

Effect of bacterial isolates and phosphite compounds on disease incidence of early blight (*Alternaria solani*) and improve productivity of some potato cultivars

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Abstract: Early blight disease caused by *Alternaria solani* is considered a serious challenge for potato production. Four bacterial strains as biocontrol agents (*Brevibacillus brevis*, *Pseudomonas putida*, *Pseudomonas aerogenosa*, *Bacillus subtilis*) and potassium phosphite (kPhi), copper (Cu) as well as difenoconazole (Score) were tested to study their effects against potato early blight disease under greenhouse and field conditions. Also the bacterial strains were tested in vitro to study their antifungal activity on growth of *A. solani*. Both strains significantly reduced the fungal growth. Higher inhibition was obtained with *Bacillus subtilis* while the lowest inhibition was with *Brevibacillus brevis*. Under greenhouse conditions, the highest reduction was achieved with the fungicide (active ingredient difenoconazole) where disease severity reduced by 25% to 37.8% and disease incidence reduced by 36.3% to 42.9% compared to control. *Bacillus subtilis* (Bsu) was the best biological agent with all potato varieties. Under field conditions, the highest reduction was obtained with treatment by fungicide and potassium phosphite followed by *Bacillus subtilis*. Potato growth, yield and its component significantly increased by fungicide and potassium phosphite compared to other treatments. Also, Picasso cv. gave the highest shoot fresh weight and yield followed by Burren compared with other cultivars. Potato plant fresh weight (g), average tuber weight (g) and potato yield (kg/plot) were highly negative correlated with early blight disease incidence percent and disease severity in both seasons.

Keywords: Early blight, biological control, *Alternaria solani*, potassium phosphite, integrated control, foliar spray, potato cultivars.

I. Introduction

Total world potato production is estimated at 364.8 million tonnes in 2012 (FAOSTAT, 2014). In Egypt, potato crop has an important position among all vegetable crops, where about 20% of total area devoted for vegetable production is cultivated with potato and the production of potato were 4.8 million tonnes in 2013 from an area of 178,000 hectares (FAOSTAT, 2014). This crop is economically important to Egypt and any disturbance in its production affects severely its local and export impact. (Kabeil *et al.*, 2008).

Early blight disease caused by *Alternaria solani* is considered a serious challenge for potato growing (Van *et al.*, 2001). In recent years, increase in disease severity on potato foliage has been reported in various potato growing areas (Vloutoglou and Kalogerakis, 2000; Kapsa, 2003; Simões *et al.*, 2003; Waals *et al.*, 2004; Pasche *et al.*, 2005). Primary damage by early blight is attributed to premature defoliation of the potato plants, resulting in tuber yield reduction. The pathogen can also attack potato tubers and produce shallow, dry, corky rot (Folsom and Bonde, 1925; O'Brien and Rich, 1976). Also, early blight caused by *Alternaria solani* is a major foliar disease attacking potato plants in most potato growing regions of the world. (Waals *et al.*, 2003; El-Gamal *et al.*, 2007). So, using fungicidal chemical treatments to control potatoes early blight disease mainly had been using in most regions of the world (Kapsa, 2004; Pasche *et al.*, 2005; Mantecon, 2007).

The development of fungal resistance and problems of environmental pollution have accompanied excessive reliance on fungicides. These problems can be avoided or minimized by using the natural material products such as medicinal plant extracts (Chaudhary *et al.*, 2003; Abd-El-Khair and Haggag, 2007), alkali metal salts (Abd-El-Kareem, 2007; Labato *et al.*, 2008), beneficial microorganisms (Wickramaarachchi, 2005; Terry *et al.*, 2007) and cultivars resistance (Demir and Levent, 2002; Rodriguez *et al.*, 2006; Kapsa, 2004).

Biological control agents who include effective bacterial species have been interesting as alternatives to chemical agents (Fravel, 2005). Many species of *Bacillus* are known to suppress several pathogens belonging to the genera (Cook and Baker, 1983; McKnight, 1993; Fiddaman and Rossall, 1994). On tomato plants, Srinivasa *et al.*, (2013) studied the effect of *Pseudomonas* isolates antagonistic activity against *Alternaria solani*, to determine their capacity to inhibit fungal infection. And they found that, out of twelve *Pseudomonas* isolates, five isolates viz., S4B7P (27.74), S1B8P (27.33), S2B10P (25.47), S3B3PF (23.07) and S1B1P (19.69) which showed higher mean inhibition percentage on all the isolates of *Alternaria solani*. Also

Wickramaarachchi (2005) studied the effect of twenty-six exotic (including *Pseudomonas fluorescens*, *Azospirillum sp.*, *P. aureofasciens* [*P. chlororaphis*], *Pseudomonas sp.* and *Actinomyces* [*Actinomycetales*]) and native rhizobacterial isolates for antagonistic activity against *A. solani*, and he found that, all isolates were antagonistic to *Alternaria solani*.

In recent years, many scientists interested to study the relationship between the role of fertilizers either as plant stimulator to resist or suppressor of the plant pathogens. The use of foliar fertilizers as SIR agents in conjunction with the use of resistant varieties, improves overall control of certain diseases (**Keuveni and Reuveni, 1997**). Although P fertilizer improves plant health in the majority of cases (**Perrenoud, 1990**). In many cases, economic control requires periodic applications of fungicides. However, optimal fertilization can be an important and often underappreciated component of an integrated program for disease management.

Potassium or sodium bicarbonate and Nerol showed great inhibitory effect in linear growth of *Alternaria solani*. Complete inhibition was obtained with potassium or sodium bicarbonate at 2% and Nerol at 0.50 %.(**Abd-El-Kareem, 2007**). Phosphites (Phi) are alkali metal salts of phosphorous acid, has ability to protect potato plants against different pathogens such as *Phytophthora infestans*, *Fusarium solani* and *Rhizoctonia solani* (**Labato, et al., 2008**). Also, **Keuveni and Reuveni (1998)** reported that phosphates and potassium salts effectively suppressed and controlled some diseases on various crops and they mentioned to that foliar applied phosphite appears to be effective as a nutritional supplement and a systemic induce resistant stimulant over a broad range of conditions.

It is cleared that, the relationship between early blight disease intensity and yield loss was highly significant (**Shtienberg et al., 1994**). Some potato cultivars have resistance, while other cultivars are susceptible (**Kuczynska, 1992**). So the objective of this research was to evaluate the effects of some bacterial strains as biological control agents in vitro inhibition of *Altumaria solani* in laboratory assays and determine the effect of foliar spray of some chemical, metal salts of potassium phosphite and Cupper as mineral components with most effective bacterial isolates on early blight disease development and suppression, yield and its components of some potato cultivars under field conditions.

II. Materials And Methods

Bacterial strains tested as a biocontrol agents:

Tested Strains of bacteria were obtained from Microbiology Unit, Soil Fertilization and Microbiology Dept. Desert Research Center, Egypt. These isolates were certified as highly effective biological control agents against other pathogens and as biofertilizers as mentioned in previous publication (**Omar and Ahmed, 2014**).

Isolation and identification of *Alternaria solani*:

To inoculums preparation for leaf bioassay, *Alternaria solani* isolates were obtained from infected potato leaves from different growing locations (Ismailia Governorate, North Sinai governorate and Qalyubia governorate), five isolates of *A. solani* were evaluated and used in this study (Table 1). The pathogens were cultured on potato dextrose agar (PDA) in 9 cm diameter petri dishes for 10 days of incubation, the culture covered the entire plate. The isolates were subcultured on PDA 4 days prior to use, plugs of *A. solani* were formed using sterile cork borer around the perimeter of actively growing culture just before inoculation according to (**Meenakshi et al., 2014**).

Table 1: Origin and year of isolation of *Alternaria solani* isolates

Code of isolate	Location	Year of isolation
As1	Al Qantara Shark	2014
As2	Al Qantara Shark	2014
As3	Baloza North Sinai	2015
As4	Baloza North Sinai	2015
As5	Qalyubia Governorate	2015

Evaluate of control agents effect on linear growth of *A. solani* in vitro:

Four bacterial strains as biocontrol agents (*Brevibacillus brevis*, *Pseudomonas putida*, *Pseudomonas aerogenosa*, *Bacillus subtilis*) were tested to study their inhibitory effects on growth of *A. solani* in vitro using dual culture technique (**Coskuntuna and Ozer, 2008; Alabouvette et al., 1993**). Each of bacterial biocontrol agents was streaked in center of sterile Petri dish on potato dextrose agar (PDA). One disc (0.5 cm in diameter) of 7 days – old culture of each pathogenic fungus was separately placed on each side of the same petri dish at 10 mm distance. Petri dishes containing of 15 ml PDA medium inoculated with fungal cultures and free of bacteria were used separately as control. All plates were incubated at 28 ±2°C until the growth of each pathogenic fungus in the control treatment reached to the edge of Petri dish. The inhibition growth rate was calculated by following equation: Inhibition growth (%) = (R – r) / r X 100 Where (R) is the diameter of mycelial growth fungi on the control cultures and (r) is the growth diameter of fungal mycelia on the plate treated with bacterial isolates.

Preparation of fungal suspension:

The fungus *A. solani* was grown on PDA medium at 25±2°C until an abundant heavy growth of conidia was evident. Conidia were harvested by scraping the surface of the colonies with a spatula and transferred to sterilized distilled water and filtered through nylon mesh to prepare the suspension of pathogen.

Preparation of Biocontrol agents:

Bacterial strains were grown on nutrient broth medium and incubated for 48h at room temperature in a rotary shaker (100 round/min). The bacterial culture was centrifuged (10000 rpm for 10 min) and the supernatant was discarded. The cell pellet was suspended in sterile 0.85% NaCl and centrifuged again under the same conditions. The supernatant was discarded and washed bacterial cells were re-suspended in sterile distilled water. The cell concentrations was adjusted to 10⁸CFU/ml and used for field and greenhouse experiments.

Chemical compounds:

- Potafore “a commercial compound containing 42% potassium and 38 % phosphorus as potassium phosphites (kPhi)” and sprayed by 2 ml/1L water.
- Oxy plus “a copper compound contain copper oxychloride by 47.89%” and sprayed by 2.5 gm/1L water.
- Score “a fungicide contained difenoconazole and used by 0.5 ml / 1L water.

Greenhouse experiment:

The experiment was carried out in the greenhouse of plant protection department, Desert Research Center, Egypt. Potato tubers cvs. Burren, Diamond, Picasso and Spunta were grown in plastic pots (50 cm diameter) containing sandy loam soil at 22–25°C and RH 75–80%. Five plants/pot and three pots for each treatment were used. Plant inoculation was carried out with 30 ml of fungus suspension when plants had 4–5 leaves. After inoculation, pots kept covered under plastic sheet for two days and treated with 90 ml from bacterial strain with concentration of bacterial cells (10⁸CFU/ml.) for each isolate. while the concentration of other compounds were (2 ml/L, 2.5 gm/L and 0.5 ml/L) for Potafore, Oxy plus and Score respectively. Inoculated potato plants treated with water were used as control. Disease incidence and severity were rated based on percentage of damaged potato leaf area and affected number of plants.

Field Experiments (Experimental site, cultivars and cultivation)

Field experiment was conducted under conditions of Baloza experimental station, desert research center, North Sinai governorate, Egypt, during summer growing seasons of 2014 and 2015 to evaluate the efficacy of bacterial strains of *Brevibacillus brevis* (Bbr), *Pseudomonas putida* (Ppu), *Pseudomonas aerogenosa* (Pae), *Bacillus subtilis* (Bsu) as biological control agents and Potassium phosphite (kPhi), Copper (Cu) as well as difenoconazole (Score) on incidence and severity of early blight disease on four potato commercial cultivars (Burren, Diamond, Picasso and Spunta) under natural conditions. All treatments were used as a foliar spray 30 days after emergence (4–5 true leaf stage), four times with 15 days interval, the concentration of treatments were (10⁸ CFU/ml ; 2 ml/L, 2.5 gm/L and 0.5 ml / L) for bacterial isolates, Potafore, Oxy plus and Score respectively, the same amount of water was sprayed to the control treatment to avoid interferences with different moisture levels. This experiment was conducted in split plot design with three replicates where potato cultivars assessed in main plot while foliar spray treatments were assessed in sub plot. Exported potato seed tubers of four cultivars planted at 15th January in rows 1 m width, 30 cm apart under drip irrigation system and the plot area was 10.5 m². In both seasons, all cultural practices (irrigation, fertilization, weeding, and pest control) were performed according to the recommendations of the Egyptian Ministry of Agriculture and Land Reclamation.

Plant traits and disease assessments:

Vegetative growth:

Random samples of five plants of each experimental plot were taken at 70 days after planting for vegetative growth data. Plant height, leaves number/plant, leaves chlorophyll content and plant fresh weight were recorded.

Yield and its components:

120 days after planting all experimental plots were harvested then tubers were counted, and weighed to record of total yield, tuber number and average tuber weight. At the end of each growing season, the average harvested yield was calculated as kg/plot.

Disease incidence and severity:

These reading were recorded with the appearance of natural disease symptoms on control plants (60 days after planting). Early blight incidence was recorded according to the number of infected plants showed

disease symptoms in relation to the whole number of potato plants. The average of records of the surveyed replicates for each particular treatment was calculated. Disease severity and incidence were estimated as follows; 0 = no leaf lesion; 1= lesions on < 25% of leaf area; 2 = lesion on 26–50% of leaf area; 3 = lesion on 51–75% of leaf area and 4 = lesions on 76 up to 100% of leaf area according to the scale from 0 to 4 suggested by Cohen *et al.*, (1991) then the traits calculated using the following formula:

$$D.S = \frac{\sum (n \times c)}{N}$$

Where; D.S. = intensity of attack, n= number of infected plants per category, c = category number and N = total examined plants.

Statistical analysis:

Data were subjected to statistical analysis by M-STAT C (Russel, 1991). The differences among means were performed using least significant difference (LSD) at 5% level.

III. Results And Discussion

Effect of biocontrol agents on linear growth of *Alternaria solani* in vitro:

Four bacterial strains were used to study their inhibitory effect on growth of *Alternaria solani* in vitro. Results indicated that both treatments reduced the linear growth of *Alternaria solani*. Higher inhibition was obtained with *Bacillus subtilis* in all cases of tested fungal isolates while the lowest inhibition rate with *Brevibacillus brevis*. The most affected pathogenic isolates was As2 followed by As1 in case of *Bacillus subtilis* which reduced the linear growth by 65.67% and 62.5 % respectively. On the other hand, the As1 was most resistant under stress of *Brevibacillus brevis* where recorded 25.5% inhibition rate (Fig 1). These results are in agreement with (Edwards *et al.*, 1994; Fravel, 2005; Sallam and Abo-Elyousr, 2012; Stepanović *et al.*, 2015). As preliminary results of screening for antagonism in petri plates assay, all tested bacterial isolates were selected to evaluate their antifungal effects in further experiments in compared to the effect of fungicide (with active ingredient Difenonazole, potassium phosphite (kphi) and Cupper (cu).

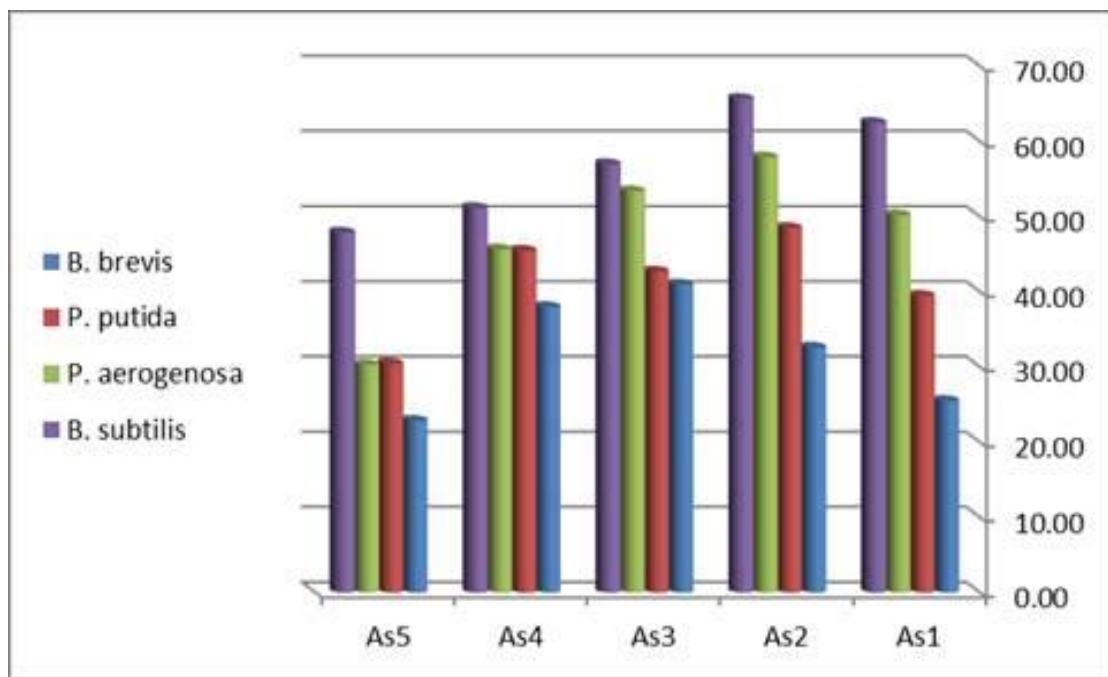


Fig. 1: Linear growth of *Alternaria solani* as affected with bacterial isolates in vitro

Influence of foliar spray treatments on early blight diseases of potato under greenhouse conditions:

To determine the activity of biocontrol agents (*Brevibacillus brevis*, *Pseudomonas putida*, *Pseudomonas aerogenosa*, *Bacillus subtilis*), Potassium phosphite (kphi), Copper (Cu) as well as difenoconazole on early blight disease under greenhouse conditions, the concentrations were applied on potato leaves after inoculation of the pathogens. The results in Tables (2 and 3) showed significant reductions in disease severity and incidence. The most effective treatments were in case of fungicide with all potato cultivars where the percentage of disease severity reduced by 37.8%, 32.5%, 32.5%, 25% with cvs. Burren, Diamond, Picasso and Spunta respectively as a reduction rates between control and treatments followed by *Bacillus subtilis* (Bsu) which gave the best biological agent effect with all potato cultivars except Diamond, where the reduction percentage of severity by Bsu were 27%, 32.5% and 25% in Burren, Picasso and Spunta, respectively compared to control. Also the disease incidence induction rates were compatible with disease severity results where the incidence has been reduced by 36.3%, 38.5%, 42.9% for cvs. Burren, Diamond, Picasso and Spunta respectively, when treated with pesticide. Also (Bsu) gave reduction rates of 42.87%, 30.80% with Picasso and Spunta, respectively. Concerning of biological agents, no significant differences between *Brevibacillus brevis* and *Pseudomonas putida*. The reduction rate was between 17% with Picasso to 7.5% with Diamond and Spunta. As expected, the chemical treatment using fungicide were the best to reduction of disease under greenhouse conditions followed by the bacterial isolates and phosphite compounds, so these results led to use all tested treatments under greenhouse to combat early blight disease and reduce the use of fungicides, these results agreed with (Westermann, 1993; Shtienberg *et al.*, 1996; Christ, 1990; Hofman *et al.*, 2003; William *et al.*, 2007).

Table (2): Evaluation of biological and chemical control agents against *Alternaria solani* under greenhouse conditions.

Treatments	Burren		Diamond		Picasso		Spunta		X ⁻	
	DI	DS	DI	DS	DI	DS	DI	DS	DI	DS
Bbr	66.7	3.3	73.3	3.7	73.3	3.7	73.3	3.7	71.7	3.6
Ppu	66.7	3.3	73.3	3.7	66.7	3.3	73.3	3.7	70.0	3.5
Pae	60.0	3.0	80.0	4.0	73.3	3.7	80.0	4.0	73.3	3.7
Bsu	53.3	2.7	66.7	3.3	53.3	2.7	60.0	3.0	58.3	2.9
(Kphi)	66.7	3.3	53.3	2.7	60.0	3.0	66.7	3.7	61.7	3.2
Cu	60.0	3.0	60.0	3.0	60.0	3.0	66.7	3.3	61.7	3.1
Score	46.7	2.3	53.3	2.7	53.3	2.7	60.0	3.0	53.3	2.7
Control	73.3	3.7	86.7	4.0	93.3	4.0	86.7	4.0	85.0	3.9
X ⁻	61.7	3.1	68.3	3.4	66.7	3.3	70.8	3.5		
LSD at 5%										
Characters	Cultivars				Foliar spray				Interaction	
Dis. Inci.	7.4				8.0				16.0	
Dis. Sev.	0.3				0.4				0.7	

Potato cultivars response towards *Alternaria solani* infection and foliar spray under field conditions:

The efficacy of four bacterial isolates and chemical components on disease incidence and severity under field conditions are shown in Tables (3 and 4). Results revealed that all cultivars have deferent susceptible to early blight disease incidence and severity recorded during two seasons. Burren cultivar exhibit less susceptibility to early blight according to disease incidence and severity compared with other cultivars.

On the other hand all foliar spray treatments gave significant results with Burren cultivar in both seasons. Picasso cultivar seems to be moderate susceptible in both seasons (54.3% and 44.8%) (3.3 and 3.0) for disease incidence and severity in first and second seasons respectively. Cultivars Diamond and Spunta showed more susceptibility to early blight in both seasons. Diamond seems to be more susceptible than Spunta in first season (63.8% and 62.9%) (4 and 3.7) for disease incidence and severity with control treatment. In the same trend, Spunta showed more susceptible for disease incidence in second season than Diamond especially with control where recorded (52.4% and 48.6%) for both cultivars.

Concerning foliar spray, all foliar spray treatments gave significantly decreased disease incidence and disease severity compared with control treatment in both seasons.

Bacterial isolates *Brevibacillus brevis* (Bbr) *Pseudomonas putida* (Ppu), *Pseudomonas aerogenosa* (Pae) and *Bacillus subtilis* (Bsu) gave significant results cambered to control. While fungicide (Score), Potassium phosphite (kphi) and copper (cu) gave the highest significant reduction in disease incidence and severity compared with bacterial isolates and control treatments. (Bsu) was more effective in decreasing the disease incidence and severity than other isolates with all cultivars through two seasons where, recorded (31.7 and 26.7) and (2.7 and 1.8) of disease incidence and severity in first and second seasons, respectively. Treated

potato plants with Potassium phosphite (kphi) and Copper (Cu) gave superior results for disease incidence and severity compared with bacterial isolates and control (Tables 3 and 4). The highly decreasing in disease incidence and severity obtained with Score treatment compared with other treatments. These results are agreement with (Kapsa and Osowski, 2003; Kapsa, 2004; Pasche *et al.*, 2005; Mantecon, 2007).

Table (3): Effect of cultivars and foliar spray treatments and their interactions on potato disease incidence during two successive growing seasons (2014 and 2015).

Treatments	Burren		Diamond		Picasso		Spunta		X ⁻	
	1 st S*	2 nd S**	1 st S	2 nd S	1 st S	2 nd S	1 st S	2 nd S	1 st S	2 nd S
Bbr	35.2	38.1	44.8	40.0	42.9	41.0	46.7	42.9	42.4	40.5
Ppu	34.3	35.2	42.9	40.0	41.0	39.0	43.8	44.8	40.5	39.8
Pae	31.4	32.4	41.0	41.9	42.9	41.9	42.9	41.9	39.5	39.5
Bsu	21.0	21.0	35.2	31.4	32.4	23.8	38.1	30.5	31.7	26.7
(Kphi)	19.0	21.0	32.4	30.5	21.0	21.0	34.3	28.6	26.7	25.2
Cu	21.9	22.9	28.6	26.7	26.7	21.0	29.5	31.4	26.7	25.5
Score	10.5	5.7	20.0	18.1	16.2	11.4	19.0	15.2	16.4	12.6
Control	43.8	41.9	63.8	48.6	54.3	44.8	62.9	52.4	56.2	46.9
X ⁻	27.1	27.3	38.6	34.6	34.6	30.5	39.6	36.0		
LSD at 5%										
	Cultivars				Foliar spray				Interaction	
1 st S	1.8				1.9				3.8	
2 nd S	2.2				3.0				5.9	

*, ** Means the first (1st S) and second (2nd S) growing seasons

Table (4): Effect of cultivars and foliar spray treatments and their interactions on potato disease severity during two successive growing seasons (2014 and 2015).

Treatments	Burren		Diamond		Picasso		Spunta		X ⁻	
	1 st S*	2 nd S**	1 st S	2 nd S	1 st S	2 nd S	1 st S	2 nd S	1 st S	2 nd S
Bbr	2.3	1.7	3.7	1.7	2.7	2.3	3.3	2.7	3.0	2.1
Ppu	2.0	2.0	3.7	2.7	2.3	2.3	2.7	3.0	2.7	2.5
Pae	2.3	2.3	4.0	3.0	3.0	2.7	3.3	2.7	3.2	2.7
Bsu	1.7	1.3	3.3	1.7	2.3	1.7	3.3	2.3	2.7	1.8
(Kphi)	1.7	1.7	3.0	2.3	2.7	1.7	3.7	2.3	2.8	2.0
Cu	1.7	1.3	3.0	2.0	2.3	1.3	3.0	2.0	2.5	1.7
Score	1.3	1.0	2.3	1.7	1.7	1.0	2.3	1.3	1.9	1.3
Control	2.7	2.7	4.0	3.3	3.3	3.0	3.7	3.0	3.4	3.0
X ⁻	2.0	1.8	3.4	2.3	2.5	2.0	3.2	2.4		
LSD at 5%										
	Cultivars				Foliar spray				Interaction	
1 st S	0.6				0.4				0.9	
2 nd S	0.2				0.4				0.9	

*, ** Means the first (1st S) and second (2nd S) growing seasons. Early Blight Disease Severity Recorded according to the Scale 0-4;

Efficacy evaluating of foliar spray application of bacterial biocontrol and chemical control agents on early blight disease under Field condition:

To evaluating the efficacy of treatments on tested potato cultivars vegetative growth, data presented in (Table 5) showed that potato shoot length, shoot fresh weight, leaf number and leaf chlorophyll content significantly affected by potato cultivars and foliar spray treatments in both seasons except the effect of cultivars in the second season. Moreover, interaction effects were not significant except their effect on shoot fresh weight in both seasons. Picasso cv produced the highest shoot length and highest shoot weight in both seasons compared with other cultivars, followed by Diamond for shoot length, Burren and Spunta for shoot fresh weight in both seasons. The lowest shoot length was obtained by cvs Burren and Spunta, while cv Diamond produced the lowest shoot fresh weight in both seasons. Burren and Diamond cultivars gave the highest leaf number compared with cv Spunta in both seasons, while the highest leaf chlorophyll content was obtained by cv Burren compared with other cultivars in both seasons.

Concerning foliar spray effects, data in (Table 5) showed that Score treatment (fungicide) significantly increased shoot length, shoot fresh weight and leaf number compared with other treatments, followed by potassium phosphite (kphi) then (Bsu) bacterial strain. While control treatment produced the lowest values followed by other bacterial strains and Cupper treatments in both seasons. Highest leaf chlorophyll content was obtained with potassium phosphite, Score and Cupper treatments compared with control and bacterial strains treatments in both seasons. Picasso cultivar treated with Score gave the highest shoot fresh weight, while other cultivars with control treatment gave the lowest value in both seasons. Similar results were obtained by (Kuczynska, 1992; Keuveni and Reuvcni, 1997; Keuveni and Reuvcni, 1998).

Table (5) Effect of cultivars, foliar spray treatments and their interactions on potato shoot length, fresh weight, leaves number and leaves chlorophyll content in two seasons.

characters		Shoot length (cm)		Shoot fresh weight (g)		Leaves number		chlorophyll (SPAD)	
Seasons	Cultivars	1 st S	2 nd S	1 st S	2 nd S	1 st S	2 nd S	1 st S	2 nd S
		Burren	58.08	60.38	304.31	266.40	21.35	17.91	46.96
Diamond	64.71	67.29	243.15	243.15	20.98	18.53	43.68	49.75	
Picasso	70.84	73.87	380.62	380.62	20.10	17.40	44.24	50.10	
Spunta	57.90	60.54	301.88	301.88	18.89	16.03	44.91	50.31	
LSD at 0.05	2.08	1.60	20.31	17.22	1.47	0.93	1.68	NS	
Foliar spray									
Bbr	61.24	64.26	291.12	281.56	18.56	15.57	44.17	50.15	
Ppu	60.57	62.37	286.73	279.18	19.08	16.09	43.66	49.56	
Pae	61.48	63.61	292.89	283.32	19.75	16.68	43.47	49.12	
Bsu	61.98	65.42	309.18	303.68	20.55	17.64	44.21	50.69	
(KPhi)	67.31	69.86	360.58	352.59	21.88	19.64	47.49	53.14	
Cu	60.33	62.21	286.08	276.43	20.33	17.34	46.30	50.86	
Score	71.07	75.29	401.65	383.66	23.90	20.41	46.92	52.58	
Control	59.08	61.13	231.70	223.69	18.61	16.37	43.36	48.76	
LSD at 0.05	2.34	2.39	13.57	14.43	1.46	1.23	1.96	1.67	
Interaction									
Burren	Bbr	55.74	58.88	307.02	268.77	20.21	16.89	46.18	52.25
	Ppu	55.09	56.89	302.00	271.80	20.58	17.26	45.80	51.87
	Pae	56.34	58.16	309.27	270.97	20.86	17.54	44.67	50.07
	Bsu	56.65	60.45	305.13	283.17	21.59	17.61	45.95	52.35
	(KPhi)	60.54	63.67	321.73	289.77	22.30	19.65	50.23	55.63
	Cu	59.70	59.50	317.43	278.80	20.58	17.59	48.21	51.28
	Score	65.05	68.18	365.23	293.27	25.61	19.62	48.68	53.42
Diamond	Control	55.50	57.30	206.70	194.67	19.08	17.09	45.97	51.37
	Bbr	65.78	68.58	220.90	220.90	20.29	16.97	43.25	49.32
	Ppu	64.29	66.09	201.83	201.83	19.97	17.31	42.19	48.26
	Pae	63.60	65.40	209.97	209.97	20.12	17.80	43.13	48.53
	Bsu	65.75	69.78	236.60	236.60	21.81	18.49	43.92	50.99
	(KPhi)	68.14	71.61	316.03	316.03	22.74	21.09	45.07	52.13
	Cu	61.35	63.15	214.93	214.93	19.80	17.48	44.23	48.63
Picasso	Score	69.72	72.18	347.00	347.00	22.76	21.44	45.99	53.06
	Control	59.02	61.48	197.97	197.97	20.32	17.67	41.68	47.08
	Bbr	68.95	71.65	351.33	351.33	17.34	14.69	43.55	49.95
	Ppu	68.60	70.40	368.87	368.87	18.93	15.61	43.57	49.63
	Pae	69.92	71.72	361.20	361.20	20.24	16.92	42.26	48.66
	Bsu	69.18	70.98	381.03	381.03	17.98	16.99	43.13	50.19
	(KPhi)	77.27	79.07	422.43	422.43	21.60	19.61	47.30	52.03
Spunta	Cu	64.22	68.36	335.77	335.77	21.29	17.97	46.27	51.67
	Score	81.62	90.08	497.20	497.20	24.22	20.90	45.24	50.64
	Control	66.92	68.72	327.10	327.10	19.19	16.54	42.60	48.00
	Bbr	54.48	57.95	285.23	285.23	16.40	13.75	43.69	49.09
	Ppu	54.31	56.11	274.20	274.20	16.84	14.19	43.07	48.47
	Pae	56.02	59.15	291.13	291.13	17.79	14.47	43.83	49.23
	Bsu	56.33	60.46	313.93	313.93	20.80	17.48	43.84	49.24
Spunta	(KPhi)	63.29	65.09	382.13	382.13	20.87	18.21	47.37	52.77
	Cu	56.02	57.82	276.20	276.20	19.63	16.31	46.48	51.88
	Score	67.91	70.71	397.17	397.17	22.99	19.67	47.79	53.19
	Control	54.87	57.01	195.03	195.03	15.83	14.18	43.21	48.61
LSD at 0.05	NS	NS	27.14	28.87	NS	NS	NS	NS	

After confirming the effectiveness of treatments in reducing the disease incidence and severity of potato late blight, the component of yield were investigated, data presented in (Table 6) showed that potato yield per plot, tuber number, average tuber weight and tuber dry matter percent significantly affected by tested potato cultivars and foliar spray application in both seasons. Only tuber number per plot in the second season was not

significant. Moreover, all interaction effects (Table 7) were not significant except total yield per plot in both seasons and tuber number per plot in the first season. Picasso cultivar gave the highest potato yield, followed by cv Burren compared with cvs Spunta and Diamond which gave the lowest yield in both seasons.

Picasso cultivar produced the highest tuber number per plot followed by Diamond in the first season compared with cv Spunta which produced the lowest tuber number per plot. On the other hand, cvs Spunta, Burren and Picasso in the first season, Spunta and Picasso in the second season produced the highest tuber weight compared with cv Diamond which gave the lowest value in this respect. Regarding dry matter percent, cvs Picasso and Diamond gave the highest percent compared with cvs Spunta and Burren in both seasons.

Concerning of foliar spray treatments, Score treatment significantly increased potato yield, tuber weight and tuber dry matter percent followed by potassium phosphite then (Bsu) bacterial strain compared with other treatments. Picasso cultivar treated with Score gave the highest potato yield compared with cv Diamond treated with Cupper, bacterial strains and control treatments in both seasons.

Table (6) Effect of cultivars and foliar spray treatments on potato yield (kg/plot), tubers number per plot, average tuber weight (g) and dry matter % in both of two growing seasons.

Cultivars	Potato yield (kg/plot)		Tubers number/plot		Average tuber weight (g)		Dry matter %	
	1St S*	2nd S**	1St S	2nd S	1St S	2nd S	1St S	2nd S
Burren	29.88	25.92	285.1	270.5	104.90	96.39	18.82	16.87
Diamond	26.50	22.54	295.4	279.1	89.58	81.50	22.11	19.51
Picasso	34.80	30.92	333.9	288.60	104.35	108.50	22.78	19.34
Spunta	28.09	24.87	261.8	245.4	106.82	101.22	20.72	18.08
LSD at 0.05	3.57	2.75	43.1	NS	2.87	9.94	0.97	0.72
Foliar spray								
Bbr	28.39	24.17	287.76	270.40	98.67	89.83	20.39	17.56
Ppu	28.18	23.63	287.32	270.58	98.53	88.49	20.66	17.87
Pae	28.44	23.89	287.74	269.31	98.94	89.05	20.59	18.07
Bsu	30.01	27.13	298.54	278.95	100.41	97.69	21.34	18.40
(KPhi)	34.78	30.82	323.81	283.23	107.76	109.32	21.94	19.61
Cu	27.68	23.55	278.56	256.89	99.39	92.13	20.94	18.15
Score	36.40	32.60	333.64	287.50	109.69	115.00	22.08	19.58
Control	24.67	22.70	254.93	250.32	97.91	93.27	20.90	18.38
LSD at 0.05	1.25	1.37	19.1	NS	4.00	8.16	0.81	0.54

*, ** Means the first (1St S) and second (2nd S) growing seasons.

Relationship between early blight disease control and yield components of tested potato cultivars:

Based on the obtained results, it may be worth to mention that potato plant fresh weight (g), average tuber weight (g) and potato yield (kg/plot) were highly negative correlated with early blight disease incidence percent in both seasons, fig.(1). A linear correlation coefficients (r) were (-0.599, -0.540), (-0.487, -0.570) and (-0.593, -0.636) for plant fresh weight, average tuber weight and potato yield in the first and second seasons, respectively. The corresponding coefficient of determination (r^2) was (0.359, 0.292), (0.337, 0.327) and (0.352, 0.404), which indicated that (35.9%, 29.2%), (23.7%, 32.7%) and (35.2%, 40.4%) of the variation in plant fresh weight, average tuber weight and potato yield were related to early blight disease incidence percent in the first and second seasons, respectively. On the other hand regression coefficients were (-3.38, -3.43), (-0.31, 0.71) and (-0.25, -0.28) for plant fresh weight (g), average tuber weight (g) and potato yield (kg/plot) in the first and second seasons, respectively. This indicated that when early blight disease incidence increased by one percent, plant fresh weight, average tuber weight and potato yield decreased by (3.38g, 3.43 g), (0.31g, 0.71g) and (0.25 kg, 0.28 kg) in the first and second season, respectively fig (2).

Moreover, the same characters were highly negative controlled with early blight disease severity in both seasons fig (2). Whereas, a linear correlation coefficients (r) were (-0.586, -0.449), (-0.573, -0.457) and (-0.561, -0.523) for plant fresh weight (g), average tuber weight (g) and potato tuber yield (kg/plot) in the first and second seasons, respectively. The corresponding coefficients determination (r^2) were (0.343, 0.201), (0.328, 0.209) and (0.315, 0.273) which indicated that (34.3, 20.1), (32.8, 20.9) and (31.5, 27.3) of the variation in plant fresh weight, average tuber weight and potato yield were related to early blight disease severity in the first and second seasons, respectively.

Table (7): The interactions between cultivars and foliar spray treatments on potato yield (kg/plot), tubers number per plot and average tuber weight (g) of two seasons

Cultivars/ treatments	Characters/ seasons	Potato yield (kg/plot)		Tubers number/plot		Average tuber weight (g)		Dry matter %	
		1 st S*	2 nd S**	1 st S	2 nd S	1 st S	2 nd S	1 st S	2 nd S
Burren	Bbr	29.33	24.78	285.80	274.51	102.34	89.86	18.22	15.58
	Ppu	29.28	24.73	288.56	279.64	102.13	89.83	18.44	16.45
	Pae	29.12	24.57	285.39	274.81	102.54	90.37	18.47	16.55
	Bsu	29.86	27.97	287.76	275.10	103.77	102.05	19.26	17.17
	(KPhi)	32.53	27.98	292.37	271.54	111.37	103.28	19.68	17.95
	Cu	29.49	24.94	285.96	274.12	103.00	90.88	18.42	16.42
	Score	34.78	31.57	309.70	284.00	112.46	111.90	19.52	18.30
	Control	24.69	20.81	244.83	230.43	101.61	92.99	18.53	16.56
Diamond	Bbr	23.50	18.95	274.59	273.38	86.68	72.42	20.33	18.38
	Ppu	22.69	18.14	263.38	257.33	86.73	72.10	21.56	18.99
	Pae	23.10	18.55	265.37	257.11	87.14	72.51	21.30	18.68
	Bsu	24.84	22.96	278.47	268.68	89.37	86.54	21.86	18.57
	(KPhi)	33.00	28.45	343.89	295.73	95.97	96.70	23.46	20.99
	Cu	23.52	19.97	270.67	258.75	87.60	79.17	22.74	19.67
	Score	35.45	30.90	365.35	318.66	97.06	97.17	23.66	20.83
	Control	25.92	22.37	301.23	302.81	86.08	75.34	21.97	19.97
Picasso	Bbr	34.31	31.10	337.14	297.53	101.59	104.66	22.76	18.64
	Ppu	35.42	30.87	351.74	316.45	101.38	99.60	22.27	18.24
	Pae	34.51	29.96	340.18	301.45	101.79	100.45	22.30	19.41
	Bsu	36.06	31.51	350.25	310.28	103.02	101.78	23.52	19.65
	(KPhi)	37.49	33.94	339.30	279.52	110.62	122.05	23.24	20.32
	Cu	32.20	27.65	314.36	270.76	102.25	101.88	22.19	18.77
	Score	37.73	34.85	333.88	270.68	113.38	131.50	23.73	20.92
	Control	30.67	27.45	304.73	262.15	100.73	106.11	22.23	18.79
Spunta	Bbr	26.42	21.87	253.51	236.17	104.06	92.40	20.24	17.63
	Ppu	25.32	20.77	245.61	228.88	103.85	92.41	20.38	17.79
	Pae	27.01	22.46	260.02	243.85	104.26	92.88	20.31	17.65
	Bsu	29.29	26.08	277.70	261.71	105.49	100.39	20.71	18.19
	(KPhi)	36.11	32.90	319.67	286.14	113.09	115.25	21.37	19.16
	Cu	25.52	21.64	243.23	223.92	104.72	96.57	20.41	17.76
	Score	37.62	33.07	325.62	276.64	115.85	119.45	21.40	18.26
	Control	17.40	20.19	168.93	205.87	103.20	98.63	20.89	18.22
LSD at 0.05		2.50	2.73	38.1	NS	NS	NS	NS	NS

The regression coefficients were (-57.29, -50.87), (-6.39, -9.99) and (-4.01, -4.06) for plant fresh weight, average tuber weight and potato yield which indicated that when early blight disease severity increased by one grade on scale, plant fresh weight, average tuber weight and potato tuber yield decreased by (57.29g,50.87g), (6.39g, 9.99g) and (4.01kg, 4.06kg) in the first and second seasons, respectively fig (2). Such decrement of potato yield /plot due mainly to average of tuber weight which in turn, decreased when plant fresh weight especially leaves (assimilative plant organs) were significantly affected by early blight disease incidence and severity. Similar results were found by (Folsom and Bonde, 1925; O'Brien and Rich, 1976; Vloutoglou and Kalogerakis, 2000; Simoes *et al.*, 2003; Waals *et al.*, 2004; Pasche *et al.*, 2005; El-Gamal *et al.*, 2007; Amitava and Devi, 2014) where they reported that primary damage by early blight is attributed to premature defoliation of the potato plants, resulting in tuber yield reduction.

IV. Conclusion

The results of this study showed that the tested biological, chemical and fertilizing factors exhibited different levels of antifungal activities on *A. solani* isolates under laboratory, greenhouse and field conditions. The highest reduction was achieved with the Score fungicide (active ingredient Difenconazole), and *Bacillus subtilis* was the most effective bacterial isolates under vitro and vivo conditions. There is a relation between yield production and rate of disease infection either severity or incidence, and the treatments were effective in reduce of infection rate and increase of potato yield, so we conclude that, using of *Bacillus subtilis* as biocontrol agents and with integrated management program with potassium phosphite or fungicide may be useful in controlling potato early blight disease under field conditions.

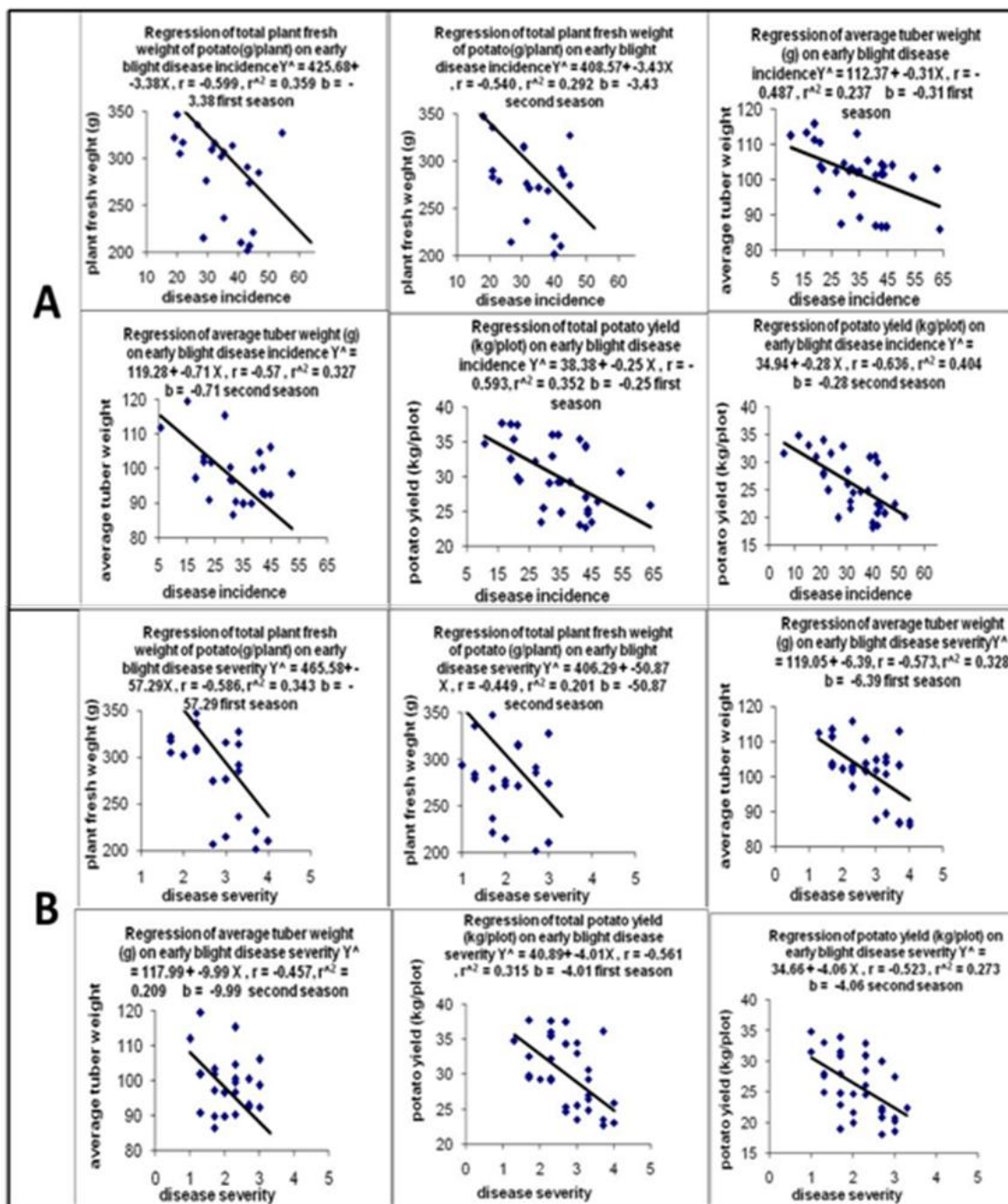


Fig.(2) Regression lines, coefficients of determination (r^2) and regression coefficients (b) for plant fresh weight (g/plant), total yield(kg/plot) and average tuber weight (g) of potato vs. early blight (A) disease incidence % and (B) disease severity for 2014 and 2015 seasons.

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