Studies on some characteristics of *Phytophthora infestans* of potato and their biocontrol

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ABSTRACT

The pathogenicity and virulence of fifteen isolates of *Phytophthora infestans,* the potato late blight agent, were tested on thirteen of the recommended potato cultivars. Rosetta, Oleva, Bolesta, Ladyflorina, Diamont and Mendaly proved to be the most susceptible among all the tested cultivars. Mating type studies proved that 36.64% of the tested isolates belonged to A_1 type, whereas A_2 type was the most common mating type (63.54%). A new wheat agar medium was tested for the first time for growth and sporulation of *P. infestans* and positive results were obtained. Significant inhibition of fungal growth of *P. infestans* was observed when cultural filterates of *Trichoderma harzianum* and *T. viride* were applied at concentration of 0.5 ml/100 ml medium. Moreover, in vitro experiments showed similar inhibitory effect when *Bacillus subtiulis* was used. Successful disease control in the field was recorded when cultural filterates of *T. harzianum* and *T. viride* were applied. Resistant Cara potato cultivar was found to contain more leaf wax content than the susceptible cultivar Rosetta. Subtantial relationship between the degree of infection and leaf wax content in potato cultivars was established.

INTRODUCTION

Different studies indicated that potato cultivars differ in their response to infection by *Phytophthora infestans* (Inglis *et al.*, 1996; Koppel, 1996; and Peters *et al.*, 1999). Ghycoski *et al.* (1996) tested the incidence and distribution of A_1 and A_2 mating types of *P. infestans* in Canada. They noted that A_2 mating type grows significantly faster than A_1, whereas in Wisconsin, USA, the A_2 isolates increased from 20% in 1993 to 74% in 1994 and to 89% in 1995. Also, Lebreton *et al.* (1998) showed that despite the appearance of the A2 mating type in Brittany in 1996 and in northern France since 1995, both populations were highly homogeneous. No evidence for sexual recombination was obtained.

Best growth rates of all *P. infestans* isolates were observed on Rye A medium (El-Korany, 1994; Hartman and Huang, 1995; and El-Korany and Amer, 1998). On the other hand, V8 juice Agar medium has proved to be the most popular and successful medium for growth and sporulation of most *Phytophthora* species (Ribeiro, 1978).

The high cost of fungicides and the difficulties in obtaining resistant cultivars make biological control a more interesting alternative for suppression of some phytopathogenic fungi (Pudy, 1979; Whipps and Budge, 1990).

Several species of *Trichoderma* have been found to be very potent biocontrol agents of several soil borne plant pathogenic fungi including *Phytophthora* species (Chat, 1987, Papavizas, 1985 and Smith *et al.*, 1990). *Trichoderma harzianum* has been shown to be effective in controlling *sclerotia* – forming plant pathogenic fungi (Hader *et al*, 1979 and Jacop Inbar *et al.*, 1996). Also some other species of *Trichoderma* were found to be an effective biological control agent for protecting a number of vegetables crops (El-Farnawany, 1996; El-Farnawany and Shama, 1996 and Diap *et al.*, 1990).

Trichoderma spp. has also been used as antagonistic microorganisms against wilt pathogenic fungi (Aboul Naser and El-Farnawany, 1995 and Abd-El-Khair *et al.*, 2004). Hyphal interaction between the microparasite *Trichoderma harzianum* and the soil borne plant pathogenic fungus *Sclerotium* were investigated in dual culture and scanning electron microscopy (Jacob *et al.*, 1969).

Mycoparaitism of some species of *Phytophthora* by *Trichoderma harzianum* was also recorded (Finlay and McCarackem, 1991; Bell *et al.*, 1982 and Hall, 1993). Roiger and Jeffers (1991) demonstrated the potential of *Trichoderma* to control *Phytophthora* disease of woody hosts. Also, Chembers *et al.* (1995) reported that *T. harzianum* and *T. pseudokononggii* appeared to inhibit *P. cinnamomi* by mycoparasitism with evidence of para growth and coiling, and *Trichoderma* spp. grew over *P. cinnamomi* in vitro, preventing further growth of this pathogen, and also reported that antibiotics produced by filtrates of young *T. harzianum* cultures inhibited growth of *P. cinnamomi* and *P. citricola*.

The developing cuticle might act as a physical barrier that inhibits fungus. Wax content play an important role in disease development in

cotton bolls of some cultivars (El-Farnawany, 1984) and in some resistant cultivars of olive fruits (El-Samra *et al.*, 1997).

The present work aims to study: (i) the response of different potato cultivars to infection with the tested isolates of *P. infestans*, the potato late blight agent, (ii) the mating type of the tested *P. infestans* isolates, (iii) the effect of different nutritional media on growth and sporulation of *P. infestans*, (iv) the effect of some biocontrol agents on *P. infestans*, (v) the relationship between potato leaf wax content and disease development.

MATERIALS AND METHODS

Isolation of *Phytophthora infestans*

Samples of potato plants showing symptoms of late blight were collected from different locations in El-Behera and Alexandria governorates. Infected tissues were washed in water, then air dried and surface disinfected with 95% ethyl alcohol. Isolation was done from a single lesion on an infected potato leaf or stem, or from a single infected potato tuber. The fungus was isolated from the diseased leaves by direct transfer of sporangia to nutrient agar according to Malcolmison (1979) as follows:

1. Diseased leaves with actively progressing Lesions were incubated at 18° C in a moist chamber overnight to encourage sporulation.
2. Next day sporangia were transferred to plates of Rye A agar by using the point of a wedge of agar medium on the tip of a scalpel to collect few sporangia. Care was taken to avoid contact with septic surfaces of the plant tissue. A colony was visible within three or four days.

T. harzianum and *T. viride* isolates used in this study were kindly obtained from institute of plant pathology, Giza, Egypt.

Characteristics of the *P. infestans* isolates

A. Determination of mating type

Hyphal inocula from isolates of unknown mating type were plated on plates of Rye A agar approximately 2 x 0.7 x 0.3 cm apart with a known A_1 mating type tester isolate (Ca 65) and onto another with a known A_2 mating type tester isolate (E 13 a) (Save *et al.*, 1968; Skidmore *et al.*, 1984). Pairing was examined after 10 days for presence of oospores.

B. Growth of different media

Six *Phytophthora infestans* isolates were randomly chosen to test their growth and sporulation on different media. Four media of Egyptian materials i.e.: Egyptian Rye agar, Lima Bean agar, Wheat agar and Barley agar, were tested in comparison with Rye A agar. The medium most highly recommended for growth and sporulation of *Phytophthora* spp. (Ribeiro, 1978), tested media were prepared as follow :

1. Rye A agar: according to Caten and Jinks (1968).
2. Egyptian Rye agar: according to Caten and Jinks (1968).
3. Lima bean agar: according to Thurston (1957).
4. Wheat agar medium: was prepared according to Caten and Jinks (1968).
5. Barely agar medium: was prepared according to Caten and Jinks (1968).
6. V8 Juice agar: was prepared according to Diener (1955).

Biological control experiments

T. harzianum and *T. viride* grown in flasks containing 50 ml of Potato Dextrose medium (PD) medium were used. Two weeks after inoculation the liquid surrounding the fungal growth was separated from three flasks of each fungus, combined together and filtrated using Seitz filter.

A. Effect of culture filter (toxin) of *Trichoderma* spp. On growth of *P. infestans*

The amended plates were prepared by adding 0.1, 0.2, 0.3 and 0.5 ml of cultural filtrate to 100 ml autoclaved and molten Rye A agar before pouring the plates. Fungal plugs of 5 mm in diameter were taken from the margins of seven days-old Rye A agar culture of the tested *P. infestans* isolates and transferred to the center of the amended plates. Three plates (replicates) for each *P. infestans* isolates were carried out. The inoculated plates were incubated in the dark at 18° C. On the seventh day, the diameter of the radial growth of the three replicates of each isolates was measured and the mean radial growth rate was calculated. The percentage of growth inhibition (%) was estimated relative to the growth of the control (unamended media). Isolates were designated according to Shattock (1988) as follows:

Sensitive, where radial growth was 10% or less of the control; intermediate, where growth was 10% ≤ 60% of the control; and fully resistant, where growth was 60% of the control or more.

B. Effect of cultural filtrate (toxin) of *Trichoderma* on sensitivity of *P. infestans*

The sensitivity of *P. infestans* to the cultural filtrate (toxin) of *Trichoderma* tested was assessed as follow:

- Potato fully expanded mature leaves (leaves No. 5-8 from the stem base) were taken from 6-8 weeks old plants of Oliva and Rosetta cultivars.
- Leaflet were soaked for 5 min. in the filtrate separately.
- Three leaflet (replicates) were taken from each culture.

C. Effect of *Bacillus subtilis* on growth of *P. infestans*

Suspension of dry *Bacillus subtilis* spores at the rate of 0.001 g/10 ml sterile distilled water was prepared by shaking well for 5 min. Suspension of the tested antagonist *B. subtilis* was streaked on the nutrient agar medium and left grow for 8 days.

The biological control test was carried out in vitro. The tested antagonist (*B. subtilis*) was streaked on the Rye A agar medium on the periphery of the Petri-dish at one side of the plate, a 5 mm disc from a 7 days-old culture of one of the tested *P. infestans* isolates was placed near the periphery of the Petri-dish directly opposite the bacterium (distances between the fungal disc and the antagonist were made standard 70 mm). Culture was incubated at 18° C without illumination for 10 days. The inhibition zone was measured. Three replicates were used for each isolate.

Determination of leaf wax content

In order to study the important role of wax content on potato late blight development, the wax surface content (mg/cm^2) of potato leaves Arenada, Cara, Diamond and Rosetta cultivars were determined according to the method described by Wang and Oinckard (1971 & 1973).

RESULTS AND DISCUSSION

Susceptibility of some potato cultivars to infection by *P. infestans* isolates

Data presented in Table (1) indicated that fungal isolates greatly differed in their pathogenic capabilities. All tested isolates were pathogenic but varied greatly in their virulence. Isolate No. 2 was considered to be the most virulent isolate, since all tested potato cultivars were highly susceptible. On the other hand, isolate No. 120 was the least virulent on the tested potato cultivars. Rosetta, Oleva, Bolesta, Mendally, Diamond and

Lady cv. were highly susceptible to most of the tested isolates. While Salyana, Cara, Spunta, Avondale and Atlas were the least susceptible cultivars, whereas Arida and Nicola were considered intermediate in their susceptibility. In this respect, many investigators showed that potato cultivars differed in their susceptibility to *P. infestans.* Kadish *et al.* (1990) found that Cara was relatively more resistant, while Nicola was the most susceptible to *P. infestans* infection. Similar results were found by Inglis *et al.* (1996) who reported that the cultivars Norship, Hilite, Rosetta Norkotah, Goldrush and Shepody were more susceptibile to *P. infestans* than Rosetta Burbank cv. Also similar results were obtained by Rowe (1993).

Characteristics of *P. infestans* isolates

The obtained isolates of *P. infestans* were tested for mating type and growth on the tested media.

(A) Mating type

All the obtained isolates were tested for mating type using A_1 and A_2 testers. Formation of oospores with one tester indicating that the isolate belongs to the opposite mating type. Data obtained clearly showed that means for mating types were 63.54% for A_2 and 36.40% for A_1 mating types, respectively. Both A_1 and A_2 mating types isolates existed in Mexico, in equal frequencies (i.e., 50% for each mating type) while in some other parts of the world, i.e., Russia, Wisconsin, and North Carolina in the USA, the A_2 mating type occurred in a percentage exceeding 50 % (Gorborova*et al.*, 1989; Marshall-Farrar *et al.*, 1998 and Fraser *et al.*, 1999). Similar results were also obtained by Shaat (1995).

Table (1): Reaction of different potato cultivars to some *P. infestans* isolates, 6 days after inoculation.

Tested cultivar	Diameter of radial growth (cm)**															Mean
	P. infestans isolates															
	107	20	121	106	205	19	103	112	120	117	2	206	5	12	24	
Arinada	1.74*	1.87	1.61	0.66	0.93	1.87	1.48	1.55	0.26	1.47	2.00	1.33	2.00	1.87	1.06	1.45^{c}
Atlas	0.79	1.61	0.93	1.06	1.20	1.35	1.20	1.55	0.65	1.33	1.20	1.35	1.61	1.61	0.93	1.23^{d}
Avondale	1.55	1.87	2.00	0.79	1.21	1.74	1.34	0.52	0.52	1.48	1.74	1.07	1.48	1.74	0.79	1.32^{d}
Bolesta	1.74	2.00	2.00	1.48	1.07	2.00	1.34	1.34	1.47	1.74	2.00	1.34	1.61	2.00	1.20	1.62^{b}
Cara	1.55	0.93	1.07	0.52	1.07	1.48	0.93	1.90	0.65	0.93	1.61	0.52	1.48	1.61	0.93	121^{a}
Diamont	1.87	1.60	2.00	1.47	1.35	1.61	1.20	1.61	1.07	1.35	2.00	1.87	1.47	2.00	1.61	1.61^{b}
Lady florina	1.61	2.00	1.74	1.20	1.07	1.74	1.47	1.47	0.80	1.74	2.00	1.87	1.87	1.87	1.60	1.60^{b}
Mendaly	1.48	1.74	1.48	0.93	1.74	1.87	1.87	1.61	1.48	1.21	2.00	1.87	1.87	2.00	1.21	1.62^{b}
Nicola	2.00	2.00	1.87	0.66	1.34	2.00	1.06	0.79	0.80	1.34	1.87	0.94	1.74	1.60	1.21	1.42^{cd}
Oleva	1.87	2.00	1.74	1.74	1.48	1.87	1.87	1.48	0.94	1.87	2.00	1.21	2.00	2.00	1.48	1.70^{ab}
Rossetta	1.74	2.00	1.74	1.48	1.61	2.00	1.74	1.87	1.74	1.33	2.00	1.74	2.00	1.87	1.74	1.77^{a}
Salyna	1.07	1.47	1.21	0.79	0.94	1.87	1.21	0.52	0.93	0.94	1.87	0.66	1.87	1.87	0.81	1.20^{d}
Spunta	1.34	2.00	1.87	0.93	1.34	1.74	1.20	0.66	0.52	1.34	2.00	0.93	1.47	1.61	0.79	1.32^{d}

L.S.D$_{0.05}$ = 0.13

* Average of 5 replicates

** A disease index was applied where :

0 = no symptoms of late blight, 1 = 35% of late blight symptoms,

2= 70% of late blight symptoms,3 = 100% of late blight symptoms

(B) Effect of the tested media on growth rate of *P. infestans* isolates

Considerable differences in radial growth of the different *P. infestans* isolates were detected between the tested media. Wheat agar, V8 juice, and Lima Bean agar medium significantly increased the growth of *P. infestans*. Table (2) showed that the means for radial growth rate were 5.74, 4.14, 3.07, 2.17 and 1.64 for Wheat agar, V8 juice, Lima Bean agar, Barley agar and Egyptian Rye agar, respectively. Wheat agar medium was considered the best for *P. infestans* growth, followed by V8 juice. On the other hand, Egyptian Rye agar medium was considered the least suitable medium for growing the fungus.

Probably, one of the reasons that hundered researches of *Phytophthora* spp. in different countries including Egypt was the lack of culture media made with available ingredients (El-Korany, 1994 and Hartman & Huang, 1995). Effect of different media on the growth rate of *P. infestans* was thoroughly studied. *P. infestans* grows poorly on most media except Rye A agar (Ribeiro, 1978; Shatt, 1995; Hartman & Huang, 1995 and El-Korany & Amer, 1998).

Consequently, efforts were made in this study to find out a substitute media for growth and sporulation of *P. infestans* using grains which are easily cultivated in Egypt. Different materials (i.e., wheat, barley, Egyptian Rye, lima bean) with different preparation methods were tested and compared to the Rye A media.

(C) Effect of the tested media on sporulation

Table (3) clearly showed that V8 juice, wheat agar and lima bean agar media were considered to be superior to all the other tested media in stimulating and enhancing sporulation of *P. infestans*. However, Egyptian Rye agar and barley agar media induced the least fungal sporulation. The yield of sporangia averaged 15.6, 8.2 and 8.8 x 10^5 sporangia/plate for V8 juice, lima bean agar and wheat agar, respectively, while Egyptian Rye agar and barley agar had 2.2 and 1.2 x10^5 sporangia/plate, respectively.

Surprisingly, wheat grains (grown in Egypt and prepared typically as Rye A agar) was a quite good medium that was not different from Rye A agar for growth and sporulation of *P. infestans*. According to the available literature, this could be considered the first record for a medium with local ingredients suitable for growth and sporulation of *P. infestans* in Egypt.

Table (2): Radial growth of *P. infestans* isolates on five different growing media.

Isolates / Medium	*P. infestans* isolates						Mean
	Type A_1			Type A_2			
	20	24	19	113	200	206	
V8 juice	4.73*	5.09	4.80	3.01	3.73	3.48	4.14^{b}
Lima bean	1.49	2.82	2.54	5.06	3.01	3.47	3.07^{c}
Wheat	6.77	5.61	6.01	6.22	4.72	5.12	5.74^{a}
Egyptian Rye	2.56	1.24	2.36	1.28	1.12	1.27	1.64^{c}
Barley	2.52	2.01	1.45	2.28	2.16	2.61	2.17^{d}
Mean	3.61^{a}	3.35^{b}	2.43^{a}	3.57^{a}	2.95^{c}	3.19^{b}	

Mean followed by the same letter are not significant

$L.S.D_{0.05}$ between media = 0.18 $L.S.D_{0.05}$ between isolates = 0.19

$L.S.D_{0.05}$ between media x isolates interaction = 0.43

* Average of three replicates

Table (3): Sporulation of P. infestans isolates on five different growing media.

Isolates / Medium	*P. infestans* isolates						Mean
	Type A_1			Type A_2			
	24	20	19	113	200	206	
V8 juice	*$14.8x10^5$	$38.9x10^5$	$22.5x10^5$	$3.7x10^5$	$8.9x10^5$	$5.1x10^5$	$15.6x10^5$
Lima bean	$2.1x10^5$	$5.1x10^5$	$3.9x10^5$	$20x10^5$	$1.1x10^5$	$16.9x10^5$	$8.2x10^5$
Wheat	$13.3x10^5$	$15.4x10^5$	$14x10^5$	$3.1x10^5$	$3.3x10^5$	$3.9x10^5$	$8.8x10^5$
Egyptian Rye	$1.6x10^5$	$1.1x10^5$	$6.9x10^5$	$0.6x10^5$	$1.1x10^5$	$1.8x10^5$	$2.2x10^5$
Barley	$1.5x10^5$	$1.7x10^5$	$0.1x10^5$	$0.2x10^5$	$1.7x10^5$	$2.2x10^5$	$1.2x10^5$
Mean	$6.7x10^5$	$12.4x10^5$	$9.5x10^5$	$5.5x10^5$	$3.2x10^5$	$5.9x10^5$	

Mean followed by the same letter are not significant

L.S.D0.05 between media = 0.57 L.S.D0.05 between isolates = 0.63

L.S.D0.05 between media x isolates interaction = 1.40

* Average of three replicates

Biological control experiments

(A) Effect of *Trichoderma* cultural filtrate on sensitivity of *P. infestans*

In order to study the effect of *Trichoderma* spp. cultural filtrate on pathogenicity of the tested isolates of *P. infestans*, cultural filtrates of two and three weeks old *T. harzianum* and *T. viride* cultures were tested on three potato cultivars leaves inoculated with *P. infestans*. Data in Table (4) and (5) clearly showed that the degree of infection (DI%) decreased in treated leaves compared with the untreated. A significant increase in the inhibitory effect of the filtrate was always associated with 3 weeks old. Besides, treatments with *Trichoderma* cultural filterate gave good control of late blight disease on two tested potato cultivars, Oleva and Rossetta.

Degrees of infection of both potato cultivars inoculated with *P. infestans* tested isolates and treated with *Trichoderma* cultural filterates from 3-weeks cultures were less than those of the 2-weeks cultivars.

Generally, the treatment with *Trichoderma* cultural filterates decreased the degree of infection of *P. infestans* on Oleva and Rossetta potato cultivar compared with untreated control. This was in good agreement with the findings of Smith *et al.* (1990), they reported that pots and field experiments are necessary to evaluate the potential of these isolates for controlling *Phytophthora* root rot of chestnut. Chamers and Scott (1995) found that colony growth of *P. cinnamomi* and *P. citricola* was strongly or completely inhibited in the culture filtrate experiments, whereas hyphal coiling was rare and no appresoria were observed in dual cultural with *P. cinnamomi*.

(B) Effect of *Trichoderma* cultural filterates on growth and development of *P. infestans* in vitro

The effect of cultural filtrates of *T. harzianum* and *T. viride* on linear growth of *P. infestans* isolates No. 19, 20, 118 and 200 was studied on artificial media. Filtrate extraction was done after growing the fungus on a liquid medium for two or three weeks. Four concentrations of the filtrate 0.1, 0.2, 0.3 and 0.5 ml/100 ml medium were used throughout this study.

Table (4): Effect of *T. harzianum* on late blight disease caused by some isolates of *P. infestans* on Rosetta and Oleva cultivars.

Isolate No.	** Degree of Infection (DI%)					
	Potato cultivars					
	Oleva			Rossetta		
	Filtrate age					
	2 Weeks	3 Weeks	Control	2 Weeks	3 Weeks	Control
19	*26.9	23.5	45.8	25.1	21.5	46.2
20	28.4	21.4	44.1	29.9	24.03	45.0
118	32.9	20.1	41.2	33.3	25.3	43.5
200	22	14.7	40.3	31.7	24.7	41.1

L.S.D $_{0.05}$ For culture filtrate age of Oleva cv. = 6.6

L.S.D $_{0.05}$ For culture filtrate age of Rossetta cv. = 4.1

** Calculation according to Horsfall and Heubergers (1942) as follows:

$$\text{Degree of infection \%} = \frac{\text{Sum of individual ratings}}{\text{No. of plants assessed}} \times \frac{100}{5}$$

* Average of three replicates

Table (5): Effect of *T. viride* on late blight disease caused by some isolates of *P. infestans* on Rosetta and Oleva cultivars.

Isolate No.	** Degree of Infection (DI%)					
	Potato cultivars					
	Oleva			Rossetta		
	Filtrate age					
	2 Weeks	3 Weeks	Control	2 Weeks	3 Weeks	Control
19	*33.6	26.9	45.8	22.06	18.5	46.2
20	29.9	23.3	44.1	23.8	19.9	45.0
118	34.4	24.06	41.2	28.03	23.4	43.5
200	24.9	17.9	40.3	20.6	23.3	41.1

L.S.D $_{0.05}$ For culture filtrate age of Oleva cv. = 6.6

L.S.D $_{0.05}$ For culture filtrate age of Rossetta cv. = 4.1

** Calculation according to Horsfall and heubergers (1942) as follows:

$$\text{Degree of infection \%} = \frac{\text{Sum of individual ratings}}{\text{No. of plants assessed}} \times \frac{100}{5}$$

* Average of three replicates

Results listed in Table (6) & (7) and Figure (1) showed that the four different concentrations of cultural filtrates used had different effects on linear growth of *P. infestans*. The concentration of 0.5 ml/100 ml medium was the most effective concentration in inhibiting growth of *P. infestans*.

Table (6) and (7) clearly showed that the cultural filtrate of *T. harzianum* and *T. viride* varied in their effect on linear growth of the tested isolates of *P. infestans*.

Table (6): Effect of *T. viride* culture filtrate on the linear growth of certain *P. infestans* isolates after 2 and 3 weeks (mm/day).

Conc.	2 Weeks					3 Weeks				
Isolates	*0.1 ml	0.2	0.3	0.5	Control	0.1	0.2	0.3	0.5	Control
19	1.8	1.4	1.3	1.05	5.9	1.1	1	0.95	0.7	5.9
20	2.3	2.05	1.9	1.3	6.8	2	1.75	1.6	1	6.8
118	1.5	1	0.9	0.75	5.3	0.85	0.8	0.65	0.6	5.3
200	1.6	1.4	1.2	0.9	5.5	1.15	1	0.85	0.65	5.5

$L.S.D_{0.05}$ for filtrate age = 0.05

- Average of three replicates

* Filtrate concentration (ml/100 ml medium).

Table (7): Effect of *T. harzianum* culture filtrate on the linear growth of certain *P. infestans* isolates after 2 and 3 weeks (mm/day).

Conc.	2 Weeks					3 Weeks				
Isolates	*0.1 ml	0.2	0.3	0.5	Control	0.1	0.2	0.3	0.5	Control
19	1.5	1.3	1.15	0.9	5.9	1.2	1	0.9	0.6	5.9
20	2.1	1.9	1.75	1.2	6.8	1.9	1.6	1.5	1.1	6.8
118	1.2	0.95	0.74	0.6	5.3	0.7	0.65	0.6	0.5	5.3
200	1.4	1.2	1.15	0.8	5.5	0.9	0.7	0.62	0.5	5.5

$L.S.D_{0.05}$ for filtrate age = 0.05

- Average of three replicates

* Filtrate concentration (ml/100 ml medium).

(C) Effect of *Bacillus subtilis* on growth of *P. infestans*

Six isolates of *P. infestans* were tested to determine their responses to the antagonistic effect of *B. subtilis*. All tested isolates of *P. infestans* proved to be sensitive to *Bacillus subtilis*, which inhibited their mycelial growth. Similar results were found by Bochow *et al.* (1991), they found that culture filtrates of Streptomyces isolates, known for their biological control activity, delayed the mycelial growth of *P. infestans* inside potato slices. On the other hand, they found that a *Bacillus subtilis* isolate had no effect despite its biological control.

Determination of leaf wax content of potato cultivars in relation to disease development

The results presented in this study clearly indicated that wax content (mg/cm_2) of potato cultivars leaves plays an important role in late blight development. Table (8) and Figure (1) clearly indicated that the surface wax content of potato leaves varied according to cultivars and the stage of leaves maturity. The differences were significant based on the mean weight of wax content percent per surface area. The highest values of surface wax content was found in mature leaves (4.33×10^{-4} mg/cm^2) of Cara cultivar and the lowest value was recorded with intermediate leaves cultivar (0.267×10^{-4} mg/cm^2) of Rossetta. Results listed in Table (8) showed that the differences in wax content between Arenada, Cara and Diamont cultivars were insignificant and the differences in wax content between the first two cultivars and the 4th one were insignificant. Also data obtained clearly showed that Cara, the less susceptible cultivar, has more wax content than Rossetta, the more susceptible cultivar, on which *P. infestans* grew faster. This indicates that there is a positive relationship between the wax content and resistance. Similar results were obtained by El-Samra *et al.* (1997), they found that the wax content of olive fruits surface was high in Otaygen cultivar and the cultivar was thus, less susceptible to all tested fungal pathogens.

Marshall and Rush (1980) reported that the cultivars of rice resistant to *Rhizoctonia solani* disease had abundant wax deposits and cultivars intermediate in resistance produced varying amounts of wax deposits and susceptible cultivars produced no wax deposits. In respect a reversible relationship was found between the radial growth rate of *P. infestans* and leaves wax content of potato cultivars (Fig. 1). The cuticule thickness and chemical composition together are partially responsible for this observed resistance to fungal penetration (Wang and Pinckard, 1973). In Egypt similar results of cotton boll resistance against some cotton boll rot fungi were also found (El-Farnawany, 1984).

Table (8): Wax content (mg/cm^2) of leaves of different potato cultivars at different leaf ages.

Cultivars	Wax content (mg/cm^2) Leaf age				Radial growth on potato leaves
	Juvenile	Intermediate	Mature	Mean	
1. Arenada	*0.887x10^{-4}	1.12x10^{-4}	1.43x10^{-4}	1.15x10^{-4}	1.45 c
2. Cara	0.907x10^{-4}	1.16x10^{-4}	4.33x10^{-4}	2.13x10^{-4}	1.21 b
3. Diamont	0.747x10^{-4}	0.443x10^{-4}	0.547x10^{-4}	0.579x10^{-4}	1.61 a
4. Rossetta	0.553x10^{-4}	0.2673x10^{-4}	0.657x10^{-4}	0.492x10^{-4}	1.77 a
Mean	0.774 x 10^{-4} b	0.748 x 10^{-4} b	1.74 x 10^{-4} b		
L.S.D $_{0.05}$	For cultivars = 0.309 x 10^{-4}				0.185
L.S.D $_{0.05}$	For maturity leaves = 0.268 x 10^{-4}				

* Average of three replicates

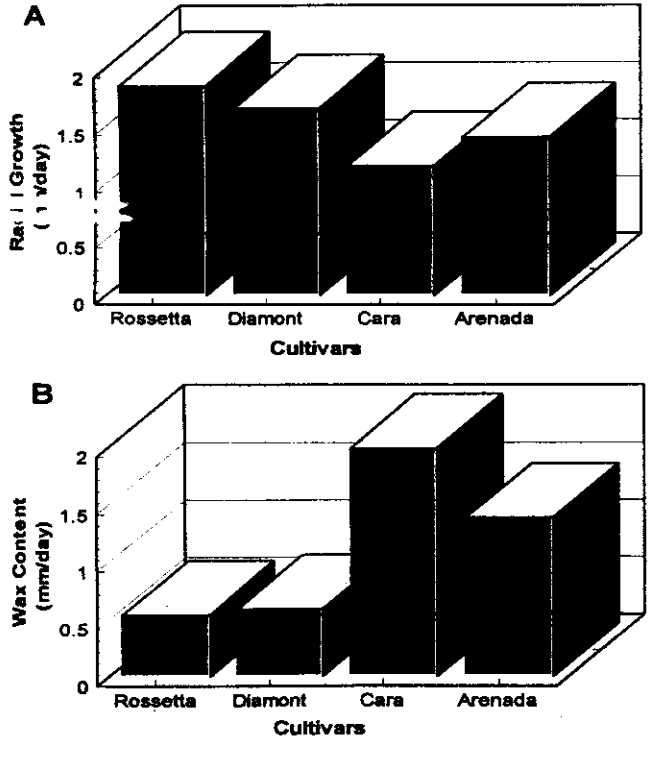

Figure (3): (A) Average radial growth of fifteen isolates of *P. infestans*. (B) Wax content of potato leaves.

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الملخص العربـــــى

دراسات على بعض خصائص فطر فيتوفثورا إنفيستانس على البطاطس ومقاومته الحيوية

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أجريت دراسات القدرة المرضية والشدة المرضية لخمسة عشرة عزلة من الفطـــر فيتوفثـــورا إنفيسانتس المسبب لمرض اللفحة المتأخرة فى البطاطس على ثلاثة عشر صنفاً تجارياً من البطاطس ولقد أثبتت الدراسة أن أصناف روسيتا ، أوليفا ، بوليستا ، ليديفلورينا ، دايمونت و ميندالى من أكثر الأصناف القابلة للإصابة ولقد أثبتت دراسات تحديد الطرز الجنسية أن 36.64 من العزلات المختبرة كانـــت تتبـــع الطراز A_1 ، بينما الطراز A_2 هو أكثر شيوعاً (63.54). ولأول مرة تمت دراسة وإختبار بيئة آجـــار القمح الجديدة على نمو و تجرثم فطر فيتوفثورا إنفيسانتس ، وقد كانت النتائج المتحصل عليها إيجابية.

هذا وقد شوهد التأثير المثبط لنمو الفطر فيتوفثورا إنفستانس وذلك عند تطبيق راشـــح الفطـــر تريكودرا هيرزيانم و تريكوديرما فيردى وكان التأثير إيجابياً وذلك عند تركيز 0.5 مل/100 مل. وقـــد شوهد نفس التأثير المثبط لنمو الفطر وذلك عند إستخدام راشح البكتيريا *Bacillus subtilis* (باســـيلس ساتلس). وقد تم تسجيل المقاومة الناجحة عن المرض فى الحقل وذلـــك عنـــد إســـتخدام راشـــح الفطـــر تريكوديرما هيرزيانم و تريكوديرما فيردى. وقد وضح من الدراسة مقاومة صنف البطاطس كارا وكـــان ذلك يرجع لمحتوى الأوراق العالى من الشمع عن الأصناف القابلة للإصابة، هذا وقد أوضحت الدراســـة العلاقة الوطيدة بين درجة الإصابة فى الأصناف المنزرعة ومحتوى الأوراق من الشمع،