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Integrated Treatments Between Humic Acid and Sulfur for Controlling Early Blight Disease of Potato Plants under Field Infection

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Abstract: Potato crop (*Solanum tuberosum* L.) is one of the most important vegetable crops in Egypt. Integrated treatments between humic acid at concentrations 6.0 or 8.0 ml / l followed by sulfur at 3.0 or 4.0 g / l were evaluated against early blight disease under sever field infection. All tested concentrations of humic acid had no inhibitory effect on *Alternaria solani*. Meanwhile, sulfur at concentrations 3.0 and 4.0 g / l caused complete reduction of linear growth of *A. solani* under laboratory conditions. In greenhouse experiments, results indicate that the integrated treatments between humic acid followed by sulfur treatments with all tested concentrations reduced early blight severity more than 85.7 %. The highest increase in chitinase activity was obtained with humic acid at concentrations 6.0 and 8.0 ml / l when applied either alone or integrated with sulfur treatments which increased the chitinase activity more than 120.0 %. In field experiments results indicate that the most effective treatments were integrated treatments between humic acid followed by sulfur with all tested concentrations which reduced the early blight severity more than 83.6 %. Potato plants treated with sulfur at concentrations 3.0 or 4.0 g / l or Redomil – plus 2g / l resulted in reducing early blight severity more than 66.7 % during two succession growing seasons. Marked increase in potato yield was obtained with integrated treatments between humic acid followed by sulfur with all tested concentrations which increased the potato yield more than 59.3 % during the two growing seasons. It could be suggested that integrated treatment between humic acid followed by sulfur might be used commercially for controlling early blight disease of potato plants under field conditions.

Key words: Potato plants - Humic acid – Sulfur – Early blight- Integrated treatments

INTRODUCTION

Potato crop (*Solanum tuberosum* L.) is one of the most important vegetable crops in Egypt. Early blight caused by *Alternaria solani* is the most important disease attacking potato plants[1,2,3,4]. Controlling this disease depends mainly on fungicidal treatments [3, 5].

In Noubareia region (Egypt) severe infection with late and early blight diseases was recorded[4]. Controlling these diseases depend mainly on fungicidal application.

Therefore, recent efforts have been directed toward new approaches for controlling plant diseases that could be effective, reliable and safe for the environment.

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[6,7], stimulation plant growth through increased cell division, as well as optimized uptake of nutrients and water[8,9]. Moreover, humic acid stimulated the soil microorganisms[8,9,10]. Furthermore, Abd- El- Kareem[11], 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.

Only sulfur and copper can be used in organic farming for controlling plant diseases. Sulfur can be used as a preventive fungicide against several plant diseases[12,13]. Sulfur prevents fungal spores from germinating, so it must be applied before the disease develops for effective results[12].

The main objective of the present research are studying the effect of integrated treatments between humic acid followed by sulfur on early blight disease of potato plants under field conditions.

MATERIALS AND METHODS

Source of Pathogenic Fungus and Potato Tubers: Pathogenic isolate of *Alternaria solani* the causal agent of early blight disease was kindly provided by Plant Pathology Department, National Research Centre, Giza,

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Egypt. Potato tubers cv. Nigola were obtained from the Department of Vegetable Crop Research, Agricultural Research Centre, -Giza, Egypt.

Laboratory Experiments:

Testing of Different Concentrations of Humic Acid and Sulfur on Linear Growth of *Alternaria Solani*: Different concentrations of humic acid and sulfur were tested to study their inhibitory effect on linear growth of *A. solani*. Five concentrations of humic acid *i,e*. 0.0,2.0,4.0, 6.0 and 8.0 ml / l and sulfur at 1.0, 2.0,3.0, and 4.0 g/ l 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 *A. solani*. Five plates were used as replicates for each particular treatment. Inoculated plates were incubated at 25 ± 2 °C.The average linear growth of fungus was calculated after 10 days.

Greenhouse Experiments:

Testing of Different Concentrations of Humic Acid and Sulfur on Early Blight Severity of Potato Plants: Different concentrations of humic acid and wettable sulfur against early blight severity of potato plants was evaluated.

- Inoclum Preparation of A. Solani: Spore suspensions of *A. solani* were prepared by inoculated sterilized DA medium with disk (6 mm diameter) taken from 10 day old culture of *A. solani*. Disk (6 mm diameter) taken from 10 days old culture grown on DA was transferred to sterilized PDA medium. Plates were incubated at 25 °C and spores suspension (10^6 spores / ml) of *A. solani* was prepared.

- Potato Plants: Potato tubers cv. Nigola grown in plastic pots (30 cm –diameter) containing a sandy loam soil under greenhouse conditions (23 - 25 ^{O}C) were used when plants had 4-5 compound leaves. Three plants / pot and ten pots for each treatment were used. Irrigation and fertilization were carried out as needed.

–Treatments: Humic acid at five concentrations *i,e*. 0.0,2.0,4.0, 6.0 and 8.0 ml / l and sulfur at 0.0, 1.0, 2.0,3.0, and 4.0 g/ l were applied as foliar spray to study their effects against early blight disease of potato plants which had 4-5 compound leaves. Plant inoculation was carried out after 5 days of chemical treatments by spraying potato plants with spore suspensions (10^6 spores / ml) of *A. solani*. Plants sprayed with tap water was served as control. Treated inoculated potato plants were incubated at 23-25 ^{O}C.

Testing of Integrated Treatment Between Humic Acid and Sulfur on Early Bight Severity of Potato Plants: Humic acid at concentrations of 6.0 & 8.0 ml / l and sulfur at 3 & 4 g / l were tested alone or integrated between them to study their effect on early blight severity of potato plants.

Potato plants had 4-5 compound leaves were sprayed with humic acid at 6 or 8 ml /l followed by sulfur at 3 or 4 g / l with 3 days interval. Plant inoculation was carried out after 5 days of sulfur treatments by spraying potato plants with spore suspensions (10^6 spores/ml) of *A. solani*. Plants sprayed with tap water were served as control. Inoculated potato plants were incubated at 23-25 ^{O}C under greenhouse conditions.

Disease Assessment: Early blight scale from 0 to 4 according to Cohen *et al*.,[14] based on the leaf area infected was used, 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.

Disease was recorded after 20 days of inoculation.

Testing of Integrated Treatments Between Humic Acid and Sulfur on Chitinase Activity of Potato Plants: Humic acid at concentrations 6.0 & 8.0 ml / l and sulfur at 3 & 4 g / l were tested alone or integrated between them to study their effect on chitinase activity of potato plants.

Extraction of Chitinase Enzyme: Chitinase activity was determined after 10 days of inoculat**ion.** Extraction of enzyme from potato leaves was done according to method of Tuzun *et al*.,[15].

Chitinase Assay: Chitinase activity was determined by colourimetric method of Boller and Mauch,[16]. Colloidal chitin was used as a substrate and dinitrosalicylic acid as reagent to measure reducing sugars.

Chitinase activity was expressed as mM N-acetylglucose amine equivalent released / gram fresh weight tissue / 60 minutes.

Field Experiments:

Testing of Integrated Treatments Between Humic Acid and Sulfur on Early Blight Severity and Tuber Yield of Potato Plants under Field Conditions: Experiments were carried out, at the Experimental Farm of National Research Centre at El-Noubareia, Behera Governorate, Egypt.

The promising treatments in pot experiments were applied under field conditions to study their effect against early blight disease in addition to their effect on potato yield during two seasons. Field experiments were conducted under natural infection in plots (4 x 10 m) each comprised of 8 rows (40 holes / row) in a randomized complete block design with three replicates (plots) for each treatment..

Treatments: Humic acid at concentrations 6.0 & 8.0 ml / l and sulfur at 3 & 4 g / l were tested alone or integrated between in addition to the Fungicides (Redomil – plus at 2 g / l) were applied as follows : -

Treatments	
Single	Integrated
1- Humic acid 6 ml/ l	1- Humic acid 6 ml / l + Sulfur 3 g / l
2- Humic acid 8 ml / l	2- Humic acid 6 ml / l + Sulfur 4 g / l
3- Sulfur 3 g / l	3- Humic acid 8 ml / l + Sulfur 3 g / l
4- Sulfur 4 g / l	4- Humic acid 8 ml / l + Sulfur 4 g / l
5-Fungicide (Redomil plus 2 g / l)	
6- Un-treated plants (control)	

Application: Single or integrated treatments were applied as foliar application on potato plants which had 4-5 compound leaves and every 15 days up to 90 days of planting. Integrated treatments between humic acid and sulfur was carried out by spraying potato plants with humic acid at 6 or 8 ml /l followed by sulfur at 3 or 4 g / l with 3 days interval.

Disease Assessment: Early blight scale was used as mentioned before and disease was recorded up to 90 days of planting.

Determination of Tuber Yield: Tuber yield of potato (kg /m^2) for each treatment was determined.

Statistical Analysis: Tukey test for multiple comparisons among means was utilized[17].

Results:

Effect of Different Concentrations of Humic Acid and Sulfur on Linear Growth of *Alternaria Solani:* Five concentrations of humic acid *i,e*. 0.0,2.0,4.0, 6.0 and 8.0 ml / l and sulfur at 1.0, 2.0,3.0, and 4.0 g/ l were tested to study their inhibitory effect against *A. solani*. Results in Table (1) indicate that all tested concentrations of humic acid had no inhibitory effect on *A. solani*. Meanwhile, sulfur at concentrations 3.0 and 4.0 g / l caused complete reduction in linear growth of *A. solani.* Sulfur at 2.0 g / l showed moderate effect which reduced the linear growth by 62.2 %.

Greenhouse Experiments:

Effect of Humic Acid and Sulfur on Early Blight Severity of Potato Plants: Humic acid at five concentrations *i,e*. 0.0,2.0,4.0, 6.0 and 8.0 ml / l and sulfur at 0.0, 1.0, 2.0,3.0, and 4.0 g/ l were applied as foliar spray to study their effects against early blight disease of potato plants. Results in Table (2) indicate that all treatments significantly reduced the disease severity. The most effective treatments are humic acid at concentrations 6.0 & 8.0 ml / l and sulfur at 3.0 & 4.0 g/ l which reduced the early blight severity more than 55.9 %. Followed by humic acid at concentrations 4.0 ml / l and sulfur at 2.0 g/ l which reduced the early blight severity more than 41.1%. Meanwhile other treatments showed less effect.

Effect of Integrated Treatment Between Humic Acid and Sulfur on Early Bight Severity of Potato Plants: Humic acid at concentrations of 6.0 & 8.0 ml / l and sulfur at 3 & 4 g / l were tested alone or integrated between them to study their effect on early blight severity of potato plants. Results in Table (3) indicate that all treatments significantly reduced the disease severity.The highest reduction in disease severity was obtained with integrated treatments between humic acid followed by sulfur treatments with all tested concentrations of both treatments which reduced the early blight severity more than 85.7 %. Meanwhile single treatments of humic acid and sulfur showed moderate effect.

Effect of Integrated Treatment Between Humic Acid and Sulfur on on Chitinase Activity of Potato Plants: Humic acid at concentrations 6.0 & 8.0 ml / l and sulfur at 3 & 4 g / l were tested alone or integrated between them to study their effect on chitinase activity of potato plants. Results in Table (4) indicate that all treatments increased the chitinase activity of potato plants. The highest increase was obtained with humic acid at concentrations 6.0 and 8.0 ml / l when applied either alone or integrated with sulfur treatments which increased the chitinase activity more than 120.0 %. Meanwhile single treatment of sulfur caused moderate increase of chitinase activity.

Field Experiments:

Effect of Integrated Treatment Between Humic Acid and Sulfur on Early Blight Severity and Tuber Yield of Potato Plants under Field Conditions: Humic acid at concentrations 6.0 & 8.0 ml / l and sulfur at 3 & 4 g / l were tested alone or integrated between them in addition to the Fungicides (Redomil – plus at 2 g / l) were applied under field conditions to study their effect against early blight disease in addition on potato yield during two seasons.

Table 1: Effect of humic acid and sulfur solution on linear growth of *Alternaria solani*

Treatments	Concentrations	Linear growth	Reduction %
Humic acid (ml / l)	2.0	90.0 a	0.0
	4.0	90.0 a	0.0
	6.0	90.0 a	0.0
	8.0	90.0 a	0.0
Sulfur (g / l)	1.0	61.5 b	31.7
	2.0	34.0c	62.2
	3.0	0.0d	100
	4.0	0.0d	100
Control	0.0	90.0a	-----

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

Table 2: Early blight severity [2] in potato plants as affected with different concentrations of humic acid and sulfur under greenhouse conditions

Treatments	Conc.	Early blight severity	Reduction %
Humic acid (ml / l)	2	2.5 b [1]	26.5
	4	2.0 c	41.1
	6	1.5 d	55.9
	8	1.4 d	58.8
Sulfur (g/ l)	1	2.3 b	32.4
	2	1.8 c	47
	3	1.3 d	61.8
	4	1.3 d	61.8
Control	0	3.4 a	---

1- Figures with the same letter are not significantly different (P= 0.05)
2 - Early blight scale from 0 to 4 according to Cohen *et al*.,(1991).

Table 3: Effect of integrated treatments between humic acid and sulfur on early blight severity of potato plants under greenhouse conditions

Treatments	Early blights severity [2]	Reduction %
Single treatments		
Humic acid 6 ml/ l	1.6 b[1]	54.4
Humic acid 8 ml / l	1.6 b	54.3
Sulfur 3 g / l	1.4 b	60
Sulfur 4 g / l	1.3 b	57.1
Integrated treatments		62.8
Humic acid 6 ml / l + Sulfur 3 g / l	0.5 c	85.7
Humic acid 6 ml / l + Sulfur 4 g / l	0.4 c	88.6
Humic acid 8 ml / l + Sulfur 3 g / l	0.4 c	88.6
Humic acid 8 ml / l + Sulfur 4 g / l	0.3 c	91.4
Control	3.5 a	

1- Figures with the same letter are not significantly different (P= 0.05)
2 - Early blight scale from 0 to 4 according to Cohen *et al*.,(1991).

a- Effect on Early Blight Severity: Results in Table (5) indicate that all treatments significantly reduced the disease severity during two growing seasons.The most effective treatments are integrated treatments between humic acid followed by sulfur with all tested concentrations of both treatments which reduced the early blight severity more than 83.6 %. Potato plants treated with sulfur at concentrations 3.0 or 4.0 g / l or Redomil – plus 2g / l resulted in reducing early blight severity more than 66.7 %. Meanwhile, single treatments of humic acid showed moderate effect.

Table 4: Effect of integrated treatments between humic acid and sulfur on chitinase activity of potato plants greenhouse conditions

Treatments	Chitinase activity [2]	Increase %
Single treatments		
Humic acid 6 ml / l	5.6 a[1]	124
Humic acid 8 ml / l	5.6 a	124
Sulfur 3 g / l	4.0 b	60
Sulfur 4 g / l	4.1 b	64
Integrated treatments		
Humic acid 6 ml / l + Sulfur 3 g / l	5.5 a	120
Humic acid 6 ml / l + Sulfur 4 g / l	5.6 a	124
Humic acid 8 ml / l + Sulfur 3 g / l	5.7 a	128
Humic acid 8 ml / l + Sulfur 4 g / l	5.7 a	128
Control	2.5 c	---

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

(2) Chitinase activity expressed as mM N-acetyl glucose amine equivalent released/ gram fresh weight/ 60 min .

Table 5: Effect of integrated treatments between humic acid and sulfur on early blight severity [2] of potato plants under field conditions

Applications	First season		Second season	
	Disease severity	Reduction %	Disease severity	Reduction %
Single treatments				
Humic acid 6ml / l	1.2 bc [1]	60	1.4 b	60
Humic acid 8ml / l	1.3 b	56.7	1.3 b	62.9
Sulfur 3 g / l	1.0 cd	66.7	1.0 c	71.4
Sulfur 4 g / l	0.8 d	73.3	1.0 c	71.4
Integrated treatments				
Humic acid 6 ml / l + Sulfur 3 g / l	0.4 e	86.7	0.6 d	83.6
Humic acid 6 ml / l + Sulfur 4 g / l	0.3 e	90	0.5 d	85.7
Humic acid 8 ml / l + Sulfur 3 g / l	0.3 e	90	0.4 d	88.6
Humic acid 8 ml / l + Sulfur 4 g / l	0.2 e	93.3	0.4 d	88.6
Redomil – plus 2g / l	0.8 d	73.3	1.0 c	71.4
Control		3		3.5 a

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

2 - Early blight scale from 0 to 4 according to Cohen *et al*.,(1991).

b- Effect on Tuber Yield: Results in Table (6) indicate that all treatments significantly increased the tuber yield during two growing seasons.The highest increase was obtained with integrated treatments between humic acid followed by sulfur treatments with all tested concentrations of both treatments which increased the potato yield more than 59.3 % during two growing seasons. Single treatments of humic acid at concentration 8.0 g / l and Redomil – plus 2g / l caused increased in tuber yield more than 48.1 %. Meanwhile both concentrations of sulfur treatments were less effective.

Discussion: Potato crop is one of the most important vegetable crops in Egypt. Early blight caused by *Alternaria solani* is the most important disease attacking potato plants[1,2,3,18]. Controlling this disease depends mainly on fungicidal treatments[5]. Severe infection with late and early blight diseases was showed in Noubareia region (Egypt)[3]. In the present study integrated treatments between humic acid as resistance inducer and sulfur as preventive safe chemical against early blight disease was evaluated.

Humic acid can be applied successfully in many areas of plant production as a plant growth stimulant, soil conditioner,*i.e.* enhanced natural resistance against plant diseases and pests[6,7]. Present results indicate that humic acid at concentrations 6 or 8 ml / l had no inhibitory effect against *Alternaria solani* and reduced early blight severity of potato plants under greenhouse and field conditions. In this respect, Scheuerell and Mahaffee[6,7] reported that the most effective treatments for suppression damping off in many plants and 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

Table 6: Effect of integrated treatments between humic acid and sulfur on tuber yield of potato plants under field conditions

Applications	First season		Second season	
	Yield %	Increase (kg /m2)	Yield %	Increase (kg /m2)
Single treatments				
Humic acid 6g/ l	3.8 bc (1)	40.7	3.5 c	40
Humic acid 8g/ l	4.0 b	48.1	4.0 b	60
Sulfur 3 g / l	3.7 c	37	3.4 c	36
Sulfur 4 g / l	3.7 c	37	3.5 c	40
Integrated treatments				
Humic acid 6 ml / l + Sulfur 3 g / l	4.4 a	59.3	4.2 a	68
Humic acid 6 ml / l + Sulfur 4 g / l	4.5 a	63	4.3 a	72
Humic acid 8 ml / l + Sulfur 3 g / l	4.5 a	63	4.4 a	76
Humic acid 8 ml / l + Sulfur 4 g / l	4.6 a	70.4	4.4 a	76
Redomil – plus 2g / l	4.0 b	48.1	4.0 b	60
Control	2.7 a		2.5 d	

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

potato plants may be due to enhanced natural resistance against plant diseases and pests[6,7]. In present study results indicate that the highest increase in chitinase activity was obtained with humic acid at concentrations 6.0 and 8.0 ml / l when applied either alone or integrated with sulfur treatments which increased the chitinase activity more than 120.0 %. In this respect, *B*-1,3-glucanases and chitinases are able to hydrolyze *B*-1,3-glucan and chitin, respectively, the major components of fungal cell walls[18,19,20,21]. Abd- El-Kareem,[11] 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.

On the other hand, humic acid stimulated plant growth through increased cell division, as well as optimized uptake of nutrients and water[8,9,22] and stimulated the soil microorganisms[8,9,23].

Sulfur can be used as a preventive fungicide against several diseases[13]. In present study, sulfur at concentrations 3.0 and 4.0 g / l caused complete reduction in linear growth of *A. solani.*

While, under field conditions, results indicate that integrated treatments between humic acid followed by sulfur reduced the early blight severity more than 83.6 % in addition to marked increase in potato yield was showed. Sulfur treatment reduced the disease severity due to its fungicidal activity. In this respect, Ellis, and Bradley[12] reported that sulfur prevents fungal spores from germinating and must be applied before the disease develops for effective results.

It could be suggested that integrated treatment between humic acid followed by sulfur might be used commercially for controlling early blight disease of potato plants under field conditions.

REFERENCES

1. Waals, J.E, L. Korsten and B. Slippers, 2004. Genetic Diversity Among *Alternaria solani* Isolates from Potatoes in South Africa. Plant Dis., 88: 959-964.
2. Pasche, J.S., C.M. Wharam and N.C. Gudmestad, 2004. Shift in Sensitivity of *Alternaria solani* in Response to Q(o)I Fungicides. Plant Dis., 88: 181-187.
3. Pasche, J.S., L.M. Piche and N.C. Gudmestad, 2005. Effect of the F129L Mutation in *Alternaria solani* on Fungicides Affecting Mitochondrial Respiration. Plant Dis., 89: 269-278.
4. El-Gamal, G. Nadia, F. Abd-El-Kareem, Y.O. Fotouh and El- Mougy, S. Nehal, 2007. Induction of systemic resistance in potato plants against late and early blight diseases using chemical inducers under greenhouse and field conditions. Research J. of Agricultural and Biological Science, 3(2): 73- 81.
5. Abd-El-Kareem, F., M.A. Abd-Alla and R.S.R. El-Mohamedy, 2002. Induced resistance in potato plants for controlling Early blight disease under field conditions. Egypt. J. App.Sci., 17(2): 51-66.
6. Scheuerell, S.J. and W.H. Mahaffee, 2004. Compost Tea as a Container Medium Drench for Suppressing Seedling Damping-Off Caused by Pythium ultimum. Phytopathology, 94: 1156-1163.
7. Scheuerell, S.J. and W.H. Mahaffee, 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.

8. Atiyeh, R.M., S. Lee, C.A. Edwards, N.Q. Arancon and J.D. Metzger, 2002. The influence of humic acids derived from earthworm processed organic wastes on plant growth. Bioresource Technology, 84: 7-14.
9. Chen, Y., M. De Nobili, T. Aviad, 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.
10. Qualls, R.G., 2004. Biodegradability of humic substances and other fractions of decomposing leaf litter. Soil Science Society of America Journal, 68: 1705-1712.
11. Abd-El-Kareem, F., 2007. 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.
12. Ellis, W. Barbara and Fern Marshall Bradley, 1992. The Organic Gardener's Handbook of Natural Insect and Disease Control. Rodale Press. Emmaus, PA., pp: 534.
13. Scherm, H. and A.T. Savelle, 2001. Control of Peach Scab with Reduced Midseason Fungicide Programs. Plant Dis., 85: 706-712.
14. Cohen, Y., U. Gisi and E. Mosinger, 1991. Systemic resistance of potato plants against *Phytophthora infestans* induced by unsaturated fatty acids. Physiol. Mol. Plant Pathol., 38: 255-263.
15. Tuzun, S., M.N. Rao, U. Vogeli, C.L. Schardl and J. Kuc, 1989. Induced systemic resistance to blue mould: Early induction and accumulation of, 1,3-β-glucanases chitinase and other pathogenesis proteins (b-proteins) in immunized tobacco. Phytopathology, 79: 979-983.
16. Boller, T. and F. Mauch, 1988. Colourimetric assay for chitinase. Methods in Enzymology, 161: 430-435.
17. Neler, J., W. Wassermann and M.H. Kutner, 1985. Applied linear statistical models. Regression, analysis of variance and experimental design: 2nd Ed. Richard, D. Irwin Inc. Homewood Illionois.
18. Abd-El-Kareem, F., 2007. Potassium or sodium bicarbonates in combination with Nerol for controlling early blight disease of potato plants under laboratory, greenhouse and field conditions. Egypt. J.of Phytopathol., 35: 73- 86.
19. Kauffmann, S., M. Legrand, P. Jeoffroy and B. Fritig, 1987. Biological function of pathogenesis-related proteins. Four PR-proteins of tobacco have *B*-1,3-glucanase activity. EMBO J., 6: 3209-3212.
20. Legrand, M., S. Kauffmann, P. Jeoffroy and B. Fritig, 1987. Biological function of pathogenesis-related proteins; Four tobacco pathogenesis-related proteins are chitinases. Proc. Natl. Acad. Sci., 84: 6750-6754.
21. Abd-El-Kareem, F., El-Mougy, S. Nehal, El-Gamal, G. Nadia and Y.O. Fotouh, 2006. Use of chitin and chitosan against tomato root rot disease under greenhouse conditions. Research J. of Agricultural and Biological Science, 2(4): 164- 169.
22. Delgado, A., A. Madrid, S. Kassem, L. Andreu, M. Campillo, 2002. Phosphorus fertilizer recovery from calcareous soils amended with humic and fulvic acids. Plant and Soil, 245: 277-286.
23. Garcia, J.C., C. Plaza, N. Senesim G. Brunetti and A. Polo, 2004. Effects of sewage sludge amendment on humic acids and microbiological properties of a semiarid Mediterranean soil. Biology and Fertility of Soils, 39: 320-328.