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Induction of Systemic Resistance in Potato Plants Against Late and Early Blight Diseases Using Chemical Inducers under Greenhouse and Field Conditions

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Abstract: Late and early blight caused by *Phytophthora infestans* and *Alternaria solani* are the most serious diseases attacking potato plants. Ascorbic acid (AA), Dichloro-isonicotinic acid (INA) ethylene diamine tetra acetic acid (EDTA) and calcium chloride were evaluated against late and early blight diseases under greenhouse and field conditions. Under greenhouse conditions, the highest reduction in diseases severity was obtained by AA at 2 & 3 g/l, and INA at 50.0 and 100 mM , they reduced late and early blight severity more than 75.0 and 82.1%, followed by EDTA and Calcium chloride, respectively. On the other hand the combined treatments between AA at 2 g/l and INA at 50 or 100 mM reduced the late and early diseases severity between (86.6-90.0 %) and (90.0-93.3 %), respectively. These treatments showed an increase in activity of both *ß*-1,3-glucanase and chitinase as 115 and 109 %, respectively. Similar trend was observed under field conditions, the most effective inducers were combination treatments between AA at 2.0g/l and INA at 50.0 or 100 mM which reduced the late and early blight severity more than 88.7 and 86.1 % and increased tuber yield from 70.0 up to 83.3 % during the two successive winter and summer cultivation seasons. Individual treatments of AA and INA showed moderate effect on reduction in diseases severity and yield increase, although they were superior to Calcium chloride as well as fungicide Redomil-plus in this concern. It could be suggested that combined treatments between ascorbic acid and INA as applicable, safe, cost effective and fungicide alternative might be used for controlling late and early blight diseases of potato plants.

Key words: Ascorbic acid-Calcium chloride-chemical inducers-Early blight- EDTA-INA-Late blight-Potato

INTRODUCTION

Potato plants (*Solanum tuberosum* L.) considered one of the most important vegetable crops overall the world. Late and early blight caused by *Phytophthora infestans* and *Alternaria solani* are the most important diseases attacking potato plants[1,2,3,4]. Control of theses diseases depends mainly on fungicidal treatments. In order to avoid the environmental pollution fungicide alternatives are needed[5,6].

Some chemicals were reported as resistance inducers against plant diseases. In this regard, the content of ascorbic acid in plant tissues has been associated with resistance to some diseases[7]. Also, Ascorbic acid was reported for inducing resistance in many plants against root rot nematodes[8] and reducing fungal diseases[9-12]. Moreover, EDTA (ethylene diamine tetra acetic acid) was reported to induce resistance in broad been against rust disease[13]. Pre and post-harvest calcium applications have been used to delay aging or ripening to reduce postharvest decay and control of many diseases in fruits and vegetables[14,15]. Foliar application of calcium chloride has been also reported to delay ripping and control mould disease in strawberries[16].

Furthermore, Dicloro-isonicotinic acid (INA) is efficient agent that protect several crops against viruses, bacteria, and fungal infection [17-19]. Abd-El-Kareem *et al.*[20] reported that INA at 100 and 150 mg/l caused high reduction in powdery mildew and Alternaria leaf spot diseases of squash plants under greenhouse and field conditions.

The main objective of the present research is to study the efficacy of different chemicals as resistance inducers against early and late blight incidence of potato plants under greenhouse and field conditions and their influence on tuber yield.

MATERIALS AND METHODS

Source of Pathogenic Fungi and Potato Tubers: One Pathogenic isolate of each *Alternaria solani* and *Phytophthora infestans* the causal agents of early and late blight diseases, respectively were kindly obtained from Plant Pathology Dept., National Research Centre, Giza, Egypt .Meanwhile, Potato tubers cv. Diamond

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were obtained from Dept., of Vegetables Crop Research, Agricultural Research Centre, Giza, Egypt.

Greenhouse Experiments:

Efficacy of Some Chemical Inducers on Early and Late Blight Incidence of Potato Plants under Greenhouse Conditions:

1- Fungal Inocula Prepation: Spores or sporangia suspension of *A. solani* and *P. infestans* were prepared by inoculated sterilized PDA or rye agar medium[21] with disk of fungal growth (6 mm diameter) taken from 10 days old cultures of *A. solani* or *P. infestans*. Plates were incubated at 25 or 18 °C, respectively. Sporangia sustention (2 x 10^4 sporangia/ml) of *P. infestans* and spores suspension (10^6 spores/ml) of *A. solani* were prepared.

2- Potato Plants: Potato seeds (*Solanum tuberosum* L.) cv. Diamond were planted in plastic pots (30 cm-diameter) containing sandy loam soil under greenhouse conditions (18-25 °C). Emerged potato plants were grown untile they had 4-5 compound leaves.

3- Chemical Applications:

A- Individual Inducers: Different concentrations of chemical inducers, *i.e.* Ascorbic acid at 1.0, 2.0 and 3.0 g/l; EDTA at 0.25, 0.50 and 1.0 g/l; Calcium chloride at 5.0, 10.0 and 15.0 g/l and INA at 50.0, 100.0 and 150.0 mM were applied as foliar spraying to study their effects against early and late blight of potato plants which had 4-5 compound leaves. Plants inoculation with the prepared fungal suspensions was carried out after 5 days of chemical treatments.

B- Combined Treatments: Different concentrations of Ascorbic acid, *i.e.* 1.0, 2.0 and 3.0 g/l combined with each of 50.0, 100.0 and 150.0 mM of INA were used to study their effects against early and late blight diseases severity of potato plants. Potato plants had 4-5 compound leaves were sprayed with ascorbic acid concentrations. After 7 days the same plants were sprayed with INA concentrations in alternative order. Plant inoculation was carried out as mentioned above. Plants sprayed with tap water was served as control. Three plants/pot and ten pots for each treatment were used. Traditional agricultural practices, *i.e.* irrigation and fertilization were carried out as needed.

Disease Assessment: Early and late blight scale from 0 to 4 according to Cohen *et al*.,[21] was followed, whereas 0 = No leaf lesions; 1 = 25% or less; 2 = 26-50 %; 3 = 51-75 % and 4 = 76-100 % infected area of plant leaf. Diseases symptoms were recorded after 20 days of inoculation.

Enzymes Activity: The previous used concentrations of chemical inducers, *i.e.* Ascorbic acid; EDTA; INA and Calcium chloride in addition to combined treatments between ascorbic acid at 2 g/l and INA at 50 or 100 mM were tested for studying their effects on chitinase and *ß*-1,3-glucanase activities of potato plants.

Extraction of Enzyme: Chitinase and *ß*1,3-glucanase activities were determined after 10 days of inoculation potato plants with *P. infestans* and *A. solani* **.** The enzyme extracted of potato leaves and the supernatant was prepared according to method of Tuzun *et al.,*[22].

Chitinase Assay: Chitinase activity was determined by colourimetric method of Boller and Mauch,[23]. Colloidal chitin was used as 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.

***ß*-1,3-glucanase Assay:** The method of Abeles *et al.*[24] was used to determine *ß*-1,3-glucanase activity. Laminarin was used as substrate and dinitrosalicylic acid as reagent to measure reducing sugars. *ß*-1, 3-glucanase activity was expressed as mM glucose equivalent released gram fresh weight tissues/60 minutes.

Field Experiments: Experiments were carried out, in Experimental Farm of National Research Centre at El-Noubareia region, Behera governorate, Egypt.

The promising treatments in pot experiments were applied under field conditions to study their effects against late and early blight diseases during winter and summer cultivation seasons under natural infection. Potato yield was also determined at the two cultivation seasons.

Field experiments consisted of plots (4x8 m) each comprised of 8 rows and 32 holes/row, was conducted in a Completely Randomized Block design with three replicates)plots(for each particular treatment.

Two concentrations of each chemical inducers, *i.e.* ascorbic acid at 2.0 and 3.0 g/l; EDTA at 0.50 and 1.0 g/l; Calcium chloride at 5.0, and 10.0 g/l and INA at 50.0 and 100.0 mM, in addition to combined treatments between ascorbic acid at 2.0 g/l and INA at 50 or 100 mM were tested. Fungicide (Redomyl-plus at 2 g/l) were applied as comparison treatment.

Individual chemical inducers were applied twice as foliar spray at 4-5 and 10-12 leaf growth stages of potato plants. The combined treatments were also applied at the same stages started with ascorbic acid followed by INA after 7 days. Meanwhile Redomyl-plus applied 4 times with 15 days intervals started at stage of 4-5 leaf.

Early and blight disease assessment scale was used as mentioned before and both diseases were recorded up to 90 days of planting. Tuber yield of potato (kg /m^2) for each treatment was determined.

Statistical Analysis: Tukey test for multiple comparisons among means was utilized after Neler *et al*.[25].

RESULTS AND DISCUSSIONS

Greenhouse Experiments: Efficacy of some chemical inducers on early and late blight severity of potato plants under greenhouse conditions was evaluated. Results in Table (1) indicate that all treatments significantly reduced the early and late blight disease severity of potato plants. The highest reduction was obtained with AA at 2 & 3 g/l and INA at 50.0 and 100 mM, they reduced late and early blight as (75.0 to 78.1 %) and (82.1 to 85.7 %), respectively. All concentrations of EDTA reduced diseases severity between (50.0 to 60.7 %). Meanwhile Calcium chloride showed less effect on the diseases severity ranged between (28.1 to 60.7 %).

The effect of combined treatments between Ascorbic acid and INA inducers against diseases severity of potato plants was recorded. Results in Table (2) indicate that all treatments reduced late and early blight severity. The great reduction was obtained with combined treatments between AA at 2 g/l with INA at 50 or 100 mM which reduced the late and early diseases as (86.6-90.0 %) and (90.0-93.3 %), respectively followed by treatments of AA at 3 g/l and INA at 50 or 100 mM which reduced the diseases severity between 56.6 to 83.3 % as compared with untreated plants. Meanwhile single treatments showed moderate effect or less.

Enzymes Activity: Chitinase and ß-1,3-glucanase activities of potato plants inoculated with *A. solani* and *P. infestanse* in response to chemical inducers were recorded. Results in Table (3) indicate that all chemical inducers stimulated the activities of both enzymes. The most effective treatments were the combined treatments between AA at 2 g/l with INA at 50 or 100 mM, they increased the *ß*-1,3-glucanase and chitinase more than 115 and 109 %, respectively in potato plants inoculated with *A. solani*. High increase was also observed with individual treatments of ascorbic acid at 2.0 and 3.0 g/l, EDTA at 1.0 g/l, and INA at 50.0 and 100.0 mM which increased the *ß*-1,3-glucanase and chitinase between 100-110 %. Meanwhile calcium chloride had moderate effect ranged between 60-70 %. Chitinase and ß-1,3-glucanase activities of potato plants inoculated with *P. infestanse* followed the same trend in lesser increase.

Table 1: Effects of different chemical inducers on late and early blight disease severity of potato plants under greenhouse conditions.

Chemical inducers	Concentration	Disease severity			
		Late blight	Reduction %	Early blight	Reduction %
Ascorbic acid	1.0 g / l	1.4 c*	56.3	1.0 bc	60.7
	2.0 g / l	0.7 d	78.1	0.5 d	82.1
	3.0 g / l	0.8 d	75.0	0.4 d	85.7
EDTA	0.25 g / l	1.6 c	50.0	1.2 b	57.1
	0.50 g / l	1.6 c	50.0	1.3 b	53.6
	1.0 g / l	1.4 c	56.3	1.0 bc	60.7
Calcium chloride	5.0 g / l	2.3 b	28.1	1.2 b	57.1
	10.0 g / l	2.2 b	31.3	1.0 bc	60.7
	15.0 g / l	2.3 b	28.1	1.4 b	50.0
INA	50 mM	0.7 d	75.0	0.4 d	85.7
	100 mM	0.7 d	75.0	0.5 d	82.1
	150 mM	1.4 c	56.3	1.2 b	57.1
Control		2.3 a	-	2.8 a	

*Figures with the same letter are not significantly different (P= 0.05)
Early and late blight scale from 0 to 4 according to Cohen *et al*.[21]

Table 2: Effects of combined treatments between ascorbic acid and INA against early and late blight severity of potato plants under greenhouse conditions.

Treatment		Disease severity			
Ascorbic acid (g/l)	INA (mM)	Late blight	Reduction %	Early blight	Reduction %
1.0	0.0	1.9 b*	36.6	2.0 b	33.3
	50	1.3 c	56.6	0.9 de	70.0
	100	1.5 bc	0.0	0.9 de	70.0
	150	1.5 bc	50.0	1.2 cd	60.0
2.0	0.0	1.2 c	60.0	0.9 de	70.0
	50	0.4 d	86.6	0.3 f	90.0
	100	0.3 d	90.0	0.2 f	93.3
	150	1.3 c	56.6	1.1 cd	63.3
3.0	0.0	1.4 cb	53.3	1.5 c	50.0
	50	1.3 c	56.6	0.4 f	86.6
	100	1.2 c	60.0	0.5 e f	83.3
	150	1.2 c	60.0	1.2 cd	60.0
0.0	0.0	3.6 a	-	3.0 a	-
	50	1.2 c	60.0	1.0 cd	66.6
	100	1.2 c	60.0	1.0 cd	66.6
	150	1.5 bc	50.0	1.3 cd	56.6

* Figures with the same letter are not significantly different (P= 0.05)
Early and late blight scale from 0 to 4 according to Cohen *et al*.[21]
Combined treatment with INA was carried out as seven days after ascorbic acid application.

Field Experiments: The promising treatments in pot experiments were applied under field conditions were evaluated against late and early blight diseases severity in addition to potato yield production during two successive winter and summer cultivation seasons. Different concentrations of chemical inducers *ie* ascorbic acid at 2.0 and 3.0 g/ l , EDTA at 0.50 and 1.0 g/l , Calcium chloride at 5.0 , and 10.0 g/l and INA at 50.0 ,and 100.0 mM ,in addition to combined treatments between ascorbic acid at 2 g/ and INA at 50 or 100 mM were used. Redomyl-plus at 2 g / l was applied as comparison treatment.

Winter Cultivation Seasons:

a- Effects on Late Blight Severity: Results in Table (4) indicate that all treatments reduced the late blight disease severity during two successive grown seasons. High reduction was obtained with combined treatments between AA at 2.0 and INA at 50.0 or 100 mM, which reduced the disease severity between 88.7 and 93.3 %. Individual treatment of AA at 2.0 & 3.0 g / l and INA at 100 mM reduced the late blight disease severity 74.3 and 80.0 %. The fungicidal treatment recorded 71.4-73.3 % reduction in disease severity, which was superior to EDTA treatment. Calcium chloride showed the lowest reduction records which were between 37.1 to 43.3 %.

b-Effects on Tuber Yield: Results in Table (5) indicate that all treatments increase potato yield during the two winter growing seasons. The highest increase was obtained with combined treatments between AA at 2.0 and either INA at 50.0 or 100 mM, which recorded as 70 % up to 81.8 %. Individual treatments of AA at 2.0 & 3.0 g/l and INA at 100 mM, increased tuber yield between (63.3-75.0 %) and (65.0-72.7), respectively. The moderate increase was obtained with the other treatments as well as fungicidal application. Calcium chloride showed the lowest increase of potato yield which was between 36.4 to 50.0 % at the two seasons.

Table 3: Effects of chemical inducers on *ß*-1,3-glucanase[1] and chitinase [2] activities of potato plants inoculated with *A. solani* and *P. infestanse* under greenhouse conditions.

Chemicla inducers	Concentrations	Enzymes activities							
		Early blight				Late blight			
		β-1,3-glucanase	Increase %	Chitinase	Increase %	β-1,3-glucanase	Increase %	Chitinase	Increase %
Ascorbic acid	1.0 g / l	3.4	70	1.7	54.5	2.5	60	1.6	56.3
	2.0 g / l	4.2	110	2	81.8	2.8	60	1.6	75
	3.0 g / l	4	100	2.1	90.9	2.7	40	1.4	68.8
EDTA	0.25 g / l	3.8	90	1.8	63.6	2.4	40	1.4	50
	0.50 g / l	3.8	90	2.2	100	2.4	40	1.4	50
	1.0 g / l	4.1	105	2.2	100	2.6	50	1.5	62.5
Calcium chloride	5.0 g / l	3.4	70	1.8	63.6	2.3	30	1.3	43.8
	10.0 g / l	3.2	60	1.7	54.4	2.2	30	1.3	37.5
	15.0 g / l	3.2	60	1.6	45.5	2.4	40	1.4	50
INA	50.0 mM	3.8	90	1.9	72.7	2.8	50	1.5	75
	100 mM	4	100	2	81.8	3	60	1.6	87.5
	150 mM	4.2	110	2.2	100	3.1	50	1.5	93.8
AA+ INA	2g/l + 50 mM	4.3	115	2.3	109	3.3	80	1.8	106.3
AA+INA	2g/l+100mM	4.3	115	2.3	109	3.5	80	1.8	118.8
Control		2	—	1.1	—	1.6	--	1	--

(1) ß-1,3-glucanase activity expressed as mM glucose equivalent released/gram fresh weight/ 60 min .
(2) Chitinase activity expressed as mM N-acetyl glucose amine equivalent released/gram fresh weight/ 60 min.

Table 4: Effects of different chemical inducers on late blight severity of potato plants during two successive winter cultivation seasons under field conditions.

Chemical inducers	Concentration	Diseases severity			
		First season		Second season	
		Late light	Reduction%	Late blight	Reduction%
Ascorbic acid	2.0 g/l	0.7 d*	76.7	0.9 d	74.3
	3.0 g/l	0.6 d	80	0.8 d	77.1
EDTA	0.50 g/l	1.0 c	63.3	1.5 c	57.1
	1.0 g/l	0.9 c	70	1.4 c	60
Calcium chloride	5.0 g/l	1.8b	40	2.2b	37.1
	10.0 g/l	1.7b	43.3	2.2b	42.6
INA	50.0 mM	1.1 c	63.3	1.1 c	68.6
	100 mM	0.6 d	80	0.8 d	77.1
AA + INA	2g/l + 50 mM	0.3 ed	90	0.4 e	88.7
AA + INA	2g/l + 100mM	0.2 e	93.3	0.3 e	91.4
Redomil-plus	2 g /l	0.8cd	73.3	1.0 d	71.4
Untreated Control		3.0 a	--	3.5 a	--

* Figures with the same letter are not significantly different (P= 0.05)
Late blight scale from 0 to 4 according to Cohen *et al.*[21]

Table 5: Potato yield in response to different chemical inducers during two successive winter cultivation seasons.

Chemical inducers	Concentration	First season		Second season	
		Yield (kg /m^2)	Increase%	Yield (kg /m^2)	Increase%
Ascorbic acid	2.0 g/ l	3.4	70	3.6	63.6
	3.0 g/l	3.5	75	3.7	68.1
EDTA	0.50g/ l	3	50	3.5	59.1
	1.0 g/l	3	50	3.6	63.6
Calcium chloride	5.0 g/l	2.9	45	3	36.4
	10.0 g /l	2.9	45	3.2	50
INA	50.0mM	3	50	3.7	68.2
	100mM	3.3	65	3.8	72.7
AA + INA	2g/l + 50 mM	3.4	70	3.9	77.3
AA+INA	2g/l + 100mM	3.5	75	4	81.8
Redomyl-plus	2 g/l	3	50	3.5	59.1
Untreated Control		2	-	2.2	-

Table 6: Effects of different chemical inducers on early blight severity of potato plants during two successive summer cultivation seasons.

Chemical inducers	Concentration	Disease severity			
		First season		Second season	
		Early blight	Reduction%	Early blight	Reduction%
Ascorbic acid	2.0 g/l	0.8 cd*	75	1.0 d	72.2
	3.0 g/ l	1.0 cd	68.8	1.0 d	72.2
EDTA	0.50 g/ l	1.1 c	65.6	1.4bc	61.1
	1.0 g/l	0.7 d	78.1	1.1 cd	69.4
Calcium chloride	5.0 g/l	1.6 b	50	1.5 bc	58.3
	10.0 g/l	1.8b	43.8	1.4bc	61.1
INA	50.0 mM	1.0 cd	68.8	1.1 cd	69.4
	100mM	1.0 cd	68.8	1.0 d	72.2
AA + INA	2g/l + 50 mM	0.4 e	87.5	0.5 e	86.1
AA + INA	2g/l + 100 mM	0.4 e	87.5	0.4 e	88.9
Redomil-plus	2 g/ l	1.0 cd	68.8	1.1cd	69.4
Untreated Control		3.2 a	--	a3.6	--

* Figures with the same letter are not significantly different (P= 0.05)
Late blight scale from 0 to 4 according to Cohen *et al*.,[21]

Summer Cultivation Seasons:

a-Effects on Early Blight Severity: The applied treatments showed similar trend that observed at winter seasons against late blight disease. Results in Table (6) indicate that all treatments reduced the early blight severity during the two successive grown seasons. Combined treatments between AA at 2.0 and either INA at 50.0 or 100 mM showed the highest reduction in disease severity ranged between 86.1 to 88.9 %. Individual treatment of AA at 2.0 & 3.0 g/l, EDTA at 1.0 g/l and INA at 50 or 100 mM reduced the late blight severity from 61.1 up to 75.0 % as compared with untreated plants. Fungicidal treatments reduced the disease severity as 68.8-69.4 %. Meanwhile Calcium chloride was the lowest effective treatment.

Table 7: Potato yield in response to different chemical inducers during two successive summer cultivation seasons.

Cemical inducers	Concentration	First season		Second season	
		Yield (kg/m^2)	Increase%	Yield (kg/m^2)	Increase%
Ascorbic acid	2.0 g/ l	3.1	72.2	3.3	65
	3.0 g/l	3.3	83.3	3.4	70
EDTA	0.50g/l	2.9	61.1	3.1	55
	1.0 g/l	3	66.7	3.2	60
Calcium chloride	5.0 g/l	2.6	44.4	2.5	25
	10.0 g/l	2.5	38.9	2.8	40
INA	50.0 mM	3	66.7	3.2	60
	100mM	3.1	72.2	3.3	65
AA + INA	2g/l + 50 mM	3.2	77.8	3.5	75
AA + INA	2g/l + 100 mM	3.3	83.3	3.6	80
Redomyl-plus	2 g/l	2.8	55.6	3.2	60
Untreated Control		1.8	--	2	--

b- Effects on Tuber Yield: Results in Table (7) indicate that all treatments increase potato yield during two grown seasons. Combined treatments between AA at 2.0 and INA at 50.0 or 100 mM showed an announced increase in obtained yield which recorded as 77.8 & 75.0 and 83.3 & 88.0 % at the two cultivation seasons, respectively. An increase in tuber yield ranged between 55.0 and 83.0 % was observed when potato plants received individual treatment of each AA, EDTA, INA as well as fungicide Redomyl. Meanwhile Calcium chloride showed less effect on the yield increase as 25.0-44.4 %.

Discussion: Late and early blight caused by *Alternaria solani and Phytophthora infestans* considered the most serious diseases attacked potato plants[1-4]. Fungicides could successfully control most of plant diseases, however they have negative effects on human health and environmental pollution, so alternatives of these fungicides are needed.

There are several fungicidal alternatives commercially used for induction of plant resistance against diseases. In this concern, ascorbic acid was reported as plant resistance inducer in many plants[7,8,12].

Al-Sayed and Thomason[26] showed that application of ascorbic acid as foliar spray on tomato plants succeeded in reduction of the root rot nematodes. Furthermore, Zacheo *et al.*[7] reported that the content of ascorbic acid in plant tissues has been associated with resistance to some diseases . Similar results are recorded in the present study that potato plants sprayed with ascorbic acid caused significant reduction in disease severity of late and early blight under greenhouse and field conditions. Ascorbic acid was successful for controlling several plant fungal diseases[9,-12].

On the other hand, Dicloro-isonicotinic acid (INA) was reported for inducing resistance in several plants against viruses, bacteria, and fungal infection[17-19]. The obtained results in the present study indicate that INA at 50.0 or 100 mM could reduce the severity of late and early blight diseases more than 60 % and increase the potato yield more than 50.0 % during the two successive cultivation seasons. The role of INA might be due to increase the enzymes activities and stimulated the defense reactions[17,19], or due to its fungicidal properties[27]. Abd-El-Kareem *et al.*[20] reported that INA at 100 and 150 mg/l caused high reduction in powdery mildew and Alternaria leaf spot diseases incidence of squash plants under greenhouse and field conditions.

Furthermore, EDTA (ethylene diamine tetra acetic acid) was reported to induce resistance in broad been against rust disease[13]. These reports confirmed the present findings that EDTA at 0.5 and 1.0 g/l reduce late and early blight severity under greenhouse and field conditions.

EDTA could still affect intracellular functions by binding apoplectic calcium, which is critical for maintaining membrane stability and selective ion permeability[28].

Foliar application of calcium chloride has been also reported to delay ripping and control mould disease in many fruits and vegetables[15,16]. Calcium chloride treatments, in present study, could reduce late and early blight disease severity and increase the activity of

chitinase and β-1,3-glucanase enzymes. In this respect, Conway *et al*.[15] reported that calcium enhanced tissue develops resistance to fungal infection by stabilizing or strengthening cell wall, thereby making them more resistant to harmful enzymes produced by fungi and that it also delays aging of fruits. Calcium ions can form salt-bridge cross-links, it makes the cell wall less accessible to the enzymes and control ripening softening as well as plant diseases[29]. Several studies have demonstrated that over expression of chitinases and β-1,3-glucanase in plants is associated with enhanced resistance to various fungal pathogens[30-32]. The present findings indicate that all applied chemical inducers stimulated the enzymes activities. The most effective treatments were the combination between AA at 2 g/l with INA at 50 or 100 mM, they increased the β-1,3-glucanase and chitinase more than 115 and 109 %, respectively. β-1,3-glucanase and chitinases are able to hydrolyze β-1,3-glucan and chitin, respectively, the major components of fungal cell walls[33,34].

On the light of the obtained results in the present study, it could be suggested that combined treatments between ascorbic acid and INA might be used for suppressing the severity of late and early blight diseases of potato plants and stimulate the increase in their produced yield under field conditions, especially it considered as safe, cost-effective and easily applied for such diseases.

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