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Control of Potato Early Blight Disease Using Biotic and a Biotic Agents

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Abstract : The present investigation aimed to evaluate the effect of foliar spray with each of chitosan and humic acid alone, or in combination with soil amendment with compost in controlling potato early blight caused by *Alternaria solani*. Under laboratory conditions, all the tested concentrations of humic acid had no inhibitory effect against the linear growth of *A. solani*. While, chitosan at the concentration of 5.0 g / L caused complete reduction in linear growth. Data also revealed that the compost water extract CWE of plant compost show antagonistic activity at all the tested concentrations (5, 10 and 15%) *via* three methods of pouring plate, well-cut diffusion and dry weight assay. Under field conditions, results indicated that all treatments significantly reduced early blight severity, during the two growing seasons. The highest reduction was obtained with the combined treatments between soil amendment with compost and foliar spray with each of humic acid, chitosan or Redomyl gold plus, which reduced the blight severity more than 75.0, 84.4 and 78.6%, respectively, during the two growing seasons. Combined treatments also reduced the spores of *A. solani* /cm^2 found on infected leaves by 95.1, 95.4 and 95.8 % as compared with untreated plants. All treatments significantly increased the chitinase activity. The highest increase in chitinase activity was obtained with combined treatments between plant compost and humic acid or chitosan, which increased the activity by 116.5 and 122.2 %, respectively. As for tuber yield, results revealed that all treatments significantly increased the tuber yield of potato plants during the two growing seasons. The highest increase in tuber yield was obtained with combined treatments between plant compost and humic acid or chitosan, which increased the tuber yield more than 95.8 and 100.0%, respectively, during the two growing seasons. Individual treatment of each compost, humic acid, chitosan and Redomil gold plus showed moderate effect.

Key words: Early blight-potato plants- plant compost- humic acid- chitosan.

Introduction

Potato (*Solanum tuberosum* L.) is a worldwide cultivated tuber-bearing plant which is the fourth main food crop in the world after rice, maize and wheat, in terms of both area cultivated and total production[1,2,3]. 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. Early blight disease is one of the most important disease that attacking potato plants [4,5,6,7,]. Early blight disease is caused by the fungus *Alternaria solani* (E.&M.) Jones and Grout. The dark-colored spores and mycelia of the pathogen survive between growing seasons in infested plant debris and soil in infected potato tubers and in overwintering debris of susceptible solanaceous crops and weeds. Spores landing on susceptible plants germinate and may penetrate tissues directly through the epidermis, through stomata and/or through wounds such as those caused by sand abrasion, mechanical injury or insect feeding. Many cycles of early blight spore productions and lesion formation occur within single growing season once primary infections are initiated. Some chemicals are effective in controlling these disease[(5)]**.** But these chemicals are expensive and not environmental friendly. Therefore, alternative control methods are needed for

managing these pathogen. Induced resistance is more effective for controlling several plant diseases [8, 9,10,11,12]**.**

Chitosan a deacetylated chitin, is currently obtained from the outer shell of crustaceans such as crabs, krills and shrimps. Chitosan exhibits a variety of antimicrobial activities[13,14]**.** On the other hands, chitosan induce host defense responses against several plant pathogens[15,16,17]. Chitosan has been extensively utilized as a foliar treatment to control the growth, spread and development of many diseases involving viruses, bacteria, fungi and pests [13,16,17]. It has also been used to increase yield and tuber quality of potatoes[7,8]. Humic acid is a suspension, based on potassium humates, which can be applied successfully in many areas of plant production as a plant growth stimulant or soil conditioner for enhancing natural resistance against plant

Diseases and pests**,** stimulation plant growth through increased cell division, as well as optimized uptake of nutrients and water[19, 20,21,22,23]. Moreover, humic acid stimulated the soil microorganisms [24,25]. Furthermore, Abd- El- Kareem *et al.* [26] reported that potato plants treated with humic acid induced resistance against early blight in addition to increased potato yield under field conditions. Moreover, the use of compost made from different raw materials to suppress many soil borne plant pathogens has been extensively reviewed by many workers [27, 28,29,30,31,32,33,]**.** Three main types of direct interactions of microorganisms found in compost toward the soil borne fungi may be characterized: parasitism, antibiosis and competition for nutrients. Recently however, another possible mechanism has been suggested namely, induced resistance in plants to fungal attack [32, 34,35]. Currently it is believed that a combination of chemicals alternative control methods with mature compost may be more efficient in inhibiting disease than using the single treatments [12,36]**.** Therefore, the main objectives of the present research are (i) to study the inhibitor effect of chitosan, humic acid and plant compost against the growth of *Alternaria solani in vitro* and (ii) to determine the effect of individual and combined treatments of each of chitosan, humic acid and plant compost on the severity of early blight disease as well as potato tuber yield of potato plants grown under field conditions.

Materials and Methods

Pathogen

Pathogenic isolate of *Alternaria solani*, the causal agent of early blight disease, was kindly provided by Plant Pathology Department, National Research Centre, Giza, Egypt.

Plant material

Potato tubers (cv. Nicola) obtained from Dept., of Vegetables Crop Research, Agricultural Research Centre, Giza, Egypt were used in this study.

Chitosan and humic acid

Chitosan powder were purchased from Sigma Chemical Co.

Plant compost

Plant compost which was composted aerobically for four months were purchased from the Egyptian company for solid waste utilization and used in this study. Chemical analysis of the tested compost (Table, 1) was kindly carried out at the Nutrition Dept., (NRC) according to[37,38]**.**

Table 1. Chemical analysis of the plant compost used in the present study.

Total Nitrogen (%)	1.53
Total Phosphorus (%)	0.29
Total Potassium (%)	1.13
Total Iron (ppm)	1368
Total Zinc (ppm)	70.0
Total Copper (ppm)	12.0
Total organic matter (%)	31.0
Organic Carbon (%)	33.4
Carbon / Nitrogen ratio	12:1
pH (1:100)	8.1
EC	1:100
Humidity (%)	8.54

Laboratory experiments

Inhibitor effect of chitosan and humic acid on the growth of A. solani in vitro

Different concentrations of chitosan and humic acid were tested to evaluate their inhibitor effect on linear growth of *Alternaria solani in vitro*. Therefore, six concentrations of each of chitosan and humic acid *viz.,* 0.0 , 1.0, 2.0, 3.0, 4.0 and 5.0 g/ L or mL/L for chitosan and humic acid respectively, were added individually to conical flasks containing sterilized PDA medium to obtain the proposed concentrations, then mixed gently and dispensed in sterilized Petri plates (9 cm – diameter). Plates were individually inoculated at the center with equal disks (6-mm- diameter) of 10-days old culture of *Alternaria solani*. Five plates were used as replicates for each particular treatment. Inoculated plates were incubated at 25 ± 2 °C. The average linear growth of the fungus was calculated after 10 days.

Inhibitor effect of compost on the growth of A. solani in vitro

The inhibitor effect of the tested compost were examined as compost water extract (CWE) against the growth of *Alternaria solani*, *in vitro* using the methods of pouring plate method and dry weight of mycelium method described by [39] and modified by [36]. The compost water extract (CWE) were prepared by vigorously shaking mature compost for 15 min in phosphate buffer (Na_2 HPO_4 8 gl-1, Na H_2PO_4 0.34 gl-1, pH 7.0) at the ratio of 1:2 (w/v). To remove the large particles from compost mixture, aliquot of 250 ml of the mixture was filtrated by 3 layers of sterile cheesecloth to obtain the active supernatant.

Pouring plate method

Crude compost water extract (CWE) of plant compost were tested against the aforementioned pathogenic fungi using the pouring plate method. Aliquots of CWE were added to sterilized PDA medium before solidification to obtain three concentrations *viz.*, 5, 10 and 15% (v/v), while only sterile distilled water was placed in the control plates. One disc (6-mm- diameter) of 10-days old culture of *Alternaria solani* was placed onto the medium in the centre of the plate. Five Petri plates were used for each treatment as well as the control treatment. All the plates were incubated at 25 ± 1°C for 10 days. The radial growth of the pathogenic fungus was measured to determine the inhibitor effect of CWE. The percentages of fungal mycelial reduction were determined according to the following formula: Fungal growth reduction (%) = (C - T / C) × 100, whereas: C = diameter of pathogenic mycelial growth (cm) of control plates and T = diameter of pathogenic mycelial growth (cm) in CWE amended plates.

Dry weight assay method

The inhibitor effect of the crude CWE was tested against the aforementioned pathogenic fungi using the fungal mycelium dry weight method. 5, 10 and 15 mililiter of CWE were added to sterile 95, 90 and 85 mL of PDB (Potato Dextrose Broth) medium in flask of 250 ml, while only sterile distilled water was used for control flasks, respectively. Then, each flask was inoculated with One disc (6-mm- diameter) of 10-days old culture of *Alternaria solani*. A blank was prepared by adding CWE to the PDB medium without any pathogenic fungus inoculation. Five flasks were used for each treatment as well as the control and the blank. The inoculated flasks were incubated at 25 ± 2°C for 10 days. The culture of PDB including the growing microorganisms, in treatments, control and blank were filtered through 0.22 µm Millipore membrane filter. Then, the dry weight of mycelial growth was determined after drying at 65 ± 1°C overnight. The dry weight reduction (%) of CWE treatment was calculated according to the following suggested formula:

$$\text{Dry weight reduction (\%)} = \frac{C - (CWE - B)}{C} \times 100$$

Where: B = Blank, dry weight of microbial growth of microorganisms in compost water extract without any pathogenic fungus, C = Control, dry weight of mycelial growth of pathogenic fungus only and CWE = Dry weight of microbial growth of microorganisms in compost water extract plus the pathogenic fungus.

Field experiments

Field experiments were carried out during two successive seasons at Omar Makram Village, El-Tahrir county, El-Behera governorate, to evaluate the effect of individual and combined treatment of each of compost, humic acid and chitosan on early blight severity of potato plants grown under field conditions. Experiments were conducted under natural infection in plots (4 × 8 m) each comprised of 8 rows (32 holes / row and one seed piece was sown in each hole) in a randomized complete block design with three replicates (plots) for each treatment. Seed tuber (cv. Necola) were cut longitudinally using sterilized knife into pieces with 2-3 sprout per piece. The potato seed pieces have been disinfected before use by deceiving in a solution of sodium hypochlorite solution (10%) for 10 min and rinsing twice with sterile distilled water. Disinfected potato seed pieces was air dried for 24 h under shadow place. Then, seed tuber pieces were planting in loamy clay well-drained soil to a depth of 10 cm. In addition, irrigation and nutrients such as phosphorus, nitrogen and potassium were added to ensure adequate plants nutrition during mid-growth and tuberization as recommended**.**

Application method

Plant compost at the rate of 10 ton / feddan were applied as a soil amendment at 15 day before tuber planting. Foliar spraying with each of humic acid at the rate of 5.0 mL / L; chitosan at the rate of 1.0 g / L and/or the fungicide Redomil - plus at the rate of 2 g / L, were applied on potato plants which had 4-5 compound leaves and every 15 days intervals up to 90 days of planting. Treatments were applied as follow:

Individual treatment	**Combined treatment**
1.Plant compost	5. Plant compost + humic acid
2.Humic acid at 5 mL/ L	6. Plant compost + chitosan
3.Chitosan at 1.0 g / L	7. Plant compost + Fungicide (Redomil plus 2g/L)
4.Fungicide (Redomil plus 2 g / L)	8- Un-treated plants (control)

Data collection and analyses

Disease assessment

Early blight disease severity (%) was recorded up to 90 days of planting by the scale from 0 to 4 according to [40] based on the infected leaf area as follows:

0 = No leaf lesions.
1 = 25 % or less.
2 = 26 to 50.
3 = 51 to 75.
4 = 76 to 100 % infected leaf area.

Effect on *A. solani* sporulation

Spores of *A. solani* / cm^2 were counted in infected potato plans. Leaves of each treatment were detached gently at the early morning and immersed in screw cap jars containing 10 ml of distilled water. Spores were released from lesions using a brush, then they were counted using hemocytometer slide. Area of lesions were detected by placing the cut lesion on millimeters quarter paper.

Biochemical studies

Determination of chitinase enzyme activities were carried out at 60 days after planting. Potato leaves were collected and to extract the enzyme, plant leaves (g) were homogenized with 0.2 M Tris HCl buffer (pH 7.8) at 0°C containing 14 m M β- mercaptoethanol at the rate of 1/3 w/v. The extracts were obtained by filtering off the debris with a clean cloth and centrifuging at 3,000 rpm for 15 min. The supernatants were recovered and kept in a tube in an ice bath until assayed. The supernatant was used to determine the activity of chitinase enzyme [41] by using UV spectrophotometer. The determination of chitinase enzyme was carried out using colloidal chitin as substrate and dinitrosalicylic acid (DNS) as reagent to measure reducing sugars according the method described by[42]. Chitinase activity was expressed as mM N-acetyl glucose amine equivalent released/ gram fresh weight/ 60 minutes at 450nm.

Potato tuber yield

Effect of individual and combined treatment with each of plant compost, humic acid and chitosan on potato tuber yield under field conditions was studied. Therefore, potato tuber were harvested after 120 days of planting. Tuber yield per each treatment was recorded and the average of the tuber yield (metric ton / hectare) was calculated for each treatment.

Statistical analysis

Tukey test for multiple comparisons among means was utilized [43].

Results

Laboratory experiments

Inhibitor effect of chitosan and humic acid on the growth of A. solani in vitro

Inhibitor effect of chitosan and humic acid against the linear growth of *Alternaria solani* are listed in **Table 2**. Data reveal that all the tested concentrations of humic acid had no inhibitor effect against the linear growth of *A. solani.* Meanwhile, chitosan at concentration of 5.0 g / L was completely inhibited the growth of *A. solani.* The highest reduction in linear growth was obtained with chitosan at 4.0 g / L, which reduced the fungal linear growth by 91.1 %.

Table 2. Reduction caused by chitosan, humic acid and Ridomil gold plus against linear mycelial growth of *Alternaria solani via* the poisoned food technique.

Treatment	Conc.	Linear mycelial growth (mm) and reduction (%)	
		Growth (mm)	Reduction (%)
Chitosan (g/L)	1.0	66.4 b[(1)]	26.2
	2.0	51.0 c	43.3
	3.0	30.0 d	66.7
	4.0	08.0 e	91.1
	5.0	00.0 f	100
Humic acid (mL/L)	1.0	90.0 a	00.0
	2.0	90.0 a	00.0
	3.0	90.0 a	00.0
	4.0	90.0 a	00.0
	5.0	90.0 a	00.0
Ridomil gold plus at 2.0g/L		00.0 f	100
Control		90.0 a	0.0

[1] Figures with the same letter are not significantly different (P= 0.05).

Inhibitor effect of compost on the growth of A. solani in vitro

The inhibitor effect of crude compost water extract (CWE) against linear growth of *A. solani* at the concentrations of 5, 10 and 15% *via* the pour plate method are shown in **Table 3.** The inhibitor effect of CWE were in the range of 75.6 to 79.7% compared to 0.0% in the control (sterilized water). The highest inhibitor effect was obtained by CWE at the concentration of 15%, where the mycelial growth reduction values reached to 79.7%. The effects of raw CWE at the concentration of 5, 10 and 15% on the fungal mycelial dry weight of *A. solani* are shown in **Table 4.** Data showed that all CWE concentrations reduced the mycelial dry weight of *A. solani*. The percentages of mycelial dry weight reduction resulted by CWE concentrations were in the range of 87.5 to 94.9%, compared to the control (sterilized water). The most inhibitor effect in mycelial dry weight of *A. solani* was obtained by CWE at the concentration of 15%, which recorded mycelial dry weight reduction estimated as 94.9%.

Table 3. The inhibitor effect of compost water extract (CWE) against the linear growth of *Alternaria solani via* the pour plate method.

Treatment	Conc. (%)	Linear growth (mm) and reduction (%)	
		Growth (mm)	Reduction (%)
CWE	5	20.2	75.6
	10	18.8	79.1
	15	18.2	79.7
Sterilized water(3)	5	90.0	00.0
	10	90.0	00.0
	15	90.0	00.0
Control		90.0	00.0

1- Figures with the same letter are not significantly different (P= 0.05).

Table 4. Effect of compost water extracts at the concentration of 5, 10 and 15% on the dry weight (g) of the pathogenic fungi via the dry weight method.

Treatment	Conc. (%)	Dry weight (g) and growth reduction (%) of *A. solani*		
		Dry weight (g)	Reduction (%)	Blank(2)
CWE	**5**	0.222	87.5	0.115
	10	0.332	88.5	0.233
	15	0.362	94.9	0.318
Sterilized water(3)	**5**	0.858	0.0	-
	10	0.858	0.0	-
	15	0.858	0.0	-

1- Figures with the same letter are not significantly different (P= 0.05).
2- Dry weight of microbial growth of microorganisms in compost without any pathogenic fungus.
3- Dry weight of mycelial growth of pathogenic fungus without compost (as control).

Field experiments

Effect on early blight severity

Effect of individual and combined treatment with each of plant compost, humic acid and chitosan on early blight severity of potato plants grown under field conditions are listed in Table 5. Results indicate that all treatments significantly reduced the disease severity during the two growing seasons. The highest reduction in early blight severity was obtained with combined treatments between soil amendment with compost and foliar spray with each of humic acid, chitosan or Ridomil gold plus, which reduced the disease severity more than 75.0, 84.4 and 78.6 % respectively during the two growing seasons. Individual treatment with each of plant compost, humic acid, chitosan and Ridomil gold plus showed moderate effect.

Table 5. Effect of individual and combined treatment with each of plant compost, humic acid and chitosan on early blight severity of potato plants grown under field conditions.

Treatment(1)	Early blight disease severity (2)			
	First growing season		Second growing season	
	Severity (%)	Reduction (%)	Severity (%)	Reduction (%)
Individual treatment				
Compost (10 ton/ feddan)	1.3 b(3)	59.4	1.7 b	48.5
Humic acid (5.0 m /L)	1.3 b	59.4	1.4 c	57.6
Chitosan (1.0 g/ L)	1.2 b	62.5	1.4 c	57.6
Ridomil gold plus (2g/L)	1.4 b	56.3	1.5 c	54.5
Combined treatment				
Compost + humic acid	0.8 c	75.0	0.8 d	75.8
Compost + chitosan	0.5 c	84.4	0.5 d	84.8
Compost + Ridomil	0.6 c	81.3	0.7 d	78.6
Control	3.2 a	-	3.3 a	-

(1) Plant compost were applied as soil amendment, while humic acid, chitosan or Fungicide (Redomil gold plus) were applied as foliar application. (2) Early blight scale from 0 to 4 according to Cohen *et al.*,(1991).
(3) Figures with the same letter are not significantly different (P= 0.05).

Effect on Alternaria solani sporulation

Effect of individual and combined treatment with each of plant compost, humic acid and chitosan on sporulation of *A. solani* on infected leaves of potato plants grown under field conditions are listed in **Table 6.** Results reveal that all treatments significantly reduced the number of spores of *A. solani* / cm^2 of infected potato leaves. The most effective treatments in reducing the number of spores were the combined treatments between soil amendment with compost and foliar spray with each of humic acid, chitosan or Ridomil gold plus, which reduced the spores / cm^2 of infected leaves by 95.1, 95.4 and 95.8 %, respectively, as compared with untreated plants. Other treatments reduced sporulation by 86.9 % at least.

Table 6. Effect of individual and combined treatment with each of plant compost, humic acid and chitosan on the sporulation of *A. solani* on infected leaves of potato plants grown under field conditions.

Treatment(1)	Sporulation of *A. solani*	
	Number of spores / cm^2	Reduction (%)
Individual treatment		
Compost (10 ton/ feddan)	1500 b(2)	87.7
Humic acid (5.0 m /L)	1600 b	86.9
Chitosan (1.0 g/ L)	1400 b	88.5
Ridomil gold plus (2g/L)	1200 c	90.2
Combined treatment		
Compost + humic acid	600 d	95.1
Compost + chitosan	560 d	95.4
Compost + Ridomil	510 d	95.8
Control	12200 a	-

(1) Plant compost were applied as soil amendment, while humic acid, chitosan or Fungicide (Redomil gold plus) were applied as foliar application.
(2) Figures with the same letter are not significantly different (P= 0.05).

Effect on chitinase activity

Effect of individual and combined treatment with each of plant compost, humic acid and chitosan on chitinase activity in the leaves of potato plants grown under field conditions are listed in **Table 7.** Results reveal that all treatments significantly increased the chitinase activity. The highest increase in chitinase activity was obtained with the combined treatments between soil amendment with compost and foliar spray with each of humic acid and chitosan, which increased the chitinase activity by 116.5 and 122.2 % respectively. Individual treatment of foliar spray with chitosan and the combined treatments of soil amendment with plant compost and foliar spray with Redomyl gold plus, caused an increase in chitinase activity reached to 108.3 and 102.8 % respectively. Meanwhile individual treatment of foliar spray with humic acid showed moderate effect.

Table 7. Effect of individual and combined treatment with each of plant compost, humic acid and chitosan on chitinase activity in the leaves of potato plants grown under field conditions.

Treatment(1)	Chitinase enzyme	
	Activity(2)	Increase (%)
Individual treatment		
Compost (10 ton/ feddan)	6.0 d (3)	66.7
Humic acid (5.0 m /L)	7.0 c	94.4
Chitosan (1.0 g/ L)	7.5 b	108.3
Ridomil gold plus (2g/L)	6.0 d	66.7
Combined treatment		
Compost + humic acid	7.8 a	116.6
Compost + chitosan	8.0 a	122.2
Compost + Ridomil gold plus	7.3 b	102.8
Control	3.6 f	-

(1) Plant compost were applied as soil amendment, while humic acid, chitosan or Fungicide (Redomil gold plus) were applied as foliar application. (2) Chitinase activity expressed as mM N-acetyl glucose amine equivalent released/ gram fresh weight/ 60 min. (3) Figures with the same letter are not significantly different (P= 0.05).

Effect on tuber yield

Effect of individual and combined treatment with each of plant compost, humic acid and chitosan on tuber yield of potato plants grown under field conditions are listed in Table 8. Results indicate that all treatments significantly increased the tuber yield of potato plants during the two growing seasons. The highest increase in tuber yield was obtained with the combined treatments between soil amendment with compost and foliar spray with each of humic acid and chitosan, which increased the tuber yield more than 95.8 and 100.0 %, respectively during the two growing seasons. Combined treatments between soil amendment with compost and foliar spray with Redomyl gold plus increased tuber yield by 81.8 and 79.2 %, respectively during the two growing seasons. Individual treatment of plant compost, humic acid , chitosan and Redomyl gold plus showed moderate effect.

Table 8. Effect of individual and combined treatment with each of plant compost, humic acid and chitosan on tuber yield of potato plants grown under field conditions.

Treatment(1)	Potato tuber yield (metric ton/ hectare)			
	First growing season		Second growing season	
	Yield	Increase (%)	Yield	Increase (%)
Individual treatment				
Compost (10 ton/ feddan)	3.1 c (1)	40.9	3.2 c	33.3
Humic acid (5.0 m /L)	3.2 c	45.5	3.3 c	37.5
Chitosan (1.0 g/ L)	3.2 c	45.5	3.3 c	37.5
Ridomil gold plus (2g/L)	3.0 c	36.4	3.1 c	29.2
Combined treatment				
Compost + humic acid	4.4 a	100.0	4.7 a	95.8
Compost + chitosan	4.5 a	104.5	4.8 a	100.0
Compost + Ridomil	4.0 b	81.8	4.3 b	79.2
Control	2.2 c	-	2.4 c	-

(1) Plant compost were applied as soil amendment, while humic acid, chitosan or Fungicide (Redomil gold plus) were applied as foliar application.
(2) Figures with the same letter are not significantly different (P= 0.05).

Discussion

In the present study, under laboratory conditions, complete inhibition in the linear growth of *Alternaria solani* was obtained with chitosan at 5.0 g / L. This results are in harmony with those obtained by [44]. Fungicidal activity of chitosan has been documented against various species of fungi and Ooomycetes[16, 45,46,47,48]. The mechanism by which chitosan affects the growth of several pathogenic fungi has not been fully elucidated, but several hypotheses have been postulated, first: its polycationic nature, it is believed that chitosan interferes with negatively charged residues of macromolecules exposed on the fungal cell surface. This interaction leads to the leakage of intracellular electrolytes and proteinaceous constituents[45]. Second the interaction of diffused hydrolysis products with microbial DNA, which leads to the inhibition of mRNA and protein synthesis [46], third the chelating of metals, spore elements and essential nutrients [45]. Forth: the interaction of chitosan with fungal DNA and RNA [48]. Five : Malformation of fungal mycelial. Chitosan is not only effective in inhibition the growth of the pathogen fungi, but also induces marked morphological changes, structural alterations and molecular disorganization of fungal cells[49,50]. Moreover, [45] reported that, chitosan caused morphological changes such as large vesicles or empty cells devoid of cytoplasm in the mycelium of *B. cinerea*. Furthermore, [51] revealed that by microscopic observation of fungi treated with chitosan, it can affect the morphology of the hyphae. Data of the present investigation also reveal that compost water extract CWE of plant compost show inhibitor effect against the growth of *A. solani*. As shown by plate experiments, crude CWE inhibited *in vitro* the growth of the pathogen, while sterilization annulled or reduced this effect. The *in vitro* observations may be ascribed to the crucial role played by the biological composition of composts in disease suppression. This results are in accordance with those obtained by[36].

Under field conditions, individual and combined treatment with each of plant compost, humic acid and chitosan reduced the early blight severity, reduced the sporulation of *A. solani* on infected potato leaves,

increase chitinase enzyme activity and tuber yield. The combined treatment show more better than individual one. This is may be due to the integration effects between treatments. Soil amendment with compost before tuber planting reduced the pathogen propagules and spores found in plant debris. In additions chitosan foliar spray reduced sporulations and thus reduced many cycles of early blight during the growing season. On the other hands, induction of resistance against *A. solani* by each of compost, chitosan and humic acid are also involved [15,16,17]. The obtained results are in agree with those obtained by many workers [7,8,19]**.** The present results indicated that all tested concentrations of humic acid had no inhibitory effect against *A. solani* but it reduced early blight disease of potato plants under field conditions. In this respect, [22,23] reported that the most effective treatments for suppression gray mould in Geranium was compost tea plus kelp extract and humic acid. The role of humic acid for reducing early blight diseases in addition to increase yield of potato plants may be due to enhanced natural resistance against plant diseases and pests [22,23]**.** In the present study results indicated the humic acid increased the chitinase activity. In this respect, *ß*-1,3-glucanases and chitinases are able to hydrolyze *ß*-1,3-glucan and chitin, respectively, the major components of fungal cell walls [52,53,54]**.** Abd- El- Kareem [54] reported that bean plants treated with humic acid induced resistance against root rot and Alternaria leaf spot in addition to increased bean yield under field conditions. Moreover, Abd- El- Kareem *et al.,* [24] reported that potato plants treated with humic acid induced resistance against early blight in addition to increased potato yield under field conditions. On the other hand, humic acid stimulated plant growth through increased cell division, as well as optimized uptake of nutrients and water [19,20] and stimulated the soil microorganisms [19]**.**

Controlling of plant diseases depends mainly on fungicides and nematicides application such chemicals are not always desirable due to potential hazards to human beings and the environment. Alternative approaches to fungicides are needed for controlling plant diseases [54,55,56,57,58,59,60,61,62,63]**.**

It could be suggested from the present study that the combined treatments between soil amendment with compost and foliar spray with each of humic acid and/or chitosan might be used for controlling early blight disease of potato plants under field conditions.

References

1. Douches, D. S.; Maas, D.; Jastrzebski, K. and Chase, R.W. (1996). Assessment of potato breeding progress in the USA over the last century. Crop Science, 36:1544-1552.
2. FAO (2010). FAOSTAT Database, http://faostat .fao.org/
3. 3-FAO (2012). FAOSTAT Crops Production. Publisher. http://faostat3. fao.org/home/index.html#DOWNLOAD. Accessed 21 June 2012.
4. Pasche, J. S; . Wharam, C. M. and Gudmestad, N. C. 2004. Shift in sensitivity of *Alternaria solani* in response to Q(o)I Fungicides. Plant Dis. 88:181-187.
5. Pasche, J. S ; Piche, L. M. and Gudmestad, N. C. 2005. Effect of the F129L Mutation in *Alternaria solani* on Fungicides Affecting Mitochondrial Respiration. *Plant Dis.* 89:269-278.
6. Abd-El-Kareem, F., 2007 a. Potassium or sodium bicarbonates in combination with citral for controlling early blight disease of potato plants under laboratory, greenhouse and field conditions. *Egypt. J.of Phytopathol.* 35: 73-86.
7. Abd-El-Kareem , F. and Haggag, W.M. 2014. Chitosan and Citral Alone or in Combination For Controlling Early Blight Disease of Potato Plants Under Field Conditions. Research Journal of Pharmaceutical, Biological and Chemical Sciences . 5 : 941- 945.
8. Kowalski, B.; Jimenez, F. ; Herrera, L. and Agramonte, P.D. 2006. Application of soluble chitosan in vitro and in the greenhouse to increase yield and seed quality of potato minitubers. *Potato Res*.
9. Guan, Y.J.; Hu, J.; Wang, X. J. and Shao, C. X. 2009. Seed priming with chitosan improves maize germination and seedling growth in relation to physiological changes under low temperature stress. J Zhejiang Univ Sci B. 2009;10:427–433.
10. Suprakash, O. and Chatterjee, N. C. 2012. Induction of resistance in tomato plants against *Fusarium oxysporum* f. sp. *lycopersici* mediated through salicylic acid and *Trichoderma harzianum*. *Journal of Plant Protection Research*; 52: 220-225.
11. Abd-El-Kareem , F and Abd-El-Latif, F. 2012. Using bicarbonates for controlling late blight disease of potato plants under field conditions. *Life Science Journal*, (9): 280- 285.
12. Abd-El-Kareem, F. ; Abd-El- Kader, M.M .; Fotouh, Y.O. ; Abd-Alla, M.A. El-Mougy, N, S. ; El-Mohamedy, R.S. and .; El-Gamal, N.G. 2013. Induction of systemic resistance in faba bean against

chocolate spot diseases severity using chemical inducers under field conditions. Journal of Applied Sciences Research, 9(6): 4006-4014.

13. Badawy, M.T.; Rabea, M.T.; Rogge, T.M.; Stevens, C.V. and Smagghe, G. 2003. Fungicidal and insecticidal activity of chitosan derivatives. *Polymer Bull.* 2003;54:279-289.
14. El-Mohamedy, R.S. ; Abdel-Kader, M.M. ; Abd-El-Kareem, F. and El-Mougym N.S. 2013. Inhibitory effect of antagonistic bio-agents and chitosan on the growth of tomato root rot pathogens *In vitro* . *Journal of Agricultural Technology,* 9 :1521-1533.
15. Uppal, A.K.; El Hadrami, A.; Adam, L.R.; Tenuta, M. and Daayf, F. 2008. Biological control of potato Verticillium wilt under controlled and field conditions using selected bacterial antagonists and plant extracts. *Biol Control.* 44:90–100.
16. Elwagia , M. E. F. and Algam, S. 2014 . Evaluation of chitosan efficacy on tomato growth and control of early blight disease. *Third Conference of Pests Management in Sudan February* 3-4, 2014, CPRC-ARC, Wad Medani (Sudan) Page 10.
17. Mishra, S. ; Jagadeesh, K.S.; Krishnaraj, P.U. and Prem, S. 2014. Biocontrol of tomato leaf curl virus in tomato with chitosan supplemented formulations of *Pseudomonas* sp. under field conditions. *Australian Journal of Crop Science*, 8:347-355.
18. Faoro, F.; Maffi, D.; Cantu, D. and Iriti, M. 2008. Chemical-induced resistance against powdery mildew in barley: the effects of chitosan and benzothiadiazole. Bio Control. 53:387–401.
19. Ait Barka, E.; Eullaffroy, P.; Cle´ment, C. and Vernet, G. 2004. Chitosan improves development, and protects *Vitis vinifera* L. against *Botrytis cinerea*. *Plant Cell Rep*., 22:608–614.
20. Chen Y, De Nobili M.; Aviad T. 2004. Stimulatory effect of humic substances on plant growth. In 'Soil organic matter in sustainable agriculture'. (Eds F Magdoff, RR Weil) pp. 103-130. Press: Boca Raton, FL.
21. Scheuerell , S. J. and Mahaffee ,W H . 2004. Compost Tea as a Container Medium Drench for Suppressing Seedling Damping-Off Caused by *Pythium ultimum. Phytopathology* 94:1156-1163.
22. Scheuerell , S. J. and Mahaffee ,W. H. 2006.Variability Associated with Suppression of Gray Mold (*Botrytis cinerea*) on Geranium by Foliar Applications of Nonaerated and Aerated Compost Teas. *Plant Dis.* 90:1201-1208.
23. Abd-El- Kareem, F., Abd-El-Latif, Faten M. and Fotouh, Y.O. 2009. Integrated treatments between humic acid and sulfur for controlling early blight disease of potato plants under field infection. *Research J. of Agricultural and Biological Science.* 5(6): 1039-1045.
24. Atiyeh, R.M.; Lee S.; Edwards C.A; Arancon, N.Q. and Metzger, J.D. 2002. The influence of humic acids derived from earthworm processed organic wastes on plant growth. *Bioresource Technology* . 84 : 7-14.
25. Qualls, R.G. 2004. Biodegradability of humic substances and other fractions of decomposing leaf litter. *Soil Science Society of America Journal*. 68:1705-1712.
26. Abd-El-Kareem, F., Abd-El-Latif, Faten M. and Fotouh, Y.O. 2009. Integrated treatments between humic acid and sulfur for controlling early blight disease of potato plants under field infection. *Research J. of Agricultural and Biological Science.* 5(6): 1039-1045.
27. Trillas, M.I.; Casanova, E.; Corxarrera, L.; Ordovas, J.; Borrero, C. and Aviles, M. 2006. Composts from agricultural waste and the *Trichoderma asperellum* strain T-34 suppress *Rhizoctonia solani* in cucumber seedlings. *Biol Control* , 39:32–38.
28. Estrella, F. S., G. C. Vargas, M. J. Lopez, C. Capel and J. Moreno, 2007. Antagonistic activity of bacteria and fungi from horticultural compost against *Fusarium oxysporum* f. sp. *melonis*. Crop Prot., 26:46-53.
29. Youssef, Sahar A. 2007. Evaluation of composted chicken manure in biocontrolling Fusarium wilt on tomato. Egypt. J. Phytopathol., 35:61-72.
30. Abd-El-Khair, H., W. M. A. El-Nagdy, O. M. Hafez and H. H. Ameen, 2009. Effect of four composts on *Meloidogyne incognita* and *Fusarium solani* infested superior grapevine and their influence on yield production and quality. Nematol. Medit., 37:89-103.
31. Catello, P., S. Riccardo, P. Alessandro, S. Felice and B. Giuliano, 2011. Compost amendments enhance peat suppressiveness to *Pythium ultimum*, *Rhizoctonia solani* and *Sclerotinia minor*. Biological Control, 56:115-124.
32. Abd-El-Razik, Sahar A., M. H. Hassan, M. I. A. Eraky and M. A. N. Sallam, 2012. Suppressive effect of certain composts on the infection by root-rot and wilt diseases of faba bean under greenhouse conditions. Egypt. J. Phytopathol., 40:41-57.

33. Catello, P., P. Alessandro, S. Riccardo, C. Giuseppe, V. Domenica and Z. Massimo, 2013. Agricultural wastebased composts exhibiting suppressivity to diseases caused by the phytopathogenic soilborne fungi *Rhizoctonia solani* and *Sclerotinia minor*. Appl. Soil Ecol., 65:43-51.
34. Sabet, K.K., M.M. Saber, M.A. El-Naggar, N.S. El-Mougy, H.M. El-Deeb and I.E. El-Shahawy, 2013. Using commercial compost as control measures against cucumber root-rot disease. Journal of Mycology, Volume 2013, Article ID 324570, 13 pages. http://dx.doi.org/10.1155/2013/324570.
35. Sabet, K.K., M.M. Saber, M.A. El-Naggar, N.S. El-Mougy, H.M. El-Deeb and I.E. El-Shahawy, 2014. The Role of Compost and/or Bio Control Agents in Induction Resistance against Cucumber Root-Rot Pathogens. Middle East Journal of Agriculture Research, 3(4): 763-773, 2014.
36. Zhao, S.; Liu, D.; Ling , N.; Chen , F.; Fang, W. and Shen, Q. 2014. Bio-organic fertilizer application significantly reduced the *Fusarium oxysporum* population and alters the communities of watermelon Fusarium wilt rhizosphere soil. Biol. Fertil. Soil, On Line , DOI :10. 1007.
37. Ma, T. S. and Zauzage, C. (1942). Determination of nitrogen, a new indicator and an improved rapid method. Industr. Eng. Chem. Anal.,Ed., 14:280-286.
38. Isaac, R. A. and Johnson, W. C. (1984). Methodology for the Analysis of Soil, Plant, Feed, Water and Fertilizer Samples. California Fertilizer Association (CFA), 80 p.
39. El-Masry, M. H.; Khalil, A. I.; Hassouna, M. S. and Ibrahim, H. A. H. (2002). *In situ* and *in vitro* suppressive effect of agricultural composts and their water extracts on some phytopathogenic fungi. World J. of Microbiol. & Biotech., 18:551-558.
40. Cohen, Y.; Gisi, U. and Mosinger, E. (1991). Systemic resistance of potato plants against *Phytophthora infestans* induced by unsaturated fatty acids. Physiol. Mol. Plant Pathol., 38: 255-263.
41. Tuzun, S.; Rao, M.N.; Vogeli, U.; Schardl, C.L. and Kuc, J. 1989. Induced systemic resistance to blue mould : Early induction and accumulation of ß-1,3 -glucanase, chitinase and other pathogenesis proteins (b-proteins) in immunized tobacco .*Phytopathology, 79 : 979-983*.
42. Monreal, J. and Reese, E. T. (1969). The chitinase of *Serratia marcescens*. Can. J. Microbiol., 15:689-696.
43. Neler, J.; Wassermann, W. and Kutner, M.H. 1985. *Applied linear statistical models. Regression, analysis of variance and experimental design* : 2nd ed. Richard, D. Irwin Inc. Homewood, Illionois.
44. Kulikov, S. N.; Chirkov, S. N.; Lopatin, S. and Varlamov V. P. 2006. Effect of the molecular weight of chitosan on its antiviral activity in plants. *Prik Biokhim Mikrobiol.*;42(2):224–228.
45. El Hassni, M.; El Hadrami. A.; Daayf, F.; Chérif, M. and El Hadrami, I. 2004. Chitosan, antifungal product against *Fusarium oxysporum* f. sp. *albedinis* and elicitor of defence reactions in date palm roots. *Phytopathol Mediterr.* 43:195–204.
46. Vasyukova, N.I.; Chalenko, G.I.; Gerasimova, N.G.; Perekhod, E.A.; Ozeretskovskaya, O.L. and Albulov, A.I. 2005 Chitin and chitosan derivatives as elicitors of potato resistance to late blight. *Appl Biochem Microbiol*. 36:372–376.
47. Rabea , M.T.; Rogge, T.M.; Stevens, C.V. and Smagghe, G.2005. Insecticidal and fungicidal activity of new synthesized chitosan derivatives. *Pest Manag Sci*. 61:951-960.
48. Palma-Guerrero, J.; Huang, I.C.; Jansson, H.B.; Salinas, J. ; Lopez, L.V. and Read, N.D. 2008. Effect of chitosan on hyphal growth and spore germination of plant pathogenic and biocontrol fungi. *J. Appl Microbiol*. 104:541-553.
49. El- Ghaouth, A.; Arul, J. ; Asselin, A.and Benhamou. N. 2002. Antifungal activity of chitosan on post-harvest pathogens: induction of morphological and cytological alterations in *Rhizopus stolonifer*. *Mycol Res.* 1992;96:769–779.
50. Ait Barka, E.; Eullaffroy, P.; Cle´ment, C. and Vernet, G. 2004. Chitosan improves development, and protects *Vitis vinifera* L. against *Botrytis cinerea*. *Plant Cell Rep*., 22:608–614.
51. Banos,S. B.; Hernandez-Lauzardo, A.N.; Velazquez-del Valle, M. G.; Hernandez-lopez, M.; Ait Barka, E.; Bosquez-Molina, E. and Wilson, C. L. 2006. Chitosan as apotential natural compound to control pre and post harvest diseases of horticultural commodities. *Crop Protection*, 25:108-118.
52. Kauffmann, S.; Legrand, M.; Jeoffroy, P. and Fritig, B. 1987. Biological function of pathogenesis-related proteins. Four PR-proteins of tobacco have *B*-1,3-glucanase activity. *EMBO J.,* 6:3209-3212.
53. Legrand, M.; Kauffmann, S.; Jeoffroy, P. and Fritig, B. 1987. Biological function of pathogenesis-related proteins; Four tobacco pathogenesis-related proteins are chitinases. *Proc. Natl. Acad. Sci.,* 84:6750-6754.

54. Abd-El-Kareem , F. 2007 b. Induced resistance in bean plants against root rot and Alternaria leaf spot diseases using biotic and abiotic inducers under field conditions. *Research J. of Agricultural and Biological Science* 3 (6) :767-774.
55. Hammam,M.M.A.; El-Mohamedy, R.S.R.; Abd-El-Kareem, F. and Abd-Elgawad, M.M.M. 2016 .Evaluation of soil amended with bio-Agents and compost alone or in combination for controlling citrus nematode *Tylenchulus semipenetrans* and Fusarium dry root rot on Volkamer lime under greenhouse conditions. International Journal of ChemTech Research, 9, No.7 : 86- 96 .
56. El-Mohamedy, R.S.R.; Hammam,M.M.A.; Abd-El-Kareem, F. and Abd-Elgawad, M.M.M.2016.Biological soil treatment to control *Fusarium solani* and *Tylenchulus semipenetrans* on sour orange seedlings under greenhouse conditions. International Journal of ChemTech Research, 9, No.7 : 73-85.
57. Elshahawy, I.E. Lashin, S.L.; Saied, N.M. and Abd-El-Kareem, F. 2015. Evaluation of safe postharvest treatments for controlling Valencia orange green and blue moulds. International Journal of ChemTech Research, 8, No.9 : 237-244.
58. Mawardika, H. and Suharjono, S. 2015. Antagonist Assay and Molecular Identification of Soil Molds Antagonist to Pathogenic Fusarium on Tomato Plants (Lycopersicum esculentum Mill.) in Bocek East Java Tomato Field. International Journal of ChemTech Researchm, 8, No.8,: 01-07.
59. Hathout, A.N. ; Abo-Sereih, N. A. ; Sabry, B.A. ; Sahab, A.F. and Aly, S.A. 2015. Molecular identification and control of some pathogenic Fusarium species isolated from maize in Egypt. International Journal of ChemTech Research, 7, No.1,: 44- 54.
60. Ghoname, A.A. ; Riad, G.S. ; El-basiouny, A.M. ; Hegazi, A.M. and El-Mohamady, R.S. 2015.Finding natural alternatives to methyl bromide in greenhouse cantaloupe for yield, quality and disease control. International Journal of ChemTech Research, 8, No.9: 84-92.
61. Abdalla M.Y.; Haggag, W.M. and Rayan, M.M.2015. Using Bioproducts Made with Native Microorganisms to Limit the Damage for Some Sugar Beet Cultivars Seeds. International Journal of ChemTech Research, 8, No.9: 245-260.
62. Vimala Kumari, T.G.; Basu, K.; Nithya, T.G. ; and Kharkwa, K.S. 2015. Study of Bio-efficacy of Alkali tolerant Trichoderma against damping off and rotting diseases of Tomato and Cauliflower caused by Pythium spp. and Sclerotina spp. . International Journal of ChemTech Research, 8, No.6: 628-634.
63. El-Sayed, S. M. and Mahdy, M. E.2015. Effect of chitosan on root-knot nematode, *Meloidogyne javanica* on tomato plants. International Journal of ChemTech Research, 7- 22-- 22-36

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