Youssef, 2000).

Bazzalo *et al.*, (1985) found that the corresponding methanolic extracted from stems of sunflower plants showed a strong inhibitory effect on the mycelial growth of *S. sclerotiorum.* Singh *et al.*, (1994) cleared that alkaloids (protopine nitrate) extracted from *Fumaria indica* were inhibitors to chickpea white stem rot disease. It may show promise for controlling the disease under field conditions.

The present study was conducted to focus on chickpea white stem rot disease through evaluation the efficacy of some newly introduced non-fungicidal chemicals such as crude extracts as safe alternatives to conventional synthetic fungicides, investigation the effect of intercropping of garlic or onion with chickpea on stem white rot disease incidence and studying the antagonistic effect of some biocontrol agents.

MATERIALS AND METHODS

Isolation, Purification, and Identification of the Pathogen:

Chickpea plants, showing white stem rot symptoms were collected from Etay El-Baroud Agricultural Research Station, El-Behera Governorate, where chickpea white stem rot disease is naturally occurred during 2001 growing season. The sterilized diseased parts were put on chickpea dextrose agar (CDA) and incubated for 5 days at 26° C. Pure cultures of the recoverd fungus were obtained by using the hyphal tip technique. Fungal discs were kept on CDA slants.

Effect of the Systemic Fungicide, Vitavax-200, on *S. sclerotiorum in vitro*:

A systemic fungicide, vitavax-200 (thiram + carboxine) at concentrations of (0, 1, 5, and 10 ppm) active ingredients were tested *in vitro*. The radial colony diameter was measured 2, 4 and 6 days after incubation. Number of sclerotia per plate was determined.

Activity of Some Biocontrol Agents Against *S. sclerotiorum* :

A- Effect of *Trichoderma harzianum* on the mycelial growth of *S. sclerotiorum* :

The *in vitro* antagonism between *T. harzianum* and *S. sclerotiorum* was studied. Inhibition area between the two organisms was measured five days after incubation. *T. harzianum* isolate was kindly provided by Plant Pathology Research Institute, Giza.

B- Effect of *Bacillus subtilis* on the mycelial growth of *S. sclerotiorum* :

B. subtilis (24 hours old) liquid formulation at approximatly 10^6 cfu/ml was kindly provided by Desert Research Center and streaked as a single straight line at the upper part of plates of CDA medium. Twenty four hours after bacterial inoculation, discs of 5 mm in diameter were taken from the advancing margin of 7-days-old culture of the tested fungus and put at the opposite side of the plate. Plates were left un-treated with bacterium inoculum to serve as control. Three replicates were used. The inhibition area between the two organisms was measured.

Effect of Some Plant Extracts on *S. sclerotiorum* :

1. Absinthe leaves extract :

Fifty gms dry leaves of absinthe (*Artemisia absinthium* L.) were boild in 200 ml of distilled water for 30 minutes. Obtained extract was filtered through two layers of cloth. The extract was taken as a stock solution, then kept in the refrigerator till using. Different concentrations (0, 25, 50, 75 and 100 %) were used. The required concentrations of the extract were adjusted and added to CDA medium. Each concentration was put individually in conical flask. The five conical flasks of the different concentrations were sterilized. The content of each flask was poured onto sterilized 9-cm Petri dishes. Three replicates were used for each concentration. Plates were inoculated with 5-mm diameter discs taken from 7-days-old culture of white stem rot fungus and incubated at 26° C. The radial colony diameter was measured and number of sclerotia per plate was determined.

2. Garlic or onion leaves extracts :

Healthy leaves of garlic (*Allium sativum* L.) Chinese cv., were carfully selected from healthy garlic plants. Leaves were cut to small pieces, then crushed in the blender with distilled water (250 ml / 250 gm). The obtained extract of garlic leaves was filtered through two layers of cloth. The extract was centrifuged at 5,000 rpm for 30 minutes, the yielded extract was sterilized through Zeitz filter, then kept in the refrigerator till using, according to method given by El-Shami *et al.*, (1985). At the same time, healthy onion (*Allium cepa* L.) leaves extract was obtained by the same technique. Different extract concentrations (0, 5, 10 and 15 %) of garlic and/or onion leaves were used. The required concentrations of both extracts were individually adjusted and added to an autoclaved CDA medium. The mixture medium was poured onto sterilized Petri dishes. Three replicates were used for each concentration. Plates were inoculated with 5-mm diameter discs taken from 7-days-old culture of white stem rot fungus and incubated at 26° C. The radial colony diameter was measured and number of sclerotia per plate was determined.

Intercropping of some winter crops with chickpea :

Garlic and onion were used for intercropping with chickpea tested entries. The experiments were conducted during 2001 and

2002 growing seasons and carried out in plots of size 2 m in length and 3 rows, between both of them 0.60 m and left under natural infection in the open field. Four chickpea entries (G 1, G 88, G 195 and G 531) were intercropped by garlic or onion plants. Two methods of intercropping were used such as ridge / ridge or hill / hill for each garlic or onion to all chickpea tested entries. Infected plants were counted and percentage of infection was calculated according to the following equation :-

$$\text{Percentage of infection} = \frac{\text{No. of infected plants}}{\text{Total No. of plants}} \times 100$$

Statistical analysis :

The obtained data were statistically analyzed according to the method of Gomez and Gomez, (1984) at the Costat computer program. Means were compared using LSD test at the 0.05 level of probability.

EXPERIMENTAL RESULTS

Symptomology:

Symptoms of chickpea white stem rot disease under natural infection appear mostly on adult plants when these plants form a thick canopy and the soil remains wet for an extended period. The above-ground symptoms show drooping of petioles and leaflets without turning yellow. Ultimately, the leaves become dry and turn straw-colored prematurely. Copious amounts of white mycelium appear in the collar region and above (up to 5 cm) when the environmental conditions (temprature equal 26° C and relative humidity up to 74 %) are suitable and may cover the base of the branches (Fig. 1). Extended grayish lesions with or without mycelial coating can be seen in the upper parts of stems. Dark black sclerotia of variable sizes and shapes can be seen occasionally mingled with mycelial strands on branches (Fig. 2). The below-ground parts of the diseased plants show surface discoloration.

Identification of the pathogen:

The causal agent of white stem rot was identified in the Plant Pathology Research Institute. Agric. Res. Center, Giza, to be *Sclerotinia sclerotiorum* (Lib.) de Bary.

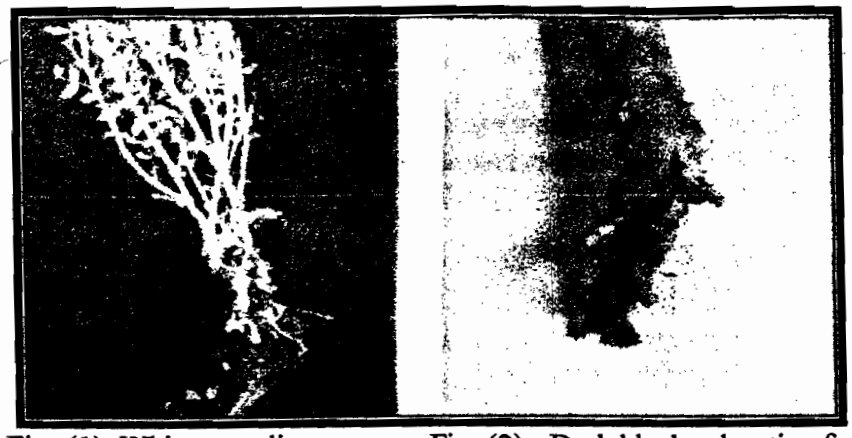


Fig. (1) :White mycelium on the crown of chickpea adult plants.

Fig. (2) : Dark black sclerotia of variable sizes and shapes on chickpea branch.

Effect of the Systemic Fungicide, Vitavax-200, on *S. sclerotiorum in vitro* :

Data in Table (1) show that all concentrations of the tested fungicide significantly reduced the mycelial growth of the fungus compared with untreated control Fig. (3). Vitavax-200 completely inhibited the mycelial growth of *S. sclerotiorum* at the rate of 10.0 ppm 2 days after inoculation. Concentration of 1.0 ppm of the tested fungicide reduced number of sclerotia formed 15 days after inoculation Table (2).

Table (1) : Effect of the tested concentrations of vitavax-200 in CDA mediumon the linear growth of *Sclerotinia sclerotiorum.*

Incubation period (days)	Colony diameter (cm) Fungicide concentrations (ppm)				Mean (cm)
	0	1	5	10	
2	3.83*	2.31	1.36	0.0	1.87
4	7.31	6.71	2.56	0.0	4.14
6	9.00	8.76	5.18	0.0	5.73
Mean	6.72	5.92	3.03	0.0	

* Data are average of three replicates

LSD at 0.05 Concentrations (C) = 0.005

Period (P) = 0.0044 C × P = 0.008

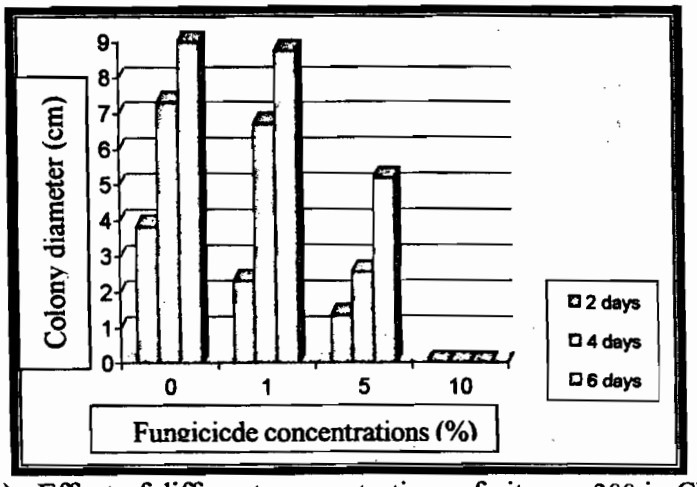


Fig.(3) : Effect of different concentrations of vitavax-200 in CDA medium on the linear growth of *Sclerotinia sclerotiorum*, 2, 4 and 6 days after inoculation.

Table (2) : Effect of different concentrations of vitavax-200 in CDA medium on the number and sclerotia formation.

Time (days)	Sclerotia number fungicide concentrations (ppm)			
	0	1	5	10
15	17 *	12	0	0

* Data are average of three replicates LSD at 0.05 = 1.153

Activity of Some Biocontrol Agents Against *S. sclerotiorum* :

1- Effect of *Trichoderma harzianum* :

Data in Table (3) show that *T. harzianum* has an antagonistic effect on *S. sclerotiorum* Fig. (4).

Table (3):Inhibition area developed between *Trichoderma harzianum* and *Sclerotinia sclerotiorum*, five days after inoculation.

Tested fungus	Inhibition area (mm)
Sclerotinia sclerotiorum only	00.0
S. sclerotiorum + *Trichoderma harzianum*	47.0

Data are average of three replicates

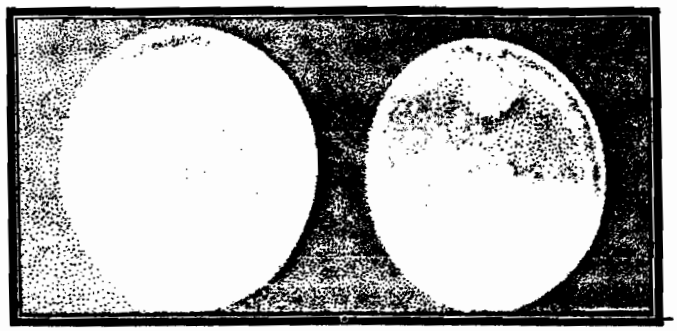


Fig. (4) : Antagonistic effect of *Trichoderma harzianum* against *S. sclerotiorum*, 5 days after inoculation.

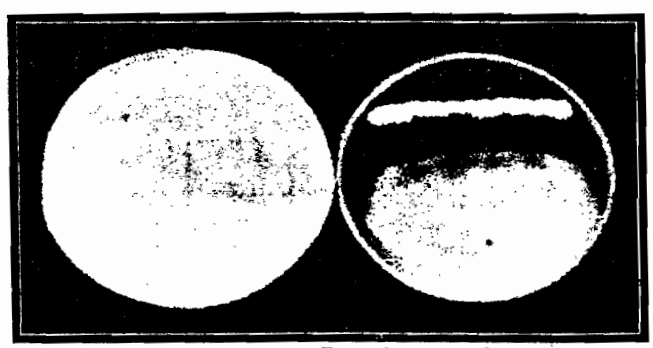
2- Effect of *Bacillus subtilis* :

Data in Table (4) show inhibition area was developed well between the two organisms Fig. (5).

Table (4) : Inhibition area developed between *Bacillus subtilis* and *Sclerotinia sclerotiorum*, seven days after inoculation.

Tested organism	Inhibition area (mm)
Sclerotinia sclerotiorum	00.0
S. sclerotiorum + *Bacillus subtilis*	28.0

Data are average of three replicates



Control *B. subtilis* + *S. sclerotiorum*

Fig.(5) : Antagonistic effect of *Bacillus subtilis* against *S. Sclerotiorum*, in solid medium, 7 days after inoculation.

Effect of Some Plant Extracts on *S. sclrotiorum* :

1. Absinthe leaves extract:

Results in Table (5) show that all the tested concentrations of absinthe leaves extract significantly reduced the mycelial growth of *S. sclerotiorum* in absinthe extract supplemented CDA media. Inhibition effect was increased by increasing extract concentrations Fig. (6). Number of sclerotia was increased on media treated with absinthe leaves extract 15 days after inoculation, compared with the untreated medium Table (6).

Table (5) : Effect of absinthe leaves extract on *Sclerotinia sclerotiorum* linear growth.

Time (days)	Colony diameter (cm) Extract concentrations (%)					Mean
	0	25	50	75	100	
2	2.85	1.75	1.30	1.05	0.00	1.39
4	6.06	3.80	3.10	2.63	1.45	3.40
6	9.00	8.68	7.31	6.83	4.23	7.21
Mean	5.97	4.74	3.90	3.50	1.89	

Data are average of three replicates LSD at 0.05

Time (T) = 0.2631 Concentrations (C) = 0.3397 C × T = 0.0058

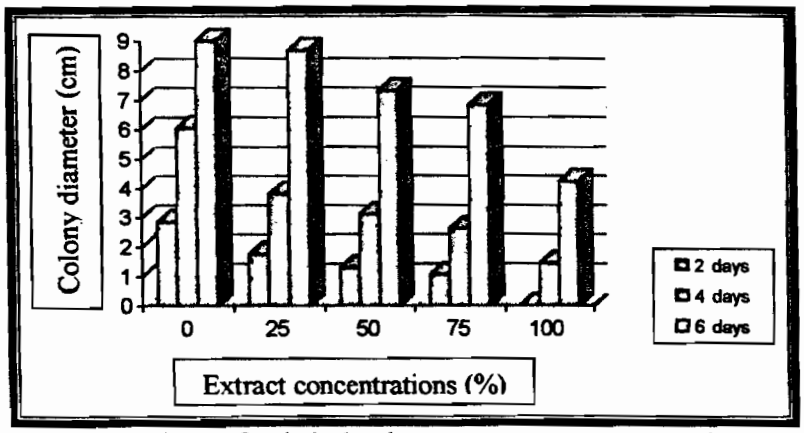


Fig.(6) : Effect of absinthe leaves extract concentrations on *Sclerotinia sclerotiorum* linear growth, 2, 4 and 6 days after inoculation.

Table (6) : Effect of absinthe leaves extract on the number and sclerotia formation of *Sclerotinia sclerotiorum*.

Time (days)	Sclerotia number Extract concentrations (%)				
	0	25	50	75	100
15	*6.33	6.66	9.66	9.66	10.00

* Data are average of three replicates LSD at 0.05 = 0.3957

2. Garlic or onion leaves extracts:

Data presented in Table (7) & Fig. (7) show that leaves extracts of garlic or onion inhibited the mycelial growth of the tested fungus. Inhibition effect of garlic or onion leaves extracts was increased by increasing the concentration of the used extract . Fungal growth was completely inhibited when media supplemented with extracts of garlic or onion leaves at concentration of 15 % under the different incubation periods. Small size sclerotia with big number were formed on media supplemented with garlic or onion leaves extracts,15 days after inoculation, at concentration rates of 5 and 10 % Table (8).

Table (7) : Effect of garlic or onion leaves extracts on *Sclerotinia sclerotiorum* linear growth.

Tested extract	Time (days)	Colony diameter (cm) Extracts concentrations (%)				Mean
		0	5	10	15	
Garlic	2	3.70	1.23	1.15	0.00	1.52
	4	6.39	3.13	2.56	0.00	3.02
	6	9.00	5.81	3.83	0.00	6.21
Onion	2	3.71	1.83	1.68	0.00	1.80
	4	6.38	4.56	3.12	0.00	3.51
	6	9.00	7.95	4.64	0.00	5.39
Mean		6.36	4.08	2.83	0.00	

Data are average of three replicates

LSD at 0.05 Time (T) = 0.0115

Concentrations (C) = 0.0133 Extracts (E) = 0.0094

C × E = 0.0188 E × T = 0.0162

C × T = 0.023 E × C × T = 0.0325

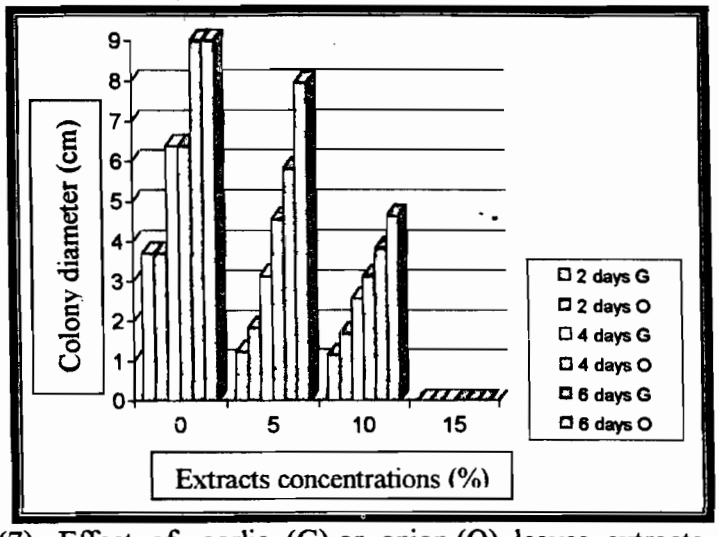


Fig. (7) : Effect of garlic (G) or onion (O) leaves extracts on *Sclerotinia sclerotiorum* linear growth, 2, 4 and 6 days after inoculation.

Table (8) : Effect of garlic or onion leaves extracts on the number and sclerotia formation of *S . sclerotiorum* .

Tested extract	Time (days)	Sclerotia number Extract concentrations (%) 0	5	10	15	Mean
Garlic	15	*5.66	8.33	10.66	0.00	6.16
Onion	15	5.66	8.00	10.33	0.00	5.99

*Data are average of three replicates LSD at 0.05

Extracts (E) = 0.004 Concentrations (C) = 0.0057 E × C = 0.0256

Intercropping of some winter crops with chickpea:

Data in Table (9) show that a significant reduction in white stem rot disease incidence was found on chickpea plants intercropped with garlic or onion plants during 2001 and 2002 growing seasons. Intercropping with garlic was more effective than did that with onion plants. In general, intercropping chickpea plants with garlic or onion significantly decreased white stem rot disease in case of hill / hill or ridge / ridge application (Fig. 8).

Table (9) : Effect of intercropping of garlic or onion with chickpea on white stem rot disease incidence during 2001 and 2002 growing seasons.

Entry		Control	Intercropping method				Mean
			Ridge/Ridge		Hill/Hill		
		Chickpea only	Garlic / chickpea	Onion / chickpea	Garlic / chickpea	Onion / chickpea	
G.1	A	5.14*	4.89	5.03	3.11	4.90	4.61
	B	9.37	8.98	9.13	7.01	8.80	8.65
G.88	A	5.09	4.88	5.04	3.00	4.17	4.43
	B	9.13	8.14	9.10	6.70	7.99	8.21
G.195A		4.87	4.17	4.81	2.98	4.01	4.16
	B	8.77	8.10	8.12	6.13	7.12	7.64
G.531A		4.23	3.08	4.03	2.79	3.78	3.58
	B	7.13	7.17	7.02	5.64	6.54	6.70
Mean	A	4.83	4.25	4.72	2.97	4.21	
	B	8.60	8.09	8.34	6.37	7.61	

A : 2001 growing season B : 2002 growing season
*Disease incidence (%) G. : Giza
LSD at 0.05 Entries (E) = 0.0159 Seasons (S) = 0.0112
Methods (M) = 0.0178 S × E = 0.0225 M × E = 0.0356
S × M = 0.0251 M × E × S = 0.0503

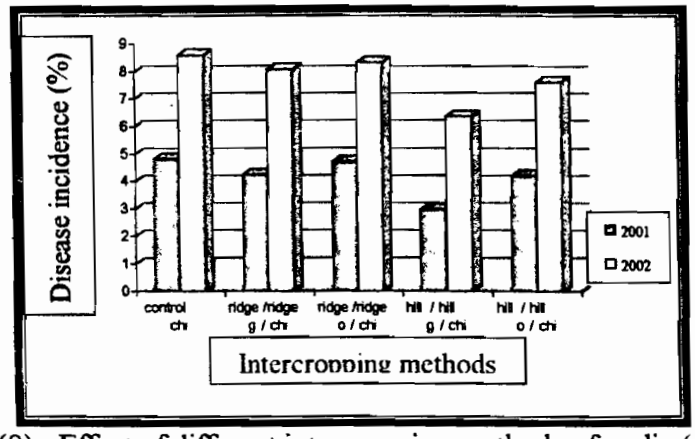


Fig. (8) : Effect of different intercropping methods of garlic (g) or onion (o) crops with chickpea (chi) on white stem rot disease incidence during 2001 and 2002 growing seasons.

DISCUSSION

Chickpea plants are attacked by several diseases, in field and during storage. The most important and destructive disease is white stem rot which occurred in El-Behera Governorate. *Sclerotinia sclerotiorum* infects over 400 species of plants including many important crop species (Boland and Hall, 1994).

Under heavy infection, chemical control emerged as alternative and quick mean for controlling the disease. In this respect, Omar *et al.*, (1992) indicated that vitavax, vitavax/captan and rhizolex were the most efficient fungicides to control pre- and post emergence damping-off and white stem rot disease of chickpea. The obtained results are in agreement with Ballantyne, (1964), Marcum et al., (1977), Ghanim, (1993) who recommended chemical control of sclerotinia stem rot disease as alternative and quick means of disease control, and Steadman, (1979) who reported that chemical fungicides can inhibit sclerotia formation.

Since, the use of some fungicides might cause hazard and pollution in the environment and ecosystem, as well as detection of benomyl-tolerant strains of *Sclerotinia* sp (Willetts and Wong, 1980), biological control has been emerged as one of the safe means of stem rot disease control. The interaction between *Trichoderma harzianum* as an antagonistic fungus and *S. sclerotiorum*, the causal fungus of chickpea white stem rot disease *in vitro* showed that *T. harzianum* inhibited the mycelial growth of *S. sclerotiorum* and inhibition area between the two organisms was developed. These results are in agreement with those obtained by Vannocci *et al.*, (1991) and Abd El-Moity *et al.*, (1993) who reported that *T. harzianum* had an antagonistic effect on *S. sclerotiorum*. In an *in vitro* test, Mazen, (1995) found that *T. viride* growth covered the growth of *S. sclerotiorum* in 9 cm Petri dishes 9 days after incubation. Variation between the two results could be due to differences between *Trichderma* species and its antagonistic effect.

Several reports indicated the efficacy of *Bacillus subtilis* isolates as antagonistic for plant pathogens (El-Neshawey, 1997). The obtained results show that an inhibition area was developed well between *B. subtilis* and *S. sclerotiorum* on the solid medium. The antagonistic effects of the bacteria as a biocontrol agent could

be due to toxic compounds, secreted enzymes or secondry metabolites (Costa *et al.*,1998) and (Conrad *et al.*,1999). The obtained results are in agreement with Tsuge et al., (1995), El-Sheikh, (1998), El-Kazzaz, (1999), Youssef, (2000) and El-Naka (2002) who mentioned that certain isolates of *B. subtilis* have an inhibitory effect against plant pathogenic fungi or bacteria.

Plant extracts show antifungal activity against a wide range of fungi (Wilson *et al.*, 1997). The present study showed that absinthe leaves extract significantly reduced the mycelial growth of *S. sclerotiorum* in CDA media. Inhibition effect on the mycelial growth was increased by increasing extract concentrations. After 15 days from incubation, number of the formed sclerotia / plate was increased on the media treated with absinthe extract compared with the untreated medium. The highly formation of sclerotia by the fungus on media treated with absinthe leaves extract may be due to fungal response to resist the extract toxicity and unfavourite conditions.

The obtained results showed that leaves extracts of both garlic or onion individualy inhibited the mycelial growth of *S. sclerotiorum*, compared with control. Inhibition effect of garlic or onion leaves extract on the fungal growth was increased by increasing the concentration of the used extract. Fungal growth was completely inhibited by extracts of garlic or onion leaves at concentration rate of 15 % under the different growth periods. On the other hand, small size sclerotia with big number were formed on media supplemented with extracts at concentrations of 5 and 10 %. This effect may be due to the fungal ability to resist the toxicity of supplemented media or the presence of some stimulatory constituents favourite the formation of sclerotia (Kurucheve *et al.*,1997). Wilson *et al.*,(1997) reported that the most plant extracts showing antifungal activity, among 345 plant extracts, were species of *Allium*. The inhibitory effect of garlic or onion extracts might be attributed to the presence of some antifungal ingredients. Generally, the obtained results of the inhibitory effect of absinthe, garlic and onion extracts against *S. sclerotiorum*, the causal agent of chickpea white stem rot disease, are in agreement with those findings by Abd El-Ghani and Heweidy, (1997), Heweidy *et al.*, (1997), Kurucheve *et al.*, (1997), Wilson *et al.*, (1997) and

Papadopoulou *et al.*, (1999), who reported that some plant extracts have a great antifungal activity against the causal agents of different diseases.

The obtained results showed that intercropping of chickpea plants with garlic or onion by both hill / hill and ridge / ridge method significantly decreased white stem rot disease incidence. This effect was probably due to that *Allium* spp may have an antifungal compounds play an important role in controlling several diseases (Kurucheve *et al.*, 1997). These results are some what agree with Omar *et al.*, (1992), Abd El-Ghani and heweidy (1997), Wilson *et al.*, (1997), Papadopoulou *et al.*, (1999) and Tripathi and Rathi (2000).

Finally, results of this study indicate that plant based compounds such as absinthe leaves, garlic or onion leaves extracts and applying of fungal and bacterial biocontrol agents may be effective safe alternatives to conventional synthetic fungicides for controlling chickpea white stem rot to minimize chemical hazard and environmental pollutions. Consequently, such non-fungicidal, non- residual treatments should be in consideration.

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استخدام بعض العوامل الحيوية ، والمستخلصات النباتية ، والتحميل لمكافحة عفن الساق الأبيض في الحمص في محافظة البحيرة

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استهدف هذا البحث التعرف على المسبب المرضي لعفن الساق الأبيض في الحمص ومحاولة تطبيق اتجاهات غير تقليدية في مقاومة المرض لمحاولة تقليل الخسائر الناجمة عنه وحماية البيئة من التلوث .
تم عزل الفطر *Sclerotinia sclerotiorum* من نباتات حمص مصابة بعفن الساق الأبيض جمعت من مناطق مختلفة من محافظة البحيرة . أظهرت نتائج دراسة تأثير المبيد فيتافاكس ٢٠٠ لمقاومة المرض أن تركيز ١٠ جزء في المليون ثبط النمو الميسليومي للفطر تماما ، بينما لم تتكون الأجسام الحجرية عند التركيزين ٥ و ١٠ جزء في المليون وتكونت بأعداد قليلة عند تركيز ١ جزء في المليون تم دراسة التأثير التثبيطي للفطر *Trichoderma harzianum* على نمو الفطر *Sclerotinia sclerotiorum* و أوضحت النتائج أن لهذا الفطر قدرة عالية على تثبيط النمو الميسليومي للفطر موضع الدراسة كما تبين أن للبكتيرة *Bacillus subtilis* قدرة كبيرة على تثبيط نمو الفطر حيث تكونت منطقة تثبيطية بين كلا الكائنين على البيئة الصلبة . كما أوضحت النتائج المتحصل عليها أن كل تركيزات مستخلص نبات الدمسيسة قللت النمو الميسليومي للفطر سكلروتنيا و أن التأثير التثبيطي للمستخلص يزداد بزيادة التركيز ، تبين كذلك أن التأثير التثبيطي للمستخلص قل مع زيادة فترات التحضين . وأن عدد الأجسام الحجرية يتزايد طرديا مع زيادة تركيز المستخلص وذلك بعد ١٥ يوما من التحضين . أكدت نتائج هذا البحث أن جميع التركيزات المستخدمة من مستخلص أوراق كل من الثوم أو البصل قللت النمو الميسليومي للفطر المسبب لمرض عفن الساق الأبيض في الحمص و أن تركيز ١٥ % لكلا المستخلصين ثبط النمو الميسليومي للفطر تماما . وتبين من النتائج أن تحميل الحمص بنباتى البصل أوالثوم سواء كان بطريقة جورة / جورة أو ريشة / ريشة قد أدى الى تقليل نسبة حدوث مرض عفن الساق الأبيض لنباتات الحمص .