BIOCONTROL OF SOME POTATO FOLIAR DISEASES

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Abstract

The efficiency of some bio-control agents against potato late blight caused by *Phytopthora infestans* , early blight caused by *Alternaria solani*, and gray mould caused by *Botrytis cinerea* were studied. *Pseudomonas fluorescens, Bacillus subtilis* and different isolates of *Trichoderma* spp. (*T. harzianum*, *T. viride* and mixture of different isolates of *Trichoderma* spp.) were evaluated. Blight stop and Plant guard were used as commercial biocides as well as compost tea and mycorrhiza were used under laboratory, greenhouse and field conditions on two potato varieties (Valor and Nicola). *P. fluroscens* and compost tea has no effect under laboratory conditions but has the highest effect under greenhouse and field conditions. All bio-control agents reduced the disease severity on three potato foliage pathogens in different seasons.

Keywords: Early and late blights, gray mould, potato.

INTRODUCTION

In most world countries, fungicides used against pathogenic fungi, mainly those belong to synthetic chemical compounds. Recently, man realized that, using highly toxic substances in agricultural led to great disturbance in biological balance. This disturbance led to the appearance of new pests because the reduction in number of natural enemies and antagonists. *Pseudomonas fluorescens* isolate (1-12) reduced 5% of the mycelial growth of *P. infestans* and *B. cinerea* (Gulati *et al.* 1998). *T. harzianum* and *T. viride* were significantly effective inhibiting the mycelial growth of *A. solani*. There was no significant difference between the effectiveness of *Trichoderma* species in pot culture studies (Babu *et al.*, 2000). Role of arbuscular mycorrhizal (AM) fungi in potato plants and foliar pathogens *Glomus intraralices* or AM showed strong multifunctional effects, *i.e.* stimulated certain AM colonization of potato roots in out door and greenhouse studies. The AM spores provide evidence effect on *Phytophthora infestans* and *Alternaria solani* on potato plants (Akhtar and Siddiqui , 2007).

MATERIALS AND METHODS

- Isolation of the causal organisms

Potato plants showed identical disease symptoms (early and late blights as well as gray mould) were collected during survey from previously mentioned governorates.

Collected samples (leaves and stems) were washed thoroughly, with tap water, surface sterilized using 5% chlorine solution for 10 minutes. Surface sterilized plants were rinsed using sterilized distilled water and dried between two sterilized filter papers. Dried sterilized plant materials (leaves and stems) were cut into small pieces using sterilized scalpel and placed on plain agar medium to isolate the causal organisms of early blight and gray mould while selective medium corn meal agar (Abd-El-Moity, 1985) was used to isolate the causal organism of late blight. Inoculated plates were incubated at 25- 28 °C. Plates were examined periodically and developed mycelia were picked up and transferred to potato dextrose agar (PDA) medium or corn meal medium according to the causal organism. The isolated fungi were purified using hyphal tip and/or single spore techniques according to (Brown, 1924 and Hawker, 1960). The purified fungi were transferred to PDA slants or corn meal agar medium. Different slants were incubated at 5 °C or 28 °C for 7 days according to the causal organism. The developed fungal cultures were stored at 5 °C for further studies. Isolated fungi of early blight and gray mould were identified according to their cultural and morphological characteristics describe by Groves (1946), Gilman (1957), Barnett and Hunter (1972), Ellis and Gibson (1975). *Phytophthora infestans* was identified according to the description of Riberio (1978), Erwin *et al.*, (1983), Ingram and Williams (1991) as well as Erwin and Riberio (1996). (Brame and Flood, 1983). *Phytophthora infestans* was grown on corn meal medium for 14 days at 18 ±2 °C in the dark. The fungal suspension was prepared by adding de-ionized distilled water to the culture and incubated at 5- 8 °C in refrigerator for 2- 3 hrs to stimulate releasing of zoospore. The zoospore suspension was adjusted to be 8×10^4 zoospore / ml using hematocytometer technique (Brame and Flood, 1983).*Alternaria solani* and *B. cinerea* were grown on Czapek Dox agar medium (Salam *et al.*, 2006) in plates 9 cm in diameter. Inoculated plates were incubated at 25 °C for 7-10 days. The developed cultures were flooded by 5 ml sterilized water. The growth (spores and mycelium) were gently rubbed using smooth brush to remove fungal growth from the medium surface. The prepared suspension was received in sterilized flasks 250 ml in capacity. Obtained suspensions of spores and mycelium suspensions were filtrated through sterilized cheese cloth to reduce the mycelial fragments. Fungal suspension of each tested fungus was collected and adjusted using sterilized water to be contain 8×10^4 (*A. solani*) and 13×10^4 (*B. cinerea*) cfu/ml using haemocytometer technique. The prepared suspension of each pathogenic fungus was used to spray potato plants and the percentages of disease incidence were calculated, compared with control treatment (sprayed with water only).

- Isolation of different biocontrol agents from potato phyloplane

Healthy three potato varieties (Diamont , Nicola and Spunta) were collected from highly infected fields with early blight caused by *A. solani*, late blight caused by *P. infestans*, as well as gray mould caused by *B. cinerea*. Healthy potato leaves obtained from El-Sharkia, El-Qalubia and EL-Behira governorates were detached and one gram of each sample was placed in bottle 600 ml in capacity containing only 99 ml of sterilized distilled water. Bottles containing distilled water and leaf samples were shacked for two hours on electric magnetic shaker (3000 rpm). Serial dilutions of 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6} were prepared from the original. One ml of each sample was used to inoculate plate contains suitable medium. The dilutions and/ or media used to isolate different microorganism groups (Fungi and Bacteria) present in Table (1). Inoculated plates were incubated at 30 ±2 °C. All plates were examined periodically and different apparently non-pathogenic microorganisms were selected and isolated. The isolated bacteria were purified, subjected for identification using manuals for bacteria (Bergy, 1975) while Barnett (1960) was used to identify the isolated fungi. Identified microorganisms were transferred to suitable slant medium and incubated at 30±2°C for 7 days and then storage in refrigerator to be used for further studies.

***- In vitro* studies**

- Effect of different bioagents on the linear growth of pathogenic fungi

Selected fungal biocontrol agents (*T. harzianum* isolates T_1 and T_2 and *T. viride* isolate T_3) and bacteria (*B. subtilis* and *P. fluorescens*) previously isolated and identified were evaluated for their effects against the growth of pathogenic fungi (*A. solani, B. cinerea* and *P. infestans*). Petri dishes 9 cm in diameter each contains 15 ml of Gliotoxin fermentation medium (Brian and Hemming, 1945) were used to determine antagonistic effect between biocontrol agents and pathogenic fungi. Different plates were inoculated by placing agar disc (4mm in diameter) obtained from 7 days old pathogenic fungus at one side of the Petri dish where the opposite side of the plate was inoculated using agar disc (4mm in diameter) obtained from 7 days old fungal bio-agents colony. Bacterial bio-control agent was inoculated by streaking loopful of three days old growth on nutrient glucose agar (NGA) medium (Dawsn, 1957) in opposite side to the disc of pathogenic fungus. Petri dishes were inoculated separately with the pathogenic fungi as control treatment. Five plates were used for each particular treatment. Inoculated plates were then incubated at 18- 25°C and examined periodically. When the mycelial growth in control treatment covers all medium surfaces, reduction percentage of mycelial growth of pathogenic fungi was calculated using the next formula:

$$X = 100 - [G2 / G1] \times 100$$

Where: X= Reduction percentage.

G_1= growth of pathogenic fungi in control plates.

G2= growth of pathogenic fungi in treated plates.

- Greenhouse studies

Evaluation of different tested treatments in controlling infection with the tested potato foliage diseases was carried out under greenhouse conditions. In these studies, plastic pots 30 cm each contain 5 kg light clay sterilizes soil. Two varieties (Valor and Nicola) were examined in summer 2005 and winter 2006 seasons periodically.

- Preparation of bio-control agents treatments

The same aforementioned antagonistic fungi *Trichoderma* spp. (*T. harzianum* T_1, T_2 and *T. viride* T_3) were grown for 10 days at 25°C on liquid gliotoxin fermentation medium (GFM) under complete darkness conditions. All cultures were blended in an electrical blender for 2 minutes. The same preparation concentrations 30 x 10^6 / ml. used *in vitro* experiment was used in this study. Mixture of *Trichoderma* spp. isolates suspension was adjusted to be containing the same number. The tested antagonistic bacteria were grown for 3 days at 25°C on liquid media. *B. subtilis* was grown on NG while, *P. fluorecens* was grown on King's B medium broth. Different antagonists were prepared as suspension and added to liquid to induce bio-agents to act their roles against pathogens at the rate (1: 1) (v: w). Formulated antagonists were used 45 and 65 days after planting. Blight stop was diluted to 1 ml: 100 ml. with distilled water while Plant guard was diluted to 2.5 ml: 100 ml. using distilled water. Mycorrhiza was added at the rate of 0.5 mg / 0.5 g potato tubers to potted soil. The mycorrhizal fungus was kindly obtained from Professor Dr. Safwat El- Hadad, Head of Brown Rot Project of Potato. The effect of adding vesicular arbscular mycorrhizal fungi (VAM) before planting to sterilize soil on tested foliage disease. Inoculation with VAM fungus, (*Glomus macrocarpums*), was carried out just before sowing.

- Preparation of compost tea

Compost was prepared by mixing one kg dry with 10 liters of water in barrel. After two weeks the mixture was turn thoroughly and the suspension of compost tea were filtered through two layers of cheese clothes. Obtained filtrates were then completed to be 10 liters (1: 10) as original filtrate. Treated leaves were then sprayed with potato foliage pathogens after using compost tea treatments. Leaves inoculated with potato foliage pathogenic fungi were used as a control. Chicken manure gets from Al Quallila Company

from El-Sharkia governorates and the analysis examination of compost were determined (Brinton *et al.*, 1996).

- *In vivo* studies

These experiments were conducted at the conditions of El Knaiat valley, El-Sharkia governorate where Valor potato variety was cultivated in summer season (January) 2004 and 2005, while Nicola potato variety was cultivated in winter season (September) 2005and 2006 in Kaffr Ayob valley. Different treatments were distributed in complete randomize plots. Three plots (3 x 3) each contained 4 rows with 48 plants were used for each treatment. The tubers of potato were planted in the deep 15 cm and length 30 cm in light clay soil. Different plant protection materials (bio-agents and compost tea) under test were sprayed 45 and 65 days after planting. Mycorrhizal fungi were applied before planting. The three isolates of *Trichoderma* spp. were grown in liquid GFM under complete darkness conditions for 9 days and mixture of three different isolates were prepared by mixing equal volume (1: 1: 1).All preparations were diluted (1: 100) water (v/v) before plant treatment. Beneficial bacteria were grown on NG liquid media as *B. subtilis* while *P. fluorescens* was grown on King's liquid media for 48 hours. Different biotic compounds as Blight stop was diluted as 1: 100 distilled water while, Plant guard were used as 2.5: 100 distilled water. Compost tea was used as described above. In all field experiments, yield percentage of diseased plants and disease severity were determined after two weeks from treatment Severity percentage was calculated using the formula of Townsend and Heuberger (1943).

$$\% \text{ Severity} = \frac{\text{sum of } n \times v \times 100}{5N}$$

Where, n= number of leaves in each symptoms category.

v= numerical value of each category.

N= total number of leaves in sample.

Different plant disease severities (7) were recorded and a plant disease index was prepared to illustrate the symptoms in each category:

0	0= no spots on the leaflet = no infection.
1	5% = few spots (1-3) on the leaflet = weak infection.
2	10% = (4-10) spots on the leaflet = moderate infection.
3	15% = (11-20) spots on the leaflet = moderate infection.
4	25% = 1/4 infected area on the leaflet = moderate infection.
5	50% = 1/2 infected area on the leaflet = severe infection.
6	75% = 3/4 infected area on the leaflet = severe infection.
7	100% = more of leaflet area infected = very severe infection.

Samples were collected from infected and non-infected VAM were examined in organic and mineral fertilization in field conditions.

Statistical analysis

The means of all treatments were compared by the least significant difference value "L.S.D." at 5% level of probability. The results of the previous experiments were statistically analyzed according to the procedures reported by (Snedecor and Cochran 1980).

RESULTS AND DISCUSSION

- Frequency of bio-agents in different governorates from healthy potato

Data in Table (1) indicated that, *B. subtilis* was frequently isolated from Nicola variety in EL- Sharkia governorate (15%) followed by Spunta variety in El-Qalubia governorate (14%) while, El-Behira governorate was recorded in Diamont variety (15%). *P. fluorescens* was frequently isolated from Nicola variety in El- Sharkia and EL-Behira governorates (14% and 16% ,respectively) followed by Spunta variety in EL-Qalubia governorate (14%). *Trichoderma* spp. was frequently isolated from Nicola variety in El-Sharkia governorate (61.7%) followed by Diamont variety in El-Qalubia governorate (31.7%) while, percentage of isolation from Spunta variety in El-Behira governorate showed only (20%).

Table 1. Frequency of bio-agents in different governorates for different healthy potato varieties.

Isolated bioagents	Varieties	Governorates.				
		El-Sharkia	El-Qalubia	EL-Behira	Means	L.S.D. 0.05
Bacillus subtilis	Diamont	12	10	15	12.33	1.3
	Nicola	15	13	13.3	13.9	3.8
	Spunta	10	14	11.3	11.66	2.39
L.S.D. 0.05		1.3	2.06	4.2	-	-
Pseudomonas fluorescens	Diamont	10.6	12	13	11.86	1.99
	Nicola	14	10	16	13.3	0.07
	Spunta	12	14	14.3	13.43	3.54
L.S.D. 0.05		1.77	1.77	2.39	-	-
Trichoderma spp.	Diamont	35	31.7	10	22.23	8.86
	Nicola	61.7	25	15	28.9	5.97
	Spunta	48.3	15	20	21.1	3.77
L.S.D. 0.05		9.2	7.55	0.03	-	-
Means		24.26	16.88	14.26	-	-

- **Effect of different bio-agents and commercial biocides on mycelial growth of three potato foliage pathogens**

Data in Table (2) indicate that, all bio-agents significantly reduced the mycelial growth of all pathogenic fungi except *P. fluorescens*. Data also clear that Blight stop as commercial biocides was significant reduced the linear growth of all pathogenic fungi followed by *T. harzianum* isolate (T_2). Blight stop reduced the mycelial growth of *A. solani* (88.24%) followed by *T. harzianum* isolate (T_2) (82.35% reduction) while, the least effect was recorded by *B. Subtilis* (23.53% reduction). Blight stop recorded the highest inhibitory effect on *B. cinerea* (94.12% reduction) followed by *T. harzianum* isolates (T_2 + T_1) caused the same reduction (70.59% reduction) also *B. subtilis* recorded the least effect (33.33% reduction). Blight stop and *T. harzianum* isolate (T_2) were recorded the same reduction in the mycelial growth of *P. infestans* followed by *T. viride* isolate (T_3) (66.7% reduction).Plant gaurd and *B. subtilis* recorded the same and least inhibitory effect (58.8%). *P. fluorescens* and compost tea were showed no and slight effect against tested pathogens. Average reduction in growth was significantly higher inhibitory in *P. infestans* (54.17% reduction) than *B. cinerea* (50% reduction) and *A. solani* (47.29% reduction). Biological control using fungi and bacteria occupied an important situation during the percents decades to avoid fungicidal utilization due to their harmful effect. *Trichoderma* spp. was the greater used bioagent against three pathogens of potato foliage diseases. Using *T. harzianum* (T_1 and T_2) and *T. viride* isolate (T_3) increased the reduction of mycelial growth of *A. solani*, *B.cinerea* and *P. infestans* when the commercial bioagents products were used *in vivo* and *in vitro* as Blight stop and Plant guard (*T. harzianum* $30x10^6$ cells/g). They were more effective compared with control. Effect of *Trichoderma* spp. was confirmed as bio-agents fungus from more than 200 years by Salgado *et al.* (1999). The present results showed also that *Trichoderma* spp. *T. harzianum* (T_{1+2}) and *T. viride* penetrate mycelium of *A. solani*, *B. cinerea* and *P. infestans* and grow within it causing malformation and complete destruction for the parasitized mycelium. *B. subtilis* also, caused destruction of three pathogens mycelial growth. These finding are disagreement with Ferreira *et al.* (1991) reported that, *B. subtilis* are antagonistic to plant pathogenic fungi and could produce at least 66 different antibiotic compounds. Several mechanisms could be suggested to interpret antagonistic potentiality of the tested antagonists for example, ability to produce lytic agents and enzymes, volatile compounds and phytotoxic substances.

Table 2. Effect of different bio-agents and commercial biocides on the mycelial growth reduction percentage of the three potato foliage pathogens.

Pathogens			*Alternaria solani*	*Botrytis cinerea*	*Phytophthora infestans*	Control	Means
Bioagents	*Bacillus subtilis*		23.53	33.33	58.8	0	28.92
	Pseudomonas fluosrcens		0	0	0	0	0
	Trichoderma harzianum	T1	58.8	70.59	60.77	0	47.54
		T2	82.35	70.59	94.12	0	61.76
	Trichoderma viride	T3	67.65	66.7	66.7	0	50.26
Commercial biocides	Plant guard		57.8	64.7	58.82	0	45.33
	Blight stop		88.24	94.12	94.12	0	69.12
Means			54.85	57.14	61.9	0	--
L.S.D. 0.05			0.65	0.97	0.95	--	--

- Effect of biocontrol agents on the disease severity caused by three pathogens on two potato varieties under greenhouse conditions

Data in Table (3) reveal that, spraying two potato varieties (Valor and Nicola) with bio-agents and commercial biocides as well as compost tea led to significant reduction in the disease severity. Reduction in disease severity was correlated with increasing yield. Regarding early blight *T. viride* isolate (T_3) showed the most reduction in disease severity on Valor variety (58.87%). On the other hand, mixture of *Trichoderma* isolates appeared as highly effectively agent on Nicola variety (48.43%). *B. subtilis* showed the highest effect and occupied first rank when used against gray mould disease on Valor variety (71.19%) followed by *P. fluorescens* recorded the effect (70.63%). On the contrary, mycorrhizal treatment reduced the disease severity on Nicola variety (57.03%) while, Plant guard recorded the least effect (27.23%). Compost tea recorded the most effective treatment in controlling late blight on Valor

variety (58.56%reduction in disease severity) while, the least effect was recorded by *P. fluorescens* (30.23%).On Nicola variety recorded the most effect by using blight stop (47.41%) whereas, the least effect was compost tea (17.33%). Data also indicate that, all potato foliage diseases are destructive for susceptible potato varieties and percentage of disease incidence in all treatments reach 100%.

Table 3. Effect of bio-control agents on the disease severity caused by three pathogens on two potato varieties under greenhouse conditions.

Bioagents	Reduction percentage of in disease severity					
	Valor (2004)			Nicola (2005)		
	Alternaria solani	*Botrytis cinerea*	*Phytophthora infestans*	*Alternaria solani*	*Botrytis cinerea*	*Phytophthora infestans*
Bacillus subtilis	21.83	71.19	36.37	28.03	41.4	40.87
Pseudomonas fluosrcens	24.69	70.63	30.23	30.72	33.29	28.56
Trichoderma harzianum T_1	48.42	58.43	52.06	20.03	38.33	37.43
T. harzianum T_2	51.42	53.64	41.73	25.13	33.29	43.66
T. viride T_3	58.87	53.52	40.95	25.18	30.86	32.24
Trichoderma mixed (T_1+T_2+T_3)	46.73	52.87	53.13	48.43	52.5	45.16
Blight stop	50.87	60.16	49.67	35.53	47.92	47.41
Plant guard	21.83	34.32	41.33	22.06	27.23	29.32
Mycorrhiza	36.03	51.61	39.58	44.33	57.03	44.48
Compost tea	31.97	39.03	58.56	26.74	35.93	17.33
Control	0	0	0	0	0	0
Means	35.66	44.72	40.32	27.85	36.15	33.31
L.S.D. 0.05	6.85	7.18	5.66	10.13	6.58	8.19

- **Effect of bio-control agents on disease incidence, disease severity and yield in organic and mineral fertilizations on potato Valor variety**

Data in Table (4) show that, spraying bio-control agents on potato plants led to significant reduction either in disease incidence and reduction of disease severity and increased yield. On potato Valor variety in season 2004 *B.subtilis* and *P. fluorescens* increased the percentage of reduction of disease incidence of *A. solani* on plants treated with organic and mineral fertilization at the same percentage (91.62%). *B.subtilis, P. fluorescens*, mixture of different isolates of *Trichoderma* spp. (T_1+T_2+T_3) and Blight stop increased the reduction of disease incidence in season (2005) in organic fertilization (77.76% reduction) while, mycorrhizal treatment was the most effect in mineral fertilization treatment (77.76% reduction).The reduction disease incidence *B. cinerea* in season (2004) was recorded (100%) in plants cultivated in field treated with organic fertilization after treating by *B. subtilis*, *P. fluorescens*, mycorrhiza while compost tea and by *B. subtilis* in field treated with mineral fertilization (88.96%) .Data recorded in season 2005 on plants cultivated either in organic or mineral fertilization fields by using *T. harzianum* (T_2) and mixture of different isolates of *Trichoderma* spp. (T_1+T_2+T_3) (100%provided reduction).The reduction of disease incidence on *P. infestans* was increased by using *B. subtilis* and *P. fluorescens* on plants cultivated in organic fertilization fields (80.53%) in season (2004) while, *P.fluorescens* was the most effect one in mineral fertilization (83.33%).

Table 4. Effect of biocontrol agents on the disease incidence, disease severity and yield against three pathogens on Volar variety in organic & mineral fertilization.

Bioagents	Fertilization	Reduction% of diseases plants						Reduction %of disease severity						Yield (kg/plot)	
		Alternaria solani		*Botrytis cinerea*		*Phytophthora infestans*		*Alternaria solani*		*Botrytis cinerea*		*Phytophthora infestans*			
		2004	2005	2004	2005	2004	2005	2004	2005	2004	2005	2004	2005	2004	2005
Bacillus subtilis	Organic	91.62	77.76	100	86.76	80.53	78.33	72.3	68.6	100	72.26	58.24	62.94	59.8	54.6
	Mineral	91.62	58.81	88.96	78.33	83.33	46.63	65.81	59.16	52.54	60.86	48.66	61.24	53	50.2
Pseudomonas fluorescens	Organic	91.62	77.76	100	80.06	80.53	63.33	53.62	69.86	100	75.37	55.99	77.92	48	59.2
	Mineral	75.03	62.18	55.51	78.33	72.26	60.01	38.42	59.3	59.86	67.05	30.71	72.59	44.13	56.16
Trichoderma harzianum (T_1)	Organic	43.45	66.64	72.26	80.06	72.26	33.93	54.92	69.53	44.23	71.19	65.47	71.06	90.2	54.9
	Mineral	31.31	49.9	66.72	78.33	69.49	26.86	50.22	68.91	65.48	81.06	26.62	51.79	60.8	42
T.harzianum (T_2)	Organic	72.26	66.66	61.11	100	43.46	49.92	65.53	68.96	63.42	78.72	52.93	74.81	64.8	70.5
	Mineral	40.65	56.63	66.72	86.76	63.91	53.36	48.15	81.33	64.85	70.82	48.66	57.26	61.8	70.53
T. viride (T_3)	Organic	61.11	72.21	63.91	80.06	46.22	63.33	36.03	72.49	56.03	52.16	35.37	61.96	35.9	57.2
	Mineral	49.99	53.26	55.51	6337	72.26	40.02	52.42	76.75	33.56	56.06	29.73	69.73	39.15	64.5
Trichoderma mixed (T_1+T_2+T_3)	Organic	72.26	77.76	83.33	86.76	72.26	71.68	69.41	75.92	68.56	68.13	69.83	63.25	71.5	100
	Mineral	66.63	58.81	66.72	100	66.63	33.43	58.56	71.86	67.93	69.36	61.76	61.86	65.03	93.9
Blight stop	Organic	72.26	77.76	83.33	86.76	72.26	33.93	58.63	66.83	64.76	66.13	40.46	62.95	66.46	74.96
	Mineral	58.36	39.97	55.51	71.68	75.03	66.63	54.73	57.73	55.43	61.52	66.23	64.96	55.36	71.9
Plant guard	Organic	35.08	16.64	44.46	20.06	44.42	20.66	59.56	58	35.38	36.66	68.64	41.99	44.15	39.73
	Mineral	31.31	54.43	44.46	27.32	31.31	20.32	39.73	55.56	41.37	39.83	24.46	47.14	40.36	37.04
Mycorrhiza	Organic	52.76	66.64	100	80.06	44.42	63.33	61.53	71.43	100	75.53	56.03	67.37	108.13	100.18
	Mineral	66.65	68.83	55.51	93.33	75.03	40.06	59.53	72.93	64.96	70.93	49.82	71.54	94.29	100.96
Compost tea	Organic	44.42	66.64	100	86.76	43.46	56.62	56.11	65.91	100	73.22	44.82	36.97	72.2	49.23
	Mineral	40.65	58.81	33.28	56.62	36.86	46.63	26.66	50.03	45.3	61.93	63.3	71.84	61.08	47.34
Control	Organic	0	0	0	0	0	0	0	0	0	0	0	0	32.2	39.21
	Mineral	0	0	0	0	0	0	0	0	0	0	0	0	31.59	37.1
Means	Organic	57.86	60.59	73.45	80.66	54.56	48.61	53.46	62.6	66.58	60.86	49.76	56.46	63.83	63.61
	Mineral	50.2	51.06	53.56	66.75	58.76	39.45	44.93	59.36	50.12	58.16	40.95	57.25	55.14	61.86
L.S.D.0.05	Organic	13.71	6.38	9.96	6.4	14.03	10.73		5.39		7.15		7.71	3.29	4.81
	Mineral	12.13	6.92	11.21	6.98	11.02	7.84	6.35	6.61	7.72	7.86	8.5	6.12	4.01	3.92

In season 2005 *B. subtilis* was the most effect bio-agent that increased the reduction of disease incidence in plants cultivated in organic fertilization (78.33%) while, Blight stop was the most bio-control agents, increased the reduction of disease incidence in mineral fertilization (66.33%).

Reduction in *A. solani* disease severity during season (2004) on plants cultivated in organic and mineral fertilization was increased by *B. subtilis* (72.33 and 65.81% respectively). In season (2005) on plants cultivated in organic fertilization fields treated with mixture of different isolates of *Trichoderma* spp. (T_1+T_2+T_3) revealed the most effect control treatment on *A. solani* (75.92%) while, *T. harzianum* isolate T_2 increased the reduction of disease severity in mineral fertilization (81.33%). The reduction of disease severity in season (2004) on plants cultivated in organic fertilization was (100%). After using *B. subtilis*, *P. fluorescens*, mycorrhiza treatment and compost tea extract while, using mixture of different isolates of *Trichoderma* spp. (T_1+T_2+T_3) was the most treatment that reduced disease severity in plants cultivated in mineral fertilization (67.93%). In season 2005 *T. harzianum* isolate T_2 was the most bio-agents increased the reduction of disease severity in potato plants cultivated in organic fertilization (78.72%) while, *T. harzianum* (T_1) was the most effect in mineral fertilization (81.06%). The reduction of disease severity of *P. infestans* was increased in season 2004 in plants cultivated in organic fertilization fields by using mixture of different isolates of *Trichoderma* spp. (T_1+T_2+T_3) (69.83%) while, Blight stop was the most effect on plants cultivated in mineral fertilization (66.23%). In season 2005, *P. fluorescens* increased the reduction of disease severity on plants cultivated in organic fertilization fields (77.92 and 72.59%). Mycorrhizal treatment was the most biocontrol agents increased the yield on Valor variety which cultivated in organic and mineral fertilization in 2004 and 2005 seasons. (108.13, 94.29 kg / pot respectively) and (100.18, 100.96 kg / pot. respectively) Plant gaurd was the least effect one on two tested seasons. Field studies indicated that, improved phosphorus uptake, engendered by mycorrhizal fungi, increased tolerance to pathogens. There are also indicated that, mycorrhizal fungi may inhibit pathogen infection by structurally and/or physiologically transforming plant roots Brendan *et al.* (1996).

- Effect of biocontrol agents on the disease incidence, disease severity and yield in organic and mineral fertilizations on Nicola variety

Data in Table (5) show 100 % reduction of *B. cinenea* and *P. infestans* disease incidence at seasons 2005 and 2006 while, the % of reduction of *A. solani* disease

incidence was increased at season 2005 on plants cultivated in organic and mineral fertilizations field by using *T. harzianum* isolate (T_1) (69.48% and 61.16% respectively). Using mixture of different isolates of *Trichoderma* spp. ($T_1+T_2+T_3$) at 2006 increased reduction of the disease incidence for the cultivated in organic fertilization (48.16%) while, using compost tea was the most effective in mineral fertilization (45.32%). Data also showed that, the reduction of disease severity was increased in mineral fertilization than organic one. The reduction of *A. solani* disease severity was recorded at seasons 2005 and 2006 in organic and mineral fertilizations by using mixture of different isolates of *Trichoderma* spp. (58.06%, 60.81% and 30.66%, 67.41% respectively). The yield content increased in Nicola variety at season 2005 cultivated in organic fertilization by using *T. harzianum* isolate (T_2) (87.3 Kg / plot) while, using *P. fluorescens* increased the yield in plants cultivated in mineral fertilization (68.4 kg / plot). Mycorrhizal treatment increased the yield in plants cultivated in organic fertilization (72.5 kg/plot) while, *P. fluorescens* was the most effect one in plants cultivated in mineral fertilization (56.6 kg / plot) at season 2006.

Table 5. Effect of biocontrol agents on disease incidence, disease severity and yield on Nicola variety in organic & mineral fertilizations under field conditions.

Bioagents	Fertilization	Reduction% of diseases plants		Reduction% of disease severity		Yield (kg/plot)	
		Alternaria solani					
		2005	2006	2005	2006	2005	2006
Bacillus subtilis	Organic	44.26	21.23	28.59	22.46	61.8	64.8
	Mineral	28.79	16.16	27.41	31.42	54.95	47.6
Pseudomonas. fluorescens	Organic	48.63	25.98	37.63	27.21	70.9	66.37
	Mineral	36.52	24.11	35.81	38.23	68.4	56.6
Trichoderma harzianu (T1)	Organic	69.48	41.11	50.26	30.33	55.8	54.7
	Mineral	61.16	33.03	42.62	42.11	57.86	49.86
T. harzianum (T2)	Organic	13.41	21.6	42.73	27.16	87.3	50.85
	Mineral	18.33	31.66	27.96	31.16	67.2	46.86
T. viride (T3)	Organic	44.26	43.11	49.32	30.5	57.8	65.9
	Mineral	21.76	44.03	43.86	48.56	51.06	51.5
Trichoderma mixed (T1+T2+T3)	Organic	48.53	48.16	58.06	30.66	70.8	61.4
	Mineral	24.07	45.15	60.81	67.41	71.01	50.7
Blight stop	Organic	8.62	27.06	50.52	14.63	66.3	57.13
	Mineral	17.16	23.46	22.33	30.46	61.5	55.9
Plant guard	Organic	11.71	34.86	52.93	26.75	43.9	42.65
	Mineral	23.51	34.32	30.42	36.86	40.1	38.9
Mycorrhiza	Organic	20.86	34.91	57.52	31.41	62.3	72.5
	Mineral	14.93	36.8	38.23	47.66	66.7	59.96
Compost tea	Organic	9.76	30.01	23.77	25.12	63.3	47.4
	Mineral	38.56	45.32	32.52	57.11	57.9	38.5
Control	Organic	0	0	0	0	37.94	36.16
	Mineral	0	0	0	0	37.1	33.36
Means	Organic	52.81	44.56	35.66	36.06	68.8	56.35
	Mineral	46.02	46.46	37.6	45.29	57.8	48.16
L.S.D. 0.05	Organic	7.54	9.89	7.54	9.89	6.1	2.87
	Mineral	7.67	6.86	7.67	6.86	3.7	3.19

Plant gaurd was the least effect one in organic and mineral fertilizations on two investigated growing Seasons. Some of these antagonists when sprayed on plant surface, prior real infection led to stimulate plant resistant and enforce treated plants to produce some to this bacterium growing very fast and occupies the court of infection and consumes all available nutrients. These actions prevent pathogens spores from reach to the susceptible tissues. *P. fluorescens* act control, either single or metabolites which depress the pathogens (Bolar *et al.*, 2000). The effect of biocides might be due to sensitivity of *A. solani, B. cinerea* and *P. infestans* to an antibiotic gliotoxin and fengymycin (fengycin) produced by *Trichoderma* spp. Therefore, it was thought that, the use of biological control, either single or combined in an integrated control program will be more success in controlling the diseases of potato. *B. subtilis* showed considerable effect in controlling potato foliage diseases. This might be due to combined in an integrated control program will be more success in controlling the diseases of potato and this bacterium grow very fast and occupies the court of infection and consumes all available nutrients. These actions prevent pathogens spores from reach to the susceptible tissues while, *P. fluorescens* act through preventing other pathogens from utilizing some essential nutrient elements i.e. iron, under starvation condition the pathogen can not proceed and cause disease symptoms (Farahat, 1998).

Mixture of *Trichderma* spp. isolates also showed good effect in controlling these diseases. The effect of mixture can be explained on the light out by Abd-El-Moity, (1985), he stated that, this synergistic effect might be due to complementary effect between different isolates included in the mixture. This means that, one isolate produces antifungal substance whereas, the second prooleices author antifungal substances (Bolar *et al.*, 2000). Mychorrhiza fungi form symbiotic association roots of potato plants able to increase plant growth and development indirectly, the enhanced availability of minerals as well as directly through the production of phytohormones. Data indicated that, improved phosphorous uptake, engendered by mycorrhiza fungi, increased tolerance to pathogens. They also indicated that, mycorrhiza fungi might inhibit pathogen infection isolate had high potentialities in mycoparasitism (Abd-El-Moity, 1981) while, the third one induce plant resistant. The combination between these different effects resulted in high effect in controlling these diseases. Data obtained showed that, compost tea was more effective against three potato pathogens. This might due to that manure extracts act through different mechanisms include nutrient and growth regulator substances in the extract by structurally and/ or physiologically transforming plant roots (Brendan *et al.*, 1996).

REFERENCES

1. Abd-El-Moity, T. H. 1981. Further studies on the biological control of white rot disease of onion. Ph.D. Thesis, Fac. Agric., Minufiya Univ., pp. 135.
2. Abd-El-Moity, T. H. 1985. Effect of single and mixture of *Trichoderma harzianum* isolates on controlling three different soil borne pathogens. Egypt. J. Microbiol., Special Issue, 111- 120.
3. Akhtar, M. S. and Z. A. Siddiqui. 2007. Biocontrol of late and early blights by *Glomus intraradics.* J. Phytopathol., 153(9):544-550.
4. Babu, S., K. Seetharaman, R. Nandakumar and I. Johnson. 2000. Effect of selected plant extracts/oils against tomato leaf blight. Internat. J. Tropical Agric., Hisar, India, 18(2):153-157. (c.f. CAB Abstracts 2000/08-2001/07).
5. Barnett, H. J. and B. B. Hunter. 1972. Illustrated Genera of Imperfect Fungi. Burgess Publishing, Minneapolis, USA, 241pp.
6. Bergey, N. 1975. Manual of Determinative Bacteriology. Williams and Wilkins Co., Baltimore.
7. Bolar, J. P., J. L. Norelli, K.W. Wong, C.K. Hayes, Q.E. Harman and H.S. Aldwinckle. 2000. Expression of endo chitinase from *Trichoderma harzianum* in transgenic apple increases resistance to apple scao and reduces vigor. Phytopathology, 90: 72-77.
8. Brame, C. and J. Flood. 1983. Antagonism of *Aureobasidum pullutans* towards *Alternaria solani.* Trans. Br. Mycol. Soc., 81:621-624.
9. Brendan, A., G. Niemira, R. Safir and C. Martha . 1996. Arbuscular mycorrhizal colonization and border cell production: A possible correlation. Am. Phytopathol. Soc. No. P. 0328-010. (c.f. Rev. Pl. Pathol. 1996. Vol. 86 No. 6.)
10. Brian, P. W. and H. G. Hemming. 1945. Gliotoxin a fungistatic metabolic product of *Trichoderma viride.* Ann. Appl. Biol., 32:214-220.
11. Brinton, W.F., A. Trankner and M. Droffner . 1996. Investigations into liquid compost extracts. Biocycle, 37(11):68-70. (c.f. CAB Abstracts 1996/06-1998/07).
12. Ellis, M.B. and I. A. S. Gibson. 1975. *Alternaria solanii:* CMI description of pathogenic fungi and bacteria No. 475. (c.f. J.W. Pscheidt and W.R. Stevenson, 1986, Early blight of potato and tomato. *Alternaria* Review, College of Agriculture, Univ. of Wisconsin, Madison, 17pp).
13. Erwin, D. C. and O. K. Riberio. 1996. Phytophthora Diseases World Wide. APS Press, the American Phytopathological Society, 562 pp.

14. Erwin, D. C., S. Bartniki-Garica and P. H. Tsao . 1983. Phytophthora, its Biology, Taxonomy, Ecology and Pathology. APS Perss, the American Phytopathological Society, 392 pp.

15. Dawsn, W. J. 1957. Plant diseases due to Bacteria, Second Ed., Cambridge the University of Press, London, pp.231.

16. Farahat, A. 1998. Biological control of some potato bacterial diseases. Ph.D. Thesis, Fac. Agric., Minufyia Univ., pp. 118.

17. Ferreira, J. H. S., F. N. Matthee and A. C. Thomas. 1991. Biologacal control of *Eupta lota* on grapevine by antagonistic strain of *Bacillus subtilis*. Phytopathology, 81: 283-287.

18. Gilman, J. C. 1957. Key to the species of the genus Botrytis. In: A Manual of Soil Fungi, 2nd ed. Iowa State College Press, pp. 299.

19. Groves, J. W. 1946. Variation in *Botrytis cinerea*. (Abst). Proc. Canada. Phytopathol. Soc., 14:18. (c.f. Rev. Appl. Mycoly 26: 406, 1997).

20. Gulati, M. K., E. Koch and W. Zeller. 1998. Isolation of antifungal metabolites produced by florscent Pseudomonas, antagonist of red core disease of strawberry. Modern Fungicides and Antifungal Compounds. 12th International Rein (Hards Brumm Symposium, May 1998).

21. Ingram, D. S. and P. H. Williams . 1991. Advance in Plant Pathology, Vol. 7. *P. infestans*, the cause of late blight of potato.Academic Press, London, San Diego, New York, Sydney, Tokyo, Tronto pp. 273.

22. Ribeiro, O. K. 1978. A source book of the genus Phytophthora. J. Cramer, FL-9490Vduz.

23. Salama, A. M., I. M. K. Ismail, M. L. A. Ali and S. A. Olif. 1990. Identification of three phenolic compounds from *Eusalyptus rostrata* leaves. Egypt J. Physiol. Sci., 14:(1-2):75-88.

24. Salgado, C. H., A. P. Arias, M. F. Flores, E. S. Sotoand and R. B. Gonez. 1999. Antagonistic activity of *Trichoderma* sp. isolated from a soil of the Crenma province, Cuba, against *Alternaria solani Alternaria solani* [Spanish] Revista de la Faculated de Agronomia, Universidad del Zulia, 16(2):167-173.

25. Snedecor, G. W. and W. G. Cochran. 1980. Statistical Methods. Oxford and J. PJ. Publishing Comp, 7th edition.

26. Townsend, G. R. and J. W. Heuberger. 1943. Methods for estimating losses caused by diseases in fungicide experiments. Dis. Rept., 27:340-343.

المقاومة الحيوية لبعض أمراض المجموع الخضرى فى البطاطس

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تم دراسة المقاومة الحيوية لأمراض المجموع الخضرى فى البطاطس وهى مرض الندوة المتأخرة الذى يسببه الفطر فيتوفثورا إنفستانس ومرض الندوة المبكرة الذى يسببه الفطر ألترناريا سولاني والعفن الرمادى الذى يسببه الفطر بوتريتس سيناريا ، وذلك باستخدام البكتريا سيدوموناس فلوريسنس وباسيلس سنتلس وأنواع من فطر التريكودرما (تريكودرما هارزيانم ، تريكودرما فردى وخليط من عزلات نفس الفطر) ، وكذلك بعض المركبات التجارية مثل البليت ستوب وبلانت جارد وكذلك الميكورهيزاو راشح الكمبوست وذلك تحت ظروف المعمل والصوبة والحقل على صنفى فالور ونيكولا ولوحظ أن البكتريا سيدوموناس لم تعطى أى تأثير فى المعمل وكذلك راشح الكمبوست بينما كان لهما تأثير فى الحقل والصوبة وأدت جميع عوامل المقاومة الحيوية لإختزال شدة المرض للفطريات الممرضة الثلاث خلال المواسم الزراعية المختلفة.