

Biochemical changes in treated cucumber plants with some elicitors against downy mildew disease in protected houses

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Abstract—This study aimed to evaluate the effect of some different biotic and abiotic inducers i.e., potassium silicate, humic acid, propolis extract, and died spores (DS) of *Pseudoperonospora cubensis* in addition to amistar fungicide in controlling cucumber downy mildew disease and induction the systemic acquired resistance of treated plants in protected houses. Spraying cucumber plants with all tested inducers reduced disease severity of downy mildew and increased the fruit weight/plant. Amistar fungicide followed by potassium silicate and DS1 were the best effective treatments without significant differences between them in reducing disease severity and increasing fruit weight/plant respectively. All treatments increased phenols and flavonoids, content in treated cucumber plants at 2, 5 and 8 days post inoculation with the downy mildew pathogen. Also, spraying cucumber plants with the tested inducers increased the activities of peroxidase (PO), polyphenoloxidase (PPO), phenylalanine ammonia lyase (PAL), chitinase and β -1,3- glucanase enzymes post inoculation the plants with downy mildew pathogen. In general, the died spores of DS1 followed by DS3 and potassium silicate were the most effective treatments in increasing activities of PO, PPO and PAL enzymes. Meanwhile, the died spores of DS3, DS1 and DS2 followed by potassium silicate were the superior in increasing the activities of chitinase and β -1,3- glucanase enzymes in leaves of treated cucumber plants in protected houses.

Key words—Cucumber, downy mildew, died spores, peroxidase, polyphenoloxidase, phenylalanine ammonia lyase, chitinase and β -1,3- glucanase.

1 Introduction

Cucumber (*Cucumis sativus* L.) is one of the most popular and favorite vegetable crops in different parts of the world. It is used either as fresh fruits or in pickling industry. Downy mildew caused by *Pseudoperonospora cubensis* (Berk and Curtis) is one of the most destructive diseases of protected cultivation, which causes considerable losses of cucurbit crops in many regions of the world (Lehman, 1991; St-Amand and Wehner, 1991; Tsai *et al.*, 1992; Reuveni and Raviv, 1997; Shama *et al.*, 1998; Chaban and Okhrimchukv, 2000) and greatly effects on both yield and quality (Thomas, 1996). As a result of the extensive using of fungicides for long time, pathogens acquired gradually resistance to those fungicides. Moreover, the fungicide residues have bad effects on human health and the environment (Zhang *et al.*, 2015). Because of fungicide residues in harvested cucumber fruits, it is interest to find new alternatives of control like natural products, mineral salts, bio-agents and others which consider more effective and safe for environment and human health (Bettiol *et al.*, 1999, Carneiro *et al.*, 2007 and Faria *et al.*, 2011). Many investigators used potassium silicate as foliar or soil applications for reducing the severity of powdery mildew on cucumber, grape, strawberry, tomato and wheat (Lee *et al.*, 2000; Yildirim *et al.*, 2002; Be' langer *et al.*, 2003; Kanto *et al.*, 2006 and Yanar *et al.*, 2011). Also, foliar and root applications of silicone reduced the severity of the powdery mildew on cucumber leaves (Lee *et al.*, 2000). On the other hand, Pereira *et al.*, (2008) demonstrated that extracts of propolis possess a high potentiality as antimicrobial, antifungal, antioxidant, antiviral and

antiprotozoal. Also, un-viable spores of some pathogens has been used as resistant inducers by many investigators like Ahmed, (2005), EL-Gammal, (2005) and Ahmed, (2015) for controlling many foliar diseases. Phenolic compounds are a class of antioxidant agents which act as free radical terminators (Om Prakash and Yamini, 2007). The mechanisms for phenolic toxicity to microorganisms include substrate deprivation, membrane disruption, and enzyme inhibition by the oxidized compounds, possibly through reaction with sulfhydryl groups or through more nonspecific interactions with the proteins (Cowan, 1999). Flavonoids are hydroxylated phenolic substances while, phenylalanine ammonia lyase (PAL) plays an important role in the biosynthesis of phenolic phytoalexins (Daayf *et al.*, 1997). The enhanced induction of Peroxidase (PO) and Polyphenoloxidase (PPO) might have contributed for the induced systemic resistance triggered by various biotic and abiotic inducers (Tian *et al.*, 2006; Imran *et al.*, 2007; Barilli *et al.*, 2010). The lytic enzymes have hydrolytic action and degrade the fungal cell wall (Haran *et al.*, 1996; Mathivanan *et al.*, 1997). Combinations of chitinase and β -1,3-glucanase inhibit growth of several pathogenic fungi. Activities of chitinase and β -1,3-glucanase are known to be induced in many plants in response to infection with fungal pathogens (Xue *et al.*, 1998).

This research aimed to evaluate the efficacy of some biotic and abiotic substances in controlling cucumber downy mildew disease and their role in induction the systemic acquired resistance against the disease.

2 MATERIALS AND METHODS

Preparation of *Pseudoperonospora cubensis* inoculum and its died spores:

Sporangia of *P. cubensis* were collected from infected leaves using a brush and water. Sporangia were washed twice with water by centrifugation at 1000 rpm for 10 minutes and suspended in water to give final concentration 10^4 sporangium /mL (Okuno *et al.*, 1991). The prepared spore suspension was divided to 4 equal volumes as follows, the first volume was exposed to UV light (1200 nm) for 30 minutes (DS1), the second volume was exposed to temperature degree at 90°C for 30 minutes (DS2), while, the third volume was treated with chloroform at rate 1 mL/L of spore suspension till evaporation chloroform (DS3), also to kill the spores of *P. cubensis*. The fourth volume of prepared spore suspension was left without any treatment to use as a source of *P. cubensis* inoculum when needed in any artificial inoculation process during this investigation. Inoculation of plants was done by spraying cucumber seedlings with sporangium suspension 10^4 / mL (Okuno *et al.*, 1991).

Effect of some biotic and abiotic inducers on cucumber downy mildew disease in protected house:

Two experiments were conducted in a commercial protected house at the experimental farm station of Fac. Agric. Moshtohor, Benha Univ., from middle September to December. In this respect, Cucumber transplants (Barracuda hybrid f1) were transplanted and received all the recommended agriculture practices of irrigation and fertilization. Cucumber plants at 5 weeks old were sprayed with one of the following treatments:

Potassium silicate (K_2SiO_3) 10 mM (1.54 g /L)

Humic acid (5 ml/L)

(DS1), (DS2) and (DS3).

Amistar 250 SC 0.5 mL/L

Aqueous solution of propolis (5 g/L).

Water extract of propolis was prepared by adding five grams of propolis to 100 ml of deionized water in an Erlenmeyer flask (250 mL), then shacked at 95°C for 2 hrs for mixing, cooled to about 25°C (room temperature) and then the aqueous extract was centrifuged at 7000 rpm for 15 minutes to obtain the supernatant. The extracted propolis supernatant was completed to one liter using the deionized water. Ten mL of each one of the tested biotic or abiotic inducers were sprayed on each plant. Three sprayings were applied every two weeks as interval. The sprayed plants with distilled water served as control. Ten plants represent a replicate and three replicates were used for each treatment. Disease severity of downy mildew was assessed with the score chart of 0 to 5 scale (0) No infection, (1) 0-10, (2) 10.1-15, (3) 15.1-25, (4) 25.1-50 and (5) More than 50 percent of leaf area being covered with mildew growth as described by Jamadar and Desai (1997).

$$\text{Disease Severity \%} = \Sigma (a \times b) / N \times K \times 100$$

Where: a = Number of infected leaves in each category.

b = Numerical value of each category.

N = Total number of examined leaves.

K = The highest degree of infection category.

$$\text{Efficacy (\%)} = \frac{\text{Treatment} - \text{Control}}{\text{Control}} \times 100$$

Average weight of fruits/plant also was measured.

Biochemical changes in treated cucumber plants with some biotic and abiotic inducers:

The effect of potassium silicate, humic acid, propolis extract, DS1, DS2, DS3 and amistar fungicide in addition to untreated control treatment on phenols and flavonoids content, peroxidase (PO), polyphenol-oxidase (PPO), phenylalanine ammonia lyase (PAL), chitinase and β -1,3-glucanase activities were determined. Cucumber seeds of Barracuda hybrid f1 were sown in plastic pots (20 cm ϕ) containing sand, clay and peat-moss (1:1:1, v/v/v). Cucumber seedlings 21 days old were sprayed with the different tested treatments 2 days before inoculation with downy mildew spores (Strobel and Kuc, 1995). The samples were taken at 2, 5 and 8 days post inoculation.

Determination of total phenols content:

Half gram of fresh plant tissue was ground using a pestle and mortar with 10 mL of 80% ethanol then, filtered and centrifuged at 10,000 rpm for 20 min. The supernatant was evaporated till dryness. The residue was dissolved in 5 mL of 80% ethanol and used as the extract. Ten drops of concentrated hydrochloric acid were added to 0.2 mL of the prepared sample in a test tube, then, heated rapidly to boiling point over a free flame, with provision for condensation. Then, the tubes were placed in water bath at 100°C for 10 minutes. After cooling, 1 mL of the reagent and 2.5 mL of 20% Na_2CO_3 were added to each tube. The mixture was diluted to 50 mL with distilled water and determined after 20 minutes using Spectrophotometer (SPECTRONIC 20-D) at 520 nm against a reagent blank (Bary and Thorpe, 1954).

Determination of total flavonoids content:

To quantify the flavonoids, 500 μ L of the previously prepared extract for determination of phenol content were transferred to a test tube. Then, 500 μ L of the acetic acid solution, 2 mL of the pyridine solution, 1 mL of the reagent aluminium chloride solution and 6 mL of 80% methanol were added. The samples remain at room temperature for 30 minutes. The spectrophotometer should be adjusted to a wavelength of 420 nm and the equipment must be rinsed with distilled water. The flavonoid content is expressed as milligrams of rutin equivalents per gram of sample (mg RE/g) (Peixoto Sobrinho *et al.*, 2008).

Determination the activities of oxidative and catalyzed enzymes:

Treated leaf samples were ground with 0.2 M Tris HCl buffer (pH 7.8) containing 14 mM β -mercaptoethanol at the rate 1/3 w/v. The extracts were

centrifuged at 10,000 rpm for 20 min at 4°C. The supernatant was used to determine enzyme activities (Tuzun *et al.* 1989).

Determination of Peroxidase (PO):

Peroxidase activity was determined according to the method described by Allam and Hollis (1972), The cuvette contained 0.5 mL of 0.1 M potassium phosphate buffer at pH 7.0, 0.3 mL of enzyme extract, 0.3 mL 0.05 M pyrogallol, 0.1 mL 1.0% H₂O₂ and distilled water to bring cuvette contents to 3.0 mL. The reaction mixture was incubated at 25°C for 15 minutes, and then the reaction was inactivated by adding 0.5 mL of 5.0% (v/v) H₂SO₄ (Kar and Mishra, 1976). Peroxides activity was expressed as the increase in absorbance at 425 nm/gram fresh weight/15 minutes.

Determination of Polyphenoloxidase (PPO):

The polyphenoloxidase activity was determined according to the method described by Matta and Dimond (1963). The reaction mixture contained 0.2 ml enzyme extract, 1.0 ml of 0.2 M sodium phosphate buffer at pH 7.0 and 1.0 ml 10⁻³ M Catechol and complete with distilled water up to 6.0 ml. The reaction mixture was incubated for 30 minutes at 30°C. Polyphenoloxidase activity was expressed as the increase in absorbance at 420 nm/g fresh weigh/30 min.

Determination of phenylalanine ammonia lyase (PAL):

Activity of PAL was determined as the rate of conversion of L-phenylalanine to *trans*-cinnamic acid as described by Dickerson *et al.*, (1984). A sample containing 0.4 mL of enzyme extract was incubated with 0.5 mL of 0.1 M borate buffer, pH 8.8, and 0.5 mL of 12 mM L-phenylalanine in the same buffer for 30 min at 30°C. The optical density (O.D.) value was recorded at 290 nm and the amount of *trans*-cinnamic acid formed calculated using its extinction coefficient of 9.630M⁻¹cm⁻¹ as described by Dickerson *et al.*, (1984). Enzyme activity was expressed as μmol *trans*-cinnamic acid min⁻¹ g⁻¹ protein.

Determination of Chitinase:

Determination the activity of chitinase was carried out according to the method of Boller and Mauch, (1988). In this respect, 1 mL of 1% colloidal chitin was added to 0.05 M citrate phosphate buffer (pH 6.6) in a test tube, then, 1 mL of enzyme extract was added and mixed by shaking. The tubes were kept in a water bath at 37°C for 60 minutes, then cooled and centrifuged before assaying. Reducing sugar was determined in 1 mL of the supernatant by dinitrosalicylic acid (DNS). Optical density was determined at 540 nm. Chitinase activity was expressed as mM N-acetylglucose amine equivalent released/gram fresh weight tissue/60 minutes.

The substrate colloidal chitin was prepared from chitin powder according to the method described by Ried and Ogryd-Ziak (1981). Twenty five grams of chitin were milled, suspended in 250 mL of 85% phosphoric acid (H₃PO₄) and stored at 4°C for 24 hr, then blended in 2 Liter of distilled water and the suspension was centrifuged. The washing procedure was repeated twice.

The colloidal chitin suspension in the final wash was adjusted to pH 7.0 with (1 N) NaOH, separated by centrifugation and the pelted colloidal chitin was stored at 4°C. Chitinase was expressed as mM N-acetylglucose amine equivalent released / gram fresh weight tissue / 60 minutes.

Determination of β -1,3-Glucanase:

The enzyme solution (100 μL) was mixed with 200 μL of 0.2 % (w/v) laminarin dissolved in 0.1 M sodium phosphate buffer (pH 6.0) and incubated at 30°C for 30 min. The reaction was terminated by adding dinitrosalicylic acid solution and boiling the reaction mixture for 5 min. The absorbance at 540 nm was measured and the unit was defined as the amount of the enzyme that released reducing sugar equivalent to 1 μg glucose per min under the above conditions (Sun *et al.*, 2006.). β-1,3-glucanase was expressed as mM glucose equivalent released /gram fresh weight tissue /60 minutes.

Statistical analyses:

Statistical analyses of all the previously designed experiments have been carried out according to the procedures (ANOVA) reported by Snedecor and Cochran (1989). Treatment means were compared by the least significant difference test "L.S.D" at 5% level of probability.

3 RESULTS

Effect of foliar application with some biotic and abiotic inducers on cucumber downy mildew disease severity in protected house:

The results in Table (1) reveal that, all tested inducers significantly effective in reducing disease severity of cucumber downy mildew compared with the control treatment during the two growing seasons (2013 and 2014). The results of the two seasons indicated clearly that amistar fungicide followed by potassium silicate and DS1 were the best effective treatments in reducing disease severity of cucumber downy mildew where the averages of recorded reduction% during the two growing seasons were 69.2, 65.4 and 63.2% respectively. Meanwhile, the least effective treatment was DS2 where the average of recorded reduction% was 51.10% during the two growing seasons. Generally, the recorded reduction% during the second season was more than those recorded in the first season with all treatments except DS2 treatment.

Effect of foliar application with some biotic and abiotic inducers on cucumber fruit weight/plant.

Data in Table (2) show that all tested treatments were significantly effective in increasing the fruit weight/plant compared with control treatment during the two growing seasons (2013 and 2014). In this respect, amistar fungicide followed by potassium silicate and DS1 were the most effective treatments in increasing fruit weight/plant during the two growing seasons where the averages of recorded increase in the fruit weight/plant during the two growing seasons were 71.6, 61.2 and 49.3% respectively. On the other hand, the least increase in

fruit weight/plant during the two growing seasons was recorded with DS2 treatment where the recorded increase of fruit weight/plant was 29.6% during the two growing seasons. Also, the recorded increase in cucumber fruit

weight/plant were higher in the second season than those recorded in the first season except humic acid and DS2 treatments which were higher in first season than the second.

Table (1): Effect of foliar application with some biotic and abiotic inducers on cucumber downy mildew disease severity in protected house

Treatment	Season 2013		Season 2014		Mean	% Reduction
	Disease severity %	% Reduction	Disease severity %	% Reduction		
Potassium silicate	3.3	64.9	3.0	65.9	3.15	65.4
Humic acid	4.2	55.3	3.7	58.0	3.95	56.6
Propolis extract	5.3	43.6	4.9	44.3	5.10	44.0
DS1	3.5	62.8	3.2	63.6	3.35	63.2
DS2	4.5	52.1	4.4	50.0	4.45	51.1
DS3	4.0	57.4	3.3	62.5	3.65	59.9
Amistar fungicide	3.0	68.1	2.6	70.5	2.80	69.2
Control	9.4	0.0	8.8	0.0	9.10	0.0

L.S.D at 0.05 0.83 0.72
DS1= *P. cubensis* spores exposed to UV light, DS2= *P. cubensis* spores treated with temperature DS3= *P. cubensis* spores exposed to Chloroform

Table (2): Effect of foliar application with some biotic and abiotic inducers on cucumber fruit weight/plant.

Treatment	Season 2013		Season 2014		Mean	Increase %
	Average fruits weight/plant	Increase %	Average fruits weight/plant	Increase %		
Potassium silicate	3.0	52.28	3.64	69.30	3.32	61.2
Humic acid	2.74	39.09	2.88	33.95	2.81	36.4
Propolis extract	2.52	27.92	2.97	38.14	2.75	33.3
DS1	2.76	40.10	3.39	57.67	3.08	49.3
DS2	2.58	30.96	2.76	28.37	2.67	29.6
DS3	2.67	35.53	3.15	46.51	2.91	41.3
Amistar fungicide	3.31	68.02	3.76	74.88	3.54	71.6
Control	1.97	0.00	2.15	0.00	2.06	0.0

L.S.D at 0.05 0.40 0.29
DS1= *P. cubensis* spores exposed to UV light, DS2= *P. cubensis* spores treated with temperature DS3= *P. cubensis* spores exposed to Chloroform

Changes in total phenols and flavonoids content:

Results in Table (3) reveal that treating cucumber plants with the tested biotic and abiotic inducers increased phenols and total flavonoid contents compared with control. In this respect, the highest increase in total phenols was recorded with amistar fungicide treatment followed by potassium silicate and DS1 at 2, 5 and 8 days post inoculation with *P. cubensis* compared with control. This increase reached its maximum at 8 days post inoculation where the recorded increases of total phenols were 48.7, 36.1 and 27.7% over control. However, the least recorded increase in total phenols was at 8 days post inoculation with DS2.

As for flavonoids content, the highest increase was recorded in case of treating cucumber plants with amistar fungicide followed by DS3 and propolis respectively at 2, 5 and 8 days post inoculation with *P. cubensis* sporangia with highly efficacy % compared to the other tested inducers and control. In this respect, the highest increase was recorded at 5 days post inoculation where amistar fungicide followed by DS3 and propolis gave 104.7, 100.0 and 83.7% of increase over control. Meanwhile, the least increase in flavonoids content was recorded with DS2 treatment at all tested inoculation periods.

Changes in peroxidase and polyphenoloxidase activities:

Data in Table (4) show that spraying cucumber plants with biotic and abiotic inducers post inoculation with *P. cubensis* increased peroxidase (PO) and polyphenoloxidase (PPO) activities compared to untreated control. In this respect, spraying cucumber plants with potassium silicate, DS1 and DS3 gave the highest increase in PO activity at 2 and 5 days post inoculation with *P. cubensis* sporangia respectively. However, the highest increase in PO activity was induced with amistar fungicide, potassium silicate and DS1 at 8 days post inoculation. On the other hand, the least increase in PO activity was recorded with spraying cucumber plants with DS2 treatment at all tested inoculation periods.

As for polyphenoloxidase enzyme, spraying cucumber plants with the tested biotic and abiotic inducers increased the activity of PPO enzyme in treated plants compared with the untreated control. In this respect, all tested inducers incited remarkable high activities of PPO enzyme at 2, 5 and 8 days post inoculation comparing to the untreated control. However,

the highest increase in determined activities of PPO was recorded at 8 days post inoculation. Also, the best inducer treatments in increasing the activities of PPO

were amistar fungicide, DS3, DS1, Potassium silicate and humic acid respectively. While, the least effective treatment was propolis extract.

Table (3): Changes in total phenols and flavonoids content in treated cucumber plants with biotic and abiotic inducers as mg/g fresh weight of leaves.

Treatment	Phenols			Flavonoids			Increase (%)					
	Days post inoculation			Days post inoculation			Phenols			Flavonoids		
	2	5	8	2	5	8	2	5	8	2	5	8
Potassium silicate	13.6	15.4	16.2	5.5	7.5	8.4	36.0	37.5	36.1	34.1	74.4	61.5
Humic acid	11.1	13.0	13.2	5.6	6.9	7.3	11.0	16.1	10.9	36.6	60.5	40.4
Propolis extract	12.4	13.2	14.5	6.9	7.9	8.7	24.0	17.9	21.8	68.3	83.7	67.3
DS1	13.2	14.1	15.2	6.5	7.7	8.6	32.0	25.9	27.7	58.5	79.1	65.4
DS2	11.3	12.9	12.7	5.4	5.7	6.9	13.0	15.2	6.7	31.7	32.6	32.7
DS3	13.1	13.7	15.0	7.1	8.6	9.5	31.0	22.3	26.1	73.2	100.0	82.7
Amistar fungicide	13.5	16.2	17.7	7.6	8.8	9.9	35.0	44.6	48.7	85.4	104.7	90.4
Control	10.0	11.2	11.9	4.1	4.3	5.2	0.0	0.0	0.0	0.0	0.0	0.0

DS1= *P. cubensis* spores exposed to UV light, DS2= *P. cubensis* spores treated with temperature DS3= *P. cubensis* spores exposed to Chloroform

Table (4): Changes in peroxidase and polyphenoloxidase activities in treated cucumber plants with the tested biotic and abiotic inducers.

Treatment	PO			PPO			Increase (%)					
	Days post inoculation			Days post inoculation			PO			PPO		
	2	5	8	2	5	8	2	5	8	2	5	8
Potassium silicate	17.7	30.9	26.9	7.0	8.5	7.4	80.4	200.0	120.5	337.5	269.6	335.3
Humic acid	15.9	28.8	23.9	5.8	7.8	7.2	61.9	179.6	95.9	262.5	239.1	323.5
Propolis extract	14.6	27.1	21.7	4.3	6.3	5.3	48.5	163.1	77.9	168.8	173.9	211.8
DS1	16.8	30.1	25.8	8.1	8.9	7.4	71.4	192.2	111.5	406.3	287.0	335.3
DS2	13.9	22.2	21.3	4.1	6.6	7.0	41.8	115.5	74.6	156.3	187.0	311.8
DS3	16.0	29.3	25.0	7.0	8.7	8.6	63.3	184.5	104.9	337.5	278.3	405.9
Amistar fungicide	14.7	25.0	28.5	6.9	7.6	8.8	50.0	142.7	133.6	331.3	230.4	417.6
Control	9.8	10.3	12.2	1.6	2.3	1.7	0.0	0.0	0.0	0.0	0.0	0.0

DS1= *P. cubensis* spores exposed to UV light, DS2= *P. cubensis* spores treated with temperature DS3= *P. cubensis* spores exposed to Chloroform

Changes in phenylalanine ammonia lyase (PAL) activity:

Results in Table (5) show that all tested biotic and abiotic inducer treatments increased PAL activity in treated leaves of cucumber plants. The highest increase % of PAL activity was recorded with DS3 treatment at 2 days post inoculation with *P. cubensis* sporangia where the recorded increase was 519.61% compared with untreated control. Meanwhile, DS1 followed by amistar

fungicide treatments came in the second rank. Also, the same trend of PAL activity was recorded at 5 and 8 days post inoculation with *P. cubensis* where the highest activity was recorded with DS3 followed by DS1 and amistar fungicide treatments. On the other hand, humic acid was the least effective treatment on PAL activity at 2 and 5 days post inoculation while, propolis extract was least effective one on PAL activity at 8 days post inoculation.

Table (5): Changes in phenylalanine ammonia lyase (PAL) activities in treated cucumber plants with the tested biotic and abiotic inducers.

Treatment	PAL			Increase (%)		
	Days post inoculation			Days post inoculation		
	2	5	8	2	5	8
Potassium silicate	19.6	31.7	37.9	284.31	86.47	47.47
Humic acid	8.3	19.0	34.0	62.75	11.76	32.30
Propolis extract	15.2	23.0	32.1	198.04	35.29	24.90
DS1	24.3	35.1	61.3	376.47	106.47	138.52
DS2	14.3	25.5	38.0	180.39	50.00	47.86
DS3	31.6	47.7	66.8	519.61	180.59	159.92
Amistar fungicide	20.2	32.5	47.8	296.08	91.18	85.99
Control	5.1	17.0	25.7	0.00	0.00	0.00

DS1= *P. cubensis* spores exposed to UV light, DS2= *P. cubensis* spores treated with temperature DS3= *P. cubensis* spores exposed to Chloroform

Changes in chitinase and β -1,3-glucanase activity.

Data in Table (6) reveal that all inducer treatments increased chitinase and β -1,3-glucanase activities. The highest increase in chitinase activity was recorded with humic acid, DS3, DS2 and potassium silicate at 2 days post inoculation where the recorded increase of chitinase activity were 560.0, 560.0, 520.0 and 500.0% over control respectively. At 5 and 8 days post inoculation, the highest activity was recorded with DS3, DS1 and DS2 followed by potassium silicate. While, the least increase in activity of chitinase was recorded with propolis extract treatment in all time.

As for β -1,3-glucanase enzyme, β -1,3-glucanase activity was higher in treated cucumber plants with all tested inducers than untreated control. In this respect, DS1, potassium silicate and DS3 were the most effective inducer treatments in increasing the activities of β -1,3-glucanase at 2 days post inoculation. Also, DS1, DS3 and propolis extract were the most effective inducer treatments respectively in increasing the activities of β -1,3-glucanase at 5 and 8 days post inoculation with *P. cubensis* sporangia. On the other hand, amistar fungicide was the least effective treatment on β -1,3-glucanase activity at all three inoculation periods.

Table (6): Changes in chitinase and β -1,3-glucanase enzymes activity in treated cucumber plants with the tested biotic and abiotic inducers.

Treatment	Chitinase			β -1,3-glucanase			Increase (%)					
	Days post spraying			Days post spraying			Chitinase			β -1,3-glucanase		
	Days post spraying			Days post spraying			Days post spraying			Days post spraying		
	2	5	8	2	5	8	2	5	8	2	5	8
Potassium silicate	3.0	4.2	3.5	4.7	5.3	4.7	500.0	223.1	288.9	95.8	96.3	161.1
Humic acid	3.3	3.4	3.0	3.1	4.9	5.2	560.0	161.5	233.3	29.2	81.5	188.9
Propolis extract	2.4	3.2	3.2	3.5	5.8	5.3	380.0	146.2	255.6	45.8	114.8	194.4
DS1	2.9	5.0	4.1	5.7	7.0	5.5	480.0	284.6	355.6	137.5	159.3	205.6
DS2	3.1	4.3	3.7	3.7	5.7	4.3	520.0	230.8	311.1	54.2	111.1	138.9
DS3	3.3	5.9	4.3	4.3	6.1	5.4	560.0	353.8	377.8	79.2	125.9	200.0
Amistar fungicide	2.5	4.1	3.3	3.4	5.2	4.2	400.0	215.4	266.7	41.7	92.6	133.3
Control	0.5	1.3	0.9	2.4	2.7	1.8	0.0	0.0	0.0	0.0	0.0	0.0

DS1= *P. cubensis* spores exposed to UV light,

DS2= *P. cubensis* spores treated with temperature

DS3= *P. cubensis* spores exposed to Chloroform

4 Discussion

Downy mildew caused by *Pseudoperonospora cubensis* (Berk, and Curt.) Rostov., is a major foliar disease of cucumber (*Cucumis sativus* L.) in humid production areas of the world (Thomas, 1986). The obtained results indicated that spraying cucumber plants with some biotic and abiotic inducers significantly reduced downy mildew disease severity during two growing seasons. Amistar fungicide followed by potassium silicate and DS1 were the most effective treatments in reducing disease severity and increasing fruit weight/plant respectively without significant differences between them. Among all tested inducers, DS2 was the least effective treatment in reducing downy mildew disease severity and in increasing fruit weight/plant. These results are in agreement with those reported by Saberi and Panah, (2015) who reported that foliar applications with Si reduced disease severity of cucumber downy mildew. Also, Abdel-Kader *et al.*, (2012) revealed that spraying cucumber with calcium chloride + *Saccharomyces cerevisiae* or chitosan or potassium bicarbonate + thyme oil resulted in the highest reduction in incidence and severity of downy and powdery mildew diseases and increased the obtained yield of cucumber plants grown under plastic houses conditions. In similar results, application of K_2SiO_3 with 12 days interval significantly reduced the severity of tomato powdery mildew. The difference between K_2SiO_3 and fungicide treatment was not significant and both treatments reduced powdery mildew

severity on tomato leaf (Yanar *et al.*, 2011). The obtained results are also in harmony with those obtained by many investigators during controlling cucumber powdery mildew like Bettiol *et al.*, (1999) who used milk, Konstantinidou-Doltsinis and Schmitt, (1998) and Faria *et al.*, (2011) who used plant extracts or aqueous extracts of organic material and Ishida *et al.*, (2001) who controlled the cucumber powdery mildew disease using the ethanolic extract of propolis (EEP) even at the concentration of 8%. Also, Aparecida *et al.*, (2014) used the ethanolic extract of propolis (EEP) as an available alternative method for controlling cucumber powdery mildew disease. As for amistar (Azoxystrobin) fungicide that has a good efficacy in controlling cucumber downy mildew disease. This result could be interpreting in light that Azoxystrobin could be inhibits mitochondrial respiration, spore germination and mycelia growth. Also, it has antispore activity thus, it could be used as a protectant, curative and eradivative material where it has a translaminar and systemic properties (Pandolfo, 1998; Fiori and Peretto, 2000; Abd-El-Moity *et al.* 2003; Xing *et al.*, 2003; Thind *et al.*, 2004; Keinath *et al.*, 2007; Gengotti *et al.*, 2008; Anand *et al.*, 2008; Morsy, 2010 and Morsy & Belal, 2014). Concerning the effect of died spores (DS) in controlling cucumber downy mildew disease, the results could be interpreting in light the findings of Ahmed, (2005) who reported that treating cucumber with powdery mildew spores killed by UV, temperature, chloroform reduced powdery mildew disease severity by 69.84, 62.25 and 54.73% less than control and increased the number and weight of

fruits/plant by UV (25.02 and 25.00%) and temperature (18.75 and 19.88%) compared to control.

As for phenols and flavonoid contents, the tested biotic and abiotic inducers in the present study showed a remarkable accumulation of phenols and flavonoid contents in leaves of treated cucumber plants. The highest increase in total phenols was induced in treated cucumber plants with amistar fungicide followed by potassium silicate and DS1 at 2, 5 and 8 days post inoculation with *P. cubensis* sporangia compared with control. This increase reached its maximum at 8 days post inoculation. However, the least increase in total phenols was recorded in treated cucumber plants with DS2 at 8 days post inoculation with *P. cubensis* sporangia. Also, the highest increase of flavonoid content was recorded in case of treating cucumber plants with amistar fungicide followed by DS3 and propolis respectively, at 2, 5 and 8 days post inoculation with *P. cubensis* sporangia compared to the other tested inducer treatments and control. The highest increase of flavonoid content was recorded at 5 days after inoculation. These findings could be interpreting in light the findings of Alkahtani *et al.*, (2001) who reported that foliar spraying of cucumber with abiotic inducers showed remarkable accumulation of phytoalexin in treated plants and the accumulation reached its maximum at 2-6 days post application. Also, the toxic phenolic compounds in plant cells acting through: (1) the structure of bond form with cell wall components of plant tissues (Mahadevan and Sridhar, 1986), (2) enhance host resistant by stimulating host defense mechanisms (Subba Rao *et al.*, 1988), (3) prevent the extent of fungal growth in plant tissues (Soni *et al.*, 1992) and (4) penetrate the microorganisms and cause considerable damage to the cell metabolisms (Kalaichelvan and Elangovan, 1995). Moreover, Farouk *et al.*, (2008) reported that foliar application of chitosan, salicylic acid and methyl jasmonate proved to be effective in reducing downy mildew (caused by *Pseudoperonospora cubensis*) occurrence and severity in cucumber plants under field conditions and increased cucumber growth parameters in addition to some physiological aspects like nitrogen, potassium, phosphorus, calcium, total phenols and photosynthetic pigments in shoot as well as, increasing the yield and its quality.

As for the defense related enzymes, results revealed that treating cucumber plants with the tested biotic and abiotic inducer treatments post inoculation with *P. cubensis* sporangia increased peroxidase (PO), polyphenoloxidase (PPO), PAL, chitinase and β -1,3-glucanase activities compared with untreated control at all days post inoculation. The highest increase in PO activity was induced by spraying cucumber plants with potassium silicate, DS1 and DS3 at 2 and 5 days post inoculation with *P. cubensis* respectively. However, the highest increase in PO activity was induced with amistar fungicide, potassium silicate and DS1 at 8 days post inoculation. As for polyphenoloxidase, all tested inducers incited remarkable high activities of PPO enzyme at 2, 5 and 8 days post inoculation comparing to the untreated control. However, the highest increase in determined activities of PPO was recorded at 8 days post

inoculation. Also, the best inducer treatments in increasing the activities of PPO were amistar fungicide, DS3, DS1, Potassium silicate and humic acid respectively while, the least effective treatment was propolis extract. However, the highest activity of PAL was recorded by DS3 followed by DS1 and amistar fungicide at 2, 5 and 8 days post inoculation. Meanwhile, humic acid was the less effective treatment at 2 and 5 days post inoculation while, propolis extract was the least effective treatment on PAL activity at 8 days post inoculation. On the other hand, the highest increase in chitinase activity was obtained with humic acid, DS3, DS2 and potassium silicate at 2 days post inoculation. However, DS3, DS1 and DS2 followed by potassium silicate recorded the highest activity at 5 and 8 days post inoculation. However, the least increase in activity of chitinase was recorded with propolis treatment at all inoculation periods. DS1, potassium silicate and DS3 were the most effective inducer treatments in increasing the activities of β -1,3-glucanase at 2 days post inoculation. However, DS1, DS3 and propolis respectively were the most effective inducer treatments in increasing the activities of β -1,3-glucanase at 5 and 8 days post inoculation with *P. cubensis* sporangia. These obtained results are in harmony with the findings of Abd-El-Kareem, (1998) who reported that treating cucumber plants with ASA, 5.0 mM, SA 5.0 mM, K_2HPO_4 100 mM and ethephon 300 mg/L increased activity of peroxidase (PO), chitinase and β -1,3-glucanase activity compared to control. Induced resistance of cucumber with oxalic acid, potassium oxalate, salicylic acid, Bion, Fungastop and Photophor against powdery mildew recorded an increase in PR-proteins (peroxidase, polyphenoloxidase, Chitinase and β -1,3 glucanase) activity as well as an increase accumulation of phytoalexins (Alkahtani *et al.*, 2011). The oxidative enzymes play an important role in induced resistance by the oxidation of phenols to oxidized toxic products (quinone) which limit fungal activity. Peroxidases also, catalyse the final polymerisation step of lignin synthesis, which increases the ability of tissue to lignify which may restrict the fungal penetration (Tian *et al.*, 2006; Imran *et al.*, 2007; Gorovitsa and Czosnek, 2008; Barilli *et al.*, 2010). On the other hand, Chitinase and β -1, 3 glucanase enzymes play an important role in plant defense against fungi by hydrolyze their cell wall (Tian *et al.*, 2006; Imran *et al.*, 2007; Gorovitsa and Czosnek, 2008 and Barilli *et al.*, 2010). PAL is one of the key enzymes in the phenylpropanoid and the flavonoid pathway where it was increased in both incompatible and compatible interactions between plants and pathogens. Also, O'Neill and Saunders, (1994) demonstrated that the existence of phenolic phytoalexins in cucumbers may be produced through a PAL pathway.

5 Conclusion

Application of biotic and abiotic inducers increased phenols, flavonoid content and activities of related defense enzymes in cucumber plants. These substances reduced subsequently downy mildew disease severity and increased fruit yield. No significant

differences between potassium silicate, DS1, DS3 and amistar fungicide treatments were recorded concerning cucumber downy mildew disease severity. Therefore, it could be suggesting to possibility of using potassium silicate and DS1 as applicable inducers for controlling downy mildew disease on cucumber plants.

6 References

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