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Preliminary Tests on the Biological Control of some Root Rot Fungal Pathogens in Sugar Beet *In Vitro*

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ABSTRACT

Four antagonistic fungi, two antagonistic bacteria and three essential plant oils were used in vitro to control *Fusarium oxysporum, F. solani* and *Sclerotium rolfsii* causing rot root diseases in sugar beet. All antagonistic fungi showed variable antagonistic effect against sugar beet root rot pathogens. Result data showed that *T. hamatum* gave 76.3%,79.6% and 70.4% .*T. harzianum* giving 78.5%, 76.6% and 63.7% , *T. viride* gave 75.5%, 78.8% and 61.8%, while *C. minitans* gave 62.2%, 60% and 71.8% inhibition of *F. oxysporum, F. solani* and *S. rolfsii* respectively. Also all fungal culture filtrates gave highest effect against the pathogens at high concentration ranged between (76% to 93%) inhibition compared with untreated control. The antagonistic bacteria *P. fluorescence* recorded the best effect on mycelial growth of pathogens than *B. subtilis*. Where *P. fluorescence* inhibited growth of *F. oxysporum, F. solani* and *S. rolfsii* by 76%, 87.1% and 73.3% but *B. subtilis* gave 66.6%, 69.3% and 62.2% respectively. The culture filtrate of *P. fluorescens* exhibited high effect giving (79.3%, 82.6% and 75.5%) at 50% concentrations, follwed by *B. subtilis* gave (72.2%, 64.8% and 68.8%) respectively with the same three pathogens compared with control. Cinnamon oils gave the highest antagonistic effect against all pathogens and completely inhibited growth (100%) at all concentrations with *F. oxyporum* and *S. rolfsii*, while with *F. solani* gave 93.2% inhibition at high concentrations, followed by spearmint oils which showed high effect with *F. oxysporum* and *F. solani* by (74.8% and 85.3% inhibition) at high concentrations, but gave moderate effect with *S. rolfsii* by 65.2% inhibition. Garlic oils exhibited high inhibition with *S. rolfsii* giving 95%, gave 71.5% and 76% with *F. oxysporum* and *F. solani* at same concentration compared with control.

Keywords: pathogenic fungi – bioagents fungi and bacteria – essential oils - antifungal activity – Sugar beet .

INTRODUCTION

Sugar beet is an important crop for sugar production in Egypt and many countries of the world after sugar cane, considered as the most important sucrose producing plant in temperate areas covering almost 40% from sucrose production in the world. Production of ugar beet has gradually decreased during the last decades because it is susceptible to pathogens which causing root rot diseases, consequently cause the major problem in sugar beet production in the world, the most fungi involved in this disease are *Rhizoctonia solani, Sclerotium rolfsii* and *Fusarium* spp. (Eweis *et al*., 2006 ; Eweis, 2007 ; Haikal *et al*., 2008) mentioned that these pathogens attack underground parts of plant resulting in pre- and post-emergence, damping-off, seedling and leaf blight and root rot disease (Emechebe and Lagoke, 2002).This disease lead to reducing yield and quality of sugar beet in many growing areas. Biological control agents are used for treatment of the phytopathogens because fungicides are very expensive and cause enormous contamination for environment. Using microorganisms as biological control are successful (Sivan, 1987) depended on effective antagonists, costs and the method of application. *Trichoderma* spp., *Coniothyrium minitans, Bacillus subtilis* and *Psedumonas fluorescens* when used as biological control of root rot diseases showed antagonistic effects against soil-borne pathogens. Many studies investigated the mode of action of antagonistic fungi and bacteria in past two decades based on root and soil-borne pathogens (Janisiewiez *et al*., 2000). Gabr *et al*. (2003) found that *Bacillus* spp. exhibited high effect on *F. solani* and *R. solani* . Mode of action of biocontrol of root and soil-borne pathogens by antibiosis, parasitism, induced resistance of the host plant and competition for space and nutrients.

Also natural products used for management of plant diseases as an alternative method to synthetic chemical because it is no effects on human health and environmental safety (Jasso de Rodrieguez *et al*., 2006). In recent years researches depended on plants and their essential oils in controlling pathogenic fungi and mycotoxins production in crop plants (Lambert *et al*., 2001). Medicinal and aromatic plants are rich in essential and volatile oils, in addition, they contain active compounds that are toxic to several phytopathogenic fungi (Goussous *et al*., 2010). Therfore, they have potential for controlling plant diseases because they posses antagonistic activity against fungi and bacteria (Pinto *et al*., 2007 ; Hussain *et al*., 2008). Essential oils could be protection tools of plants, also attract some insects and can play role in mediating the interactions of plant with the environment (Bakkali *et al*., 2008).

The objective of this study is to evaluate the antifungal activity of *T. harzianum, T. hamatum, T. viride, C. minitans, B. subtilis* and *P. fluorescens*, three essential oils *Mentha spicata, Cinnamomum zylanicum* and *Allium sativum* against the pathogenic fungi : , *F. solani, F. oxysporum* and *Sclerotium rolfsii* which used as an alternative to synthetic chemical to reduce root rot diseases in sugar beet and the pollution of environment.

MATERIALS AND METHODS

Patogenic fungi : three pathogenic fungi which showed root rot symptoms were isolated from infested sugar beet roots in centers Dakahlia Governorate included *F. oxysporum, F. solani* and *S. rolfsii*. These isolates fungi were isolated and purified in vitro and identified by Dept. of Plant Pathology, Faculty of Agriculture, Mansoura University.

Biocontrol agents: The bioagents were used in this study included three fungi (*T. harzianum, T. hamatum* and *T. viride*) were obtained from Prof. Dr. Mohamed El-Sheshtawi laboratory, Dept. of plant pathology, Fac. Agric., Mansoura Univ. While *C. minitans* was obtained as a gift from Prof. Dr. Laszlo Vajna, Dept. of Plant Pathology, Institute of Plant Protect. Res, Budapest, Hungary. Also two bacteria (*B. subtilis* and *P. fluorescens*) were obtained from Dept. of plant pathology, Fac. Agric., Mansoura, Univ. Three essential oils (*Mentha spicata, Cinnamomum zeylanicum* and *Allium sativum*)

were collected and purchased from the market of Mansoura city.

Biological control:

1- Effect of antagonistic fungi on growth of the pathogens:

Antagonistic fungi (*T. harzianum, T. hamatum, T. viride* and *C. minitans*) were investigated against pathogenic fungi (*F. oxysporum, F. solani* and *S. rolfsii*) in vitro by using the dual culture method. Percentage of inhibition zone was determined compared with untreated control.

*** Effect of fungal filtrates on mycelial growth of the pathogens:**

The antagonistic fungi were inoculated with 5 m of fungal culture and incubated at 25°C in conical flask containing Potato Dextrose Broth (PDB) medium the fungal, then the filtrate was poured in Petri dishes containing discs (5mm) from 7 days old cultures incubated at 25°C for 7 days. Untreated control containing pathogen only. Percentage of growth reduction was determined compared with control.

2- Effect of antagonistic bacteria on inhibition growth of the pathogens:

Antagonistic effect of two bacteria (*B. subtilis* and *P. fluorescens*) were investigated against pathogenic by streaking the bacteria was streaked on surface medium containing PDA on one side of the plate (1cm from the edge of the plate) facing one disc of the pathogen. Control was done by growing one disc of the pathogen alone. Three replicates were used for each treatment then all plates incubated at 25ºC for 7 days. Percentage of growth reduction was determined.

*** Effect of bacterial filtrates on inhibition growth of the pathogens:**

The filtrates were prepared by streaking bacteria on Nutrient Agar Medium and incubated for 48 h at 30ºC, then the bacterial filtrates were inculated in flask containing 100 ml of Nutrient Broth medium and incubated at for 72 h 30ºC. The residue of filtrates was mixed with PDA to obtain concentrations (10, 25 and 50%), then poured in Petri dishes inoculated with 5 mm-diameter of pathogen placed in centre plate. Control was made on the same medium in Petri dishes containing the pathogen only. Three replicates were used for each treatment then all plates incubated at 25ºC for 7 days. Percentage of growth reduction was determined compared with control.

3- Antifungal activity of plant oils on inhibition growth of the pathogens:

Three oils (cinnamon, garlic and spearmint) were determined for antagonistic activity at three concentrations (25, 50 and 75%) against pathogenic fungi. The concentrations were poured in PDA medium containing 0.5% Tween-80. Control was made on same medium containing the pathogen without oils., then all plates inoculated with 5 mm diameter discs of pathogens. Three replicates were used for each treatment, then all plates were incubatedfor 7 days at 25ºC. Percentage of growth reduction was determined compared with control.

Statistical analysis : Obtained data were statistically analyzed according to CoStat 6.311 (2005) of analysis of variance (Gomez and Gomez, 1984) at $p \leq 0.05$ as outlined by Duncan, 1955)

RESULTS

1- Inhibitory effect of antagonistic fungi on growth of the pathogens:

The results in Table (1) showed that all antagonistic fungi have high potentiality in controlling of pathogens with different degrees. *T. harzianum, T. hamatum* and *T. viride* showed high effect giving (78.5%,76.3% and 75.5% inhibition), while *C. minitans* showed moderate effect giving (62.2% inhibition) with *F. oxysporum* .

The effect of antagonistic fungi on *F. solani* exhibited that *T. hamatum, T. viride* and *T. harzianum* gave the best effect giving (79.6%, 78.8% and 76.6% inhibition) respectively. But *C. minitans* gave 60% inhibition. With *S. rolfsii* it showed that *C. minitans* and T. *hamatum* gave high inhibition by (71.8% and 70.4%). But *T. harzianum* and *T. viride* gave moderate effect giving (63.7% and 61.8% inhibition).

Table 1. Inhibitory effect of antagonistic fungi on growth of the pathogens:

pathogens	*F. oxysporum*		*F. solani*		*S. rolfsii*	
Treatments	***R.G**	**Inh. %**	**R.G**	**Inh. %**	**R.G**	**Inh. %**
Control	9.00 a	0.00	9.00 a	0.00	9.00 a	0.00
T. viride	2.2 d	75.5	1.9 d	78.8	3.43 b	61.8
T. hamatum	2.13 d	76.3	1.83 d	79.6	2.66 c	70.4
T. harzianum	1.93 d	78.5	2.1 d	76.6	3.26 b	63.7
C. minitans	3.4 b	62.2	3.6 b	60	2.53 c	71.8

***R.G=Radial growth (cm.) Inh. %= inhibition %.**
Values within a column followed by the same letter are not significantly different according to Duncun's multiple range test (P=0.05) (Duncan, 1955).

2- Effect of fungal filtrates on radial growth of the pathogens:

Result in Table (2) showed that all fungal filtrates inhibited the mycelial growth of pathogens. *T. viride, T. hamatum* and *T. harzianum* showed high effect on radial growth of *F. oxysporum* giving (78.5%, 77.4% and 72.6% inhibition) at concentration 10%, giving (80.7%, 79.6% and 79.3% inhibition) at concentration 25% and giving (93%, 92.2% and 91.5% inhibition) at conc. 50%. On the other hand *C. minitans* gave (62.2%, 67,4% and 80% inhibition) at all concentrations respectively.

With *F. solani* it was found that *T. viride, T. harzianum* and *T. hamatum* showed high effect with inhibition (80%, 78.5% and 74.4%) at concentration 10%, (85.5%, 89.6% and 86%) at conc. 25% and giving (91.5%, 92.2% and 93.3%) at conc 50%, while *C. minitans* gave (53.3%, 63.3% and 78.5%) at threee concentrations respectively.

Respect to *S. rolfsii* all antagonistic fungi showed high inhibition on radial growth, where *C. minitans, T. harzianum, T. hamatum* and *T.viride* gave (67.7%, 66.6%, 64.4%, 55.5%) at concentration 10%, gave (71.1%, 70.4%, 70% and 68.5%) at concentration 25% and gave (82.2%, 80%, 79.6% and 76%) at concentration 50% respectively.

3- Effect of antagonistic bacteria on radial growth of the pathogens:

Data in Table (3) showed that antagonistic bacteria *P. fluorescens* showed the best effect than *B. subtilis* on the mycelial growth of all the pathogens. *P.*

fluorescens recorded 76% inhibition. This followed by *B. subtilis* which gave 66.6% inhibition with *F. oxysporum*. While *P. fluorescens* gave high inhibition (87.1%) with *F. solani*, followed by *B. subtilis* which recorded 69.3% inhibition. On the other hand, *P. fluorescens* gave 73.3% inhibition with *S. rolfsii*, *B. subtilis* showed moderate effect giving 62.2% inhibition.

Table 2. Inhibitory effect of fungal filtrates on mycelial growth of pathogens:

Treatments	Conc. %	Pathogens					
		F. oxysporum		*F. solani*		*S. rolfsii*	
		*R.G	Inh. %	*R.G	Inh. %	*R.G	Inh. %
Control	0	9.00 a	0.00	9.00 a	0.00	9.00 a	0.00
T. hamatum	10	2.03 gh	77.4	2.30 fg	74.4	3.20 d	64.4
	25	1.83 h	79.6	1.26 ij	86	2.7 e	70
	50	0.70 i	92.2	0.60 k	93.3	1.83 gh	79.6
T. viride	10	1.93 h	78.5	1.80 h	80	4.00 c	55.5
	25	1.73 h	80.7	1.30 ij	85.5	2.83 de	68.5
	50	0.63 i	93	0.76 k	91.5	2.16 fg	76
T. harzianum	10	2.46 fg	72.6	1.93 gh	78.5	3.00 de	66.6
	25	1.86 h	79.3	0.93 jk	89.6	2.66 e	70.4
	50	0.76 i	91.5	0.70 k	92.2	1.80 gh	80
C. minitans	10	3.40 d	62.2	4.20 d	53.3	2.90 de	67.7
	25	2.93 e	67.4	3.30 e	63.3	2.60 ef	71.1
	50	1.80 h	80	1.93 gh	78.5%	1.60 h	82.2

***R.G=Radial growth (cm.) conc. = concentrations Inh. %= inhibition %.**
Values within a column followed by the same letter are not significantly different according to Duncun's multiple range test (P=0.05) (Duncan, 1955).

Table 3. Effect of antagonistic bacteria on radial growth of the pathogens:

pathogens Treatments	*F. oxysporum*		*F. solani*		*S. rolfsii*	
	*R.G	Inh. %	R.G	Inh. %	R.G	Inh. %
Control	9.00 a	0.00	9.00 a	0.00	9.00 a	0.00
T. hamatum	2.13[b]	76 %	1.66[b]	81 %	2.40[c]	75 %
B. subtilis	3.00 c	66.6	2.76 c	69.3	3.40 b	62.2
P. fluorescens	2.16 d	76	1.16 e	87.1	2.40 c	73.3

***R.G=Radial growth (cm.) conc. = concentrations**
Inh. %= inhibition %.
Values within a column followed by the same letter are not significantly different according to Duncun's multiple range test (P=0.05) (Duncan, 1955).

4- Effect of culture filtrates of antagonistic bacteria on radial growth of pathogens:

Data obtained from Table (4) showed that bacterial culture filtrates were effective on inhibition of pathogens. The results exhibited that *P. fluorescence* have stronger antagonistic activity than *B. subtilis*, where *P. fluorescence* gave (50%, 62.2% and 79.3%) inhibition, followed by *B. subtilis* which gave inhibition (38.8%, 57.4% and 72.2%)) at concentrations 10%, 25% and 50% respectively against *F. oxysporum*. In case *F. solani* the high antagonistic was observed in *P. fluorescence* (51.5%, 73.3% and 82.6%) inhibition, followed by *B. subtilis* gave inhibition (33.7%, 48.5% and 64.8%) at same concentrations. Moreover, in case *S. rolfsii* it was found that *P. fluorescence* showed high effect giving (51.8%, 69.3% and 75.5%), while *B. subtilis* gave (40.7%, 51.8% and 68.8%) inhibition at the same concentrations respectively.

5- Effect of plant oils on radial growth of pathogens:

The results in Table (5) indicated that all essential oils showed high inhibition of mycelial growth of all pathogens, while cinnamon oils recorded the highest effect followed by spearmint and garlic oils. Cinnamon oil showed complete inhibition (100 %) at all concentrations, followed by spearmint oil which recorded (52.6%, 66.6% and 74.8% inhibition) at concentrations 10%, 25% and 50% respectively. Garlic oil gave inhibition (34.1%, 63% and 71.5%) at the same concentrations against *F. oxysporum*.

Also with *F. solani* cinnamon oil exhibited highest effect giving 88.8%, 91.1% and 93.2% inhibition), followed by spearmint oil which gave inhibition of (64.4%, 84.8% and 88.3%). Garlic oil showed inhibition 45.4%, 67.4% and 76%) respectively with the same concentrations. However, with *S. rolfsii* cinnamon oil showed the highest inhibition reached to 100% at all concentrations, followed by garlic oil which gave (47.6%, 78.7% and 95.3%) at concentrations 10%, 25% and 50% respectively, then spearmint gave (41.3%, 48.8% and 65.2%) at the same concentrations respectively.

Table 4. Inhibitory effect of bacterial filtrates on growth of pathogens:

Treatments	Conc. %	Pathogens					
		F. oxysporum		*F. solani*		*S. rolfsii*	
		*R.G	Inh. %	*R.G	Inh. %	*R.G	Inh. %
Control	0	9.00 a	0.00	9.00 a	0.00	9.00 a	0.00
B. subtilis	10	5.50 b	38.8	5.96 b	33.7	5.33 b	40.7
	25	3.83 d	57.4	4.63 c	48.5	4.33 c	51.8
	50	2.50 ef	72.2	3.16 e	64.8	2.80de	68.8
P. fluorescens	10	4.50 c	50	4.36 cd	51.5	4.33 c	51.8
	25	3.40 d	62.2	2.40 f	73.3	2.76 de	69.3
	50	1.86 h	79.3	1.56 hi	82.6	2.20 fg	75.5

***R.G=Radial growth (cm.) conc. = concentrations**
Inh. %= inhibition %.
Values within a column followed by the same letter are not significantly different according to Duncun's multiple range test (P=0.05) (Duncan, 1955).

Table 5. Antifungal activity of some plant oils on inhibition growth of the pathogens :

Treatments	Conc. %	Pathogens					
		F. oxysporum		*F. solani*		*S. rolfsii*	
		*R.G	Inh. %	*R.G	Inh. %	*R.G	Inh. %
Control	0	9.00 a	0.00	9.00 a	0.00	9.00 a	0.00
Cinnamon oils	25	0.00 f	100	1.00 e	88.8	0.00 d	100
	50	0.00 f	100	0.8 e	91.1	0.00 d	100
	75	0.00 f	100	0.61 e	93.2	0.00 d	100
Garlic oils	25	5.93 a	34.1	4.91 a	45.4	4.71 a	47.6
	50	3.33 c	63	2.93 bc	67.4	1.91 c	78.7
	75	2.56 de	71.5	2.15 cd	76	0.42 d	95.3
Spearmint oils	25	4.26 b	52.6	3.2 b	64.4	5.28 a	41.3
	50	3.00 cd	66.6	1.36 de	84.8	4.60 a	48.8
	75	2.27 e	74.8	1.33 de	85.3	3.13 b	65.2

***R.G=Radial growth (cm.) Inh. %= inhibition %.**
Values within a column followed by the same letter are not significantly different according to Duncun's multiple range test (P=0.05).

DISCUSSION

F. solani, *F. oxysporum* and *S. rolfsii* causes root rot diseases in sugar beet result in high crop losses, several diseases management strategies include crop rotation, application of fungicides for control plant diseases. The use chemical fungicides for controlling plant diseases lead to undesirable effects on the environment, animal and human health. Biological control may help to provied an alternative methods to fungicides in controlling phytopathogenic fungi (Ronseaux *et al*., 2013 ; Yousef *et al*., 2013).

The results mentioned indicated that all antagonistic fungi, bacteria and plant oils have potential to inhibit growth of the pathogenic fungi with varaition in their antagonistic activity. The antagonistic fungi was effective in inhibiting radial growth of pathogens, where *T. harzianum, T. hamatum, T. viride* and *C. minitans* gave the high effect on all tested pathogens. Also fungal filtrates of *T. viride, T. hamatum, T. harzianum* and *C. minitans* showed highest effect at high concentration on all tested pathogens these results are similar with (Prasad *et al*., 2002 ; Lewis and Lunsden., 2001) they reported that *Trichoderma* spp. were able to inhibit pathogenic fungi include *R. solani, S. rolfsii* and *Fusarium* spp.

The antifungal activity of Trichoderma due to many mechanisms like competition for nutrients and space, mycoparasitism, production of volatile compounds, soluble antibiotics and lytic enzymes include ß-1,3-glucanase (Wheatly *et al*., 1997). On the other hand Bruckner *et al*. (1990) reported that *Trichoderma* spp. produce high quality fungitoxic metabolities. The difference in the efficacy of one strain against various phytopathogens may be attribute to its possess high of levels of one mechanisms than anther, where mycoparasitism is a complex process and results in dissolution of the fungal cell wall by lytic enzymes especially with Sclerotium and Rhizoctonia (Scherm *et al*., 2009 ; Reithner *et al*., 2011). The competition also play an important role in the interaction between Trichoderma and Fusarium (Segarra *et al*., 2010). The competition occurs when space or nutrients is unavailable or limited, therefore the biocontrol agents produce antifungal substances able to inhibit the growth of fungi. *T. viride* produce extracellular enzymes such as chitinase, ß-glucanase and pectinase, these enzymes can degrade fungal cell wall (Markovich and Kononova, 2003). Also C. minitans have antifungal properties by mycoparasite and production lytic enzymes include chitinase and ß-1,3 glucanase

In this study we found that the antagonistic bacteria (*B. Subtilis* and *P. fluorescens*) and their supernatants exhibited good effect on the pathogens. *P. fluorescens* was more effective than *B. subtilis* which gave (76%, 87.1% and 73.3%), but *B. subtilis* gave (66.6%, 69.3% and 62.2%) inhibition with *F. oxysporum*, *F. solani* and *S. rolfsii* respectively. Also culture filtrate of *P. fluorescences* was effective than *B. subtilis* with *F. oxysporum* , *F. solani* and *S. rolfsii* at all concentrations particularly at concentration 50%.

The antagonistic bacteria play important role in controlling plant diseases by several ways include competition for nutrients and space, antibiosis, sidrophores production and induced systematic resistance in host plant (Shahraki *et al*., 2009). The bacterial strains produce one or more of antifungal antibiotics which inhibit fungal growth in vitro (Whipps, 2001). *P. fluorescence* is considered as one of the most important biocontrol agents because it can produce antifungal metabolites include 2, 4 diacetylphloroglucinol, hydrogen cyanide phenazinnes and surfactants (Hass and Defago, 2005) which inhibit various pathogenic fungi in vitro. Also *Bacillus* spp. can produce volatile metabolities that inhibited mycelial growth of *F. oxysporum* (Tehrani and Ramezani, 2003). *B. subtilis* inhibited growth *F. oxysporum* in vitro by production of several antibiotics such as bacilysin, iturin and mycobacillin (Chung *et al*., 2008).

Data obtained from essential oils referred that cinnamon oil gave the highest effect at all concentrations (100% inhibition) with *F. oxysporum* and *S. rolfsii*, while gave (88.8% to 93.2% inhibition) with *F. solani* Followed by spearmint oil which showed high effect against *F. oxysporum* and *F.solani* with inhibition (76.3 and 88.3%), while showed moderate effect with *S. rolfsii* (65.2%) at high concentration. Garlic oil gave high effect with *S. rolfsii* (95.4%), but gave 73.5% with *F. solani* and gave 71.5% with *F. oxysporum* at high concentration. These results agree

with (Seow *et al*., 2014) they reported that garlic, cinnamon and menthe have antimicrobial and antiviral activities. The results showed that three essential oils possess antifungal activity against pathogens by different degrees depending on the concentrations of these oils. Several studies reported that cinnamon and spearmint oils have inhibitory effects on the growth of different microbes (Patil *et al*., 2009; Yamamoto-Ribeiro *et al*., 2013). Cinnamon oil showed better effect on tested fungi due to it is richness in volatile phenols, eugenol and cinnamaldehyde

Spearmint oil showed high antifungal activity against pathogenic fungi may be due to high contents of carvone and cis-carveol. Also it contains menthol, menthoforan, menthon, pipriton and polgon. Yadav (2002) reported that *Mentha spicata* oil showed strong fungitoxicity against some storage fungi

Garlic oil possess antifungal and antioxidant properties due to including sulfur, phenolic compounds (Rivilin, 2001), also contain thiosulfonates, allicin, cardiac glycosids, terpenoids and saponins.

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دراسة مبدئية معملية على المكافحة الحيوية لبعض فطريات أعفان الجذور فى بنجر السكر

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أجريت هذه الدراسة لتقدير تأثير بعض عوامل المقاومة الحيوية باستخدام 4 أنواع من الفطريات وتشمل : *T. hamatum, T. harzianum, T. viride* and *C. minitans* ونوعين من البكتيريا وتشمل *B. subtilis* and *P. fluorescens* و3 أنواع من الزيوت وتشمل القرفة ، النعناع والثوم واختبرت فعاليتهم فى المعمل ضد الفطريات الممرضة المسببة لمرض عفن الجذور فى بنجر السكر . واظهرت النتائج الاتى : * بالنسبة للتضاد الفطرى :- كل الفطريات المستخدمة اعطت فاعلية عالية فى تثبيط الفطريات الممرضة ووجد الاتى :* *T. hamatum* اعطت نسبة تثبيط قدرها 76.3%, 79.6% ،70.4% *T. harzianum* *T. viride* 75.5 % 78.5%, 76.6%, 63%, 78.8 % و 61. 8 % *C. minitans* 62.2%, 60%, 71.8% مع الفطريات الممرضة *F. oxysporum, F. solani* and *S. rolfsii* على التوالى* بالنسبة للراشح الفطرى : اعطى الراشح الفطرى اعلى تاثير على الفطريات الممرضة وبخاصة عند اعلى تركيز وتتراوح نسبة التثبيط من 93% - % 76 مع كل الفطريات الممرضة عند تركيز 50 % . * بالنسبة للتضاد البكتيرى :- * اظهرت بكتيريا التضاد *P. fluorescens* اعلى تاثير على نمو الفطريات الممرضة عن بكتيريا *B. subtilis* * *P. fluorescens* اعطت نسبة تثبيط قدرها 76%, 87.1%, 73.3 % . *B. subtilis* اعطت نسبة تثبيط قدرها 66.6%, 69.3%, 62.,2% مع *F. oxysporum, F. solani* and *S. rolsii* على التوالى * ايضا اعطى الراشح البكتيرى تاثير عالى مع بكتيريا *P. fluorescens* * يليها *B. subtilis* *P. fluorescens* اعطت نسبة تثبيط قدرها 82.6%, 75.5 % *B. subtilis* 79.3% اعطت 68.8 % 72.2%, 64.8 % على التوالى مع نفس الفطريات الممرضة عند تركيز * 50% بالنسبة للزيوت النباتية :- * زيت القرفة كان افضل الزيوت المستخدمة حيث اعطى تثبيط تام عند كل التركيزات بنسبة 100 % لكل من *F. oxysporum* و *S. rolfsii* بينما اعطى نسبة تثبيط 93.2 % مع *F. solani* عند تركيز 75 % . * يلية زيت النعناع حيث اعطى نسبة تثبيط عالية 74.8 % و85.3 % مع *F. oxysporum* and *F. solani* عند تركيز 75 % بينما اعطى نسبة تثبيط متوسط 65.2 % مع * *S.* اما زيت الثوم اعطى تاثير عالى مع *S. rolfsii* بنسبة تثبيط 96.3 % يليه *F. solani* حيث اعطى نسبة تثبيط قدرها 76 % و مع *F. oxysporum* اعطى نسبة تثبيط 71.5 % وذلك عند تركيز 75 %