BIOCHEMICAL CHANGES IN SQUASH LEAVES SPRAYED WITH SOME CHEMICALS FOR INDUCING RSISTANCE TO POWDERY MILDEW

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Abstract

Foliar sprays of nine abiotic agents namely; ascorbic acid, boric acid, calcium chloride, cobalt chloride, copper sulphate, manganese sulphate, oxalic acid, potassium di-hydrogen phosphate and salicylic acid, were tested to evaluated their efficacy to induce resistance against *Sphaerotheca fuliginea*, the causal of powdery mildew of squash (*Cucurbita pepo* L.) under glasshouse conditions. All tested foliar treatments, except $CaCl_2$, were effective in inducing systemic protection against powdery mildew. However, they were less effective than penconazole which was equally effective as $MnSO_4$ at 20 mM as they caused a 100% systemic protection on the upper leaves. Among the tested agents, six have significantly increased sugar content of leaves, while all of them decreased the total phenols compared to the control. Out of the tested agents, $MnSO_4$, salicylic acid, oxalic acid and boric acid enhanced the peroxidase activity. However, polyphenoloxidase activity was affected only by oxalic acid, $MnSO_4$ and KH_2PO_4 as they highly increased it to the control. In addition, it was found that most of the tested compounds caused significant increase in the total soluble protein of the 4^{th} leaf.

Key words: *Sphaerotheca fuliginea*, squash, control, induced resistance, phenols, sugars, peroxidase, polyphenol oxidase and proteins.

Introduction

Squash (*Cucurbita pepo* L.) is one of the important vegetable crops in A.R.E. Egypt is considered one of the leading producing countries of squash in the world. It takes the fifth grade between them **(FAOStat database, 2003)**.

Powdery mildew is a common disease of squash in most areas of the world and can be a major production problem. *Sphaerotheca fuliginea* and *Erysiphe cichoracearum* are the two most commonly recorded fungi causing cucurbit powdery mildew. Recently, *S. fuliginea* is more common **(McGrath, 1997)**.

Controlling powdery mildew through inducing systemic resistance (ISR) has been extensively studied during the last fifteen years to obtain systemic protection against powdery mildew by spraying the lower leaves of plants with solutions of chemical agents that they not themselves fungicides **(Reuveni *et al.*, 1995)**. The efficacy of various chemical inducers of systemic resistance against powdery mildew disease has been tested by many investigators. Among them, **Frey and Carver (1998)** used salicylic acid at a concentration of 15 mM on pea. **Descalzo *et al.* (1990**) used oxalic acid on cucmber under simulated commercial greenhouse conditions, **Reuveni *et al.*, (1995 and 1997)** applied a solutions of K_2HPO_4, KH_2PO_4, $CuSO_4$, $MnCl_2$ and

boric acid on cucumber. **Gamil (1995)** and **Ahmed** (**2005**) foliar sprayed of $CoSO_4$ and K_2HPO_4 on squash and cucumber plants.

Gamil (1995) revealed that some biochemical-related resistance factors such as plasma membrane damage, lipid peroxidation and accumulation of phenolic compounds were increased with enhanced resistance in squash plants against CMV. **Meena *et al.* (2001)** found that foliar application of SA at a concentration of 1 mM on groundnut significantly reduced late leaf spot disease intensity, and observed an increase in phenolic content, one day after challenge inoculation with *Cercosporidium personatum*, in SA-treated leaves.

Detailed experiments proved that systemic accumulation of defence-related enzyme peroxidase can be induced in leaves treatment with chemicals for inducing resistance to diseases in cucumber **(Gottstein and Kuć, 1989)**. **Okuno *et al.* (1991)** showed that the SA treatment and localized infection with *Pseudoperonospora cubensis* induced several novel acid soluble proteins in the treated and the upper untreated leaves in correlation with induced resistance. **Avdiushko *et al.* (1993); Gamil (1995); Mosa (1997)** and **Ahmed** (**2005**) detected an increase in the activities of peroxidase, polyphenol oxidase, lipoxygenase, chitinase and α-glucosidase in cucumber and squash leaves in the vicinity of lesions caused by dipotassium phosphate application. **Orober *et al.* (1998)** found an increase in the activities of peroxidase and polyphenoloxidase in all parts of the induced plants as a further consequence to the induction of systemic acquired resistance of phosphate application in cucumber against powdery mildew.

The current study was planned to examine the efficacy of certain chemical agents for inducing systemic protection against squash powdery mildew and biochemical changes in some chemical components with the inducted leaves were also investigated.

Materials and Methods

Source of diseased samples and propagation of mildew inoculum:

Squash plants, heavily infected with powdery mildew fungus, *Sphaerotheca fuliginea* (Schltdl.) Pollacci were collected during September, 2001. Inoculum of the powdery mildew was propagated as following: conidia of *Sphaerotheca fuliginea* - from the collected mildewed plants - were gently shaken over healthy squash plants 2 weeks age previously grown in a glasshouse. The newly mildewed squash plants were used as a source of conidial inoculum for further experiments.

Growing squash plants:

Squash seeds cultivar ‘Eskandarani’ were grown in pots 15 cm in diameter, one plant per each, three plants for each treatment. These pots were put aside from the plants grown for the mildew propagation in a separate room in the glasshouse.

Induction of systemic resistance:

Chemical induction of the systemic resistance (ISR) was performed at seedling stage (14 days after sowing) by spraying the upper surface of the first two true leaves with one of the following aqueous solutions 2 days before challenge inoculation by conidia of the powdery mildew fungus **(Strobel and Kuć, 1995)**. Salicylic acid (SA), ascorbic acid (AsA)], oxalic acid (OA), boric acid (BA), manganese sulphate ($MnSO_4$), cobalt chloride ($CoCl_2$), copper sulphate ($CuSO_4$), calcium chloride [$CaCl_2.2H_2O$] and potassium di-hydrogen phosphate (KH_2PO_4) were used as chemical inducers. Aqueous solutions of 5, 10 and 20mM were used for all, except KH_2PO_4 which was used at 50, 100 and 200mM.

For comparison with the tested chemical resistance-inducers, spraying with the wide broad used fungicide Topas-100 (10.0% penconazole "w/v" [(R,S-1-(2-(2,4-dichlorophenyl) -Q pentyl)-1H-1,2,4-triazole]) at 25 ppm (the recommended dose 0.25ml/L) and spraying with tap water were used in control treatments.

Challenge inoculation:

Inoculation was accomplished by shaking diseased squash samples over plants at a height of about 30cm. Inoculated plants were incubated on glasshouse benches until disease assessment was undertaken. Inoculation was done 2 days after foliar application with resistance-inducers **(Strobel and Kuć, 1995)**.

Disease assessment:

Fourteen days after challenge inoculation, powdery mildew disease development - as affected by the different tested treatment - was evaluated by counting the number of mildew colonies on leaves surface with the naked eye.

Biochemical changes:

Samples for chemical analysis were taken 30 days after treatment from the fourth plant leaf of each treatment. Extraction from squash leaves were prepared as follows: A representative samples, 1 g of each, were cut into small portions and immediately plunged into 95% boiling ethanol for ten minutes to kill the tissues. The extraction was then resumed in a soxhlet apparatus by using 75% ethanol as an extractant until the percolate was colorless (8-10 hrs). The combined ethanolic extracts were filtered and evaporated to near dryness on a mild water bath, 60°C. The dried residue was redissolved in a known volume, 5 ml, of 50% iso-propanol and used for chemical analysis as follows:

Determination of sugar content:

Total and reducing sugars were determined spectrophotometrically with picric acid as described by **Thomas and Dutcher (1924).**

Determination of phenolic compounds:

Phenolic compounds were determined using the colourimetric method of analysis by Folin-Ciocalteu reagent described by **Bray and Thorpe (1954)**.

Activities of peroxidase and polyphenol-oxidase:

The fifth leaf of treated and non-treated plants was harvested 30 days after treatment, by cutting them at the leaf base level. Leaf extract for

protein/enzyme assay were prepared from the harvested leaves according to **Tuzun *et al.* (1989).**

Peroxidase assay:

The activity of peroxidase enzyme was measured as described by **Chance and Maehly (1955)**. The obtained enzyme extract (0.3 ml) was added to 0.1 ml of 100 mM potassium phosphate buffer (pH 7.0), prepared by mixing 38.5ml of 100mM potassium phosphate monobasic (KH_2PO_4) and 61.5ml of 100mM potassium phosphate dibasic (K_2HPO_4); 0.32 ml of 5% pyrogallol; 0.16 ml of 0.5% hydrogen peroxide in sample cuvette (final volume of 3.0 ml) and rest of distilled water. The initial rate increase in absorbance at 420 nm was regarded as an arbitrary unit of enzyme activity. Enzyme activity was expressed as Δ_{420}/min/g**.**

Polyphenol oxidase assay:

Polyphenoloxidase was assayed following the method of **Taneja and Sachar (1974)**. The reaction mixture contained 2 ml of 1% catechol solution as substrate, 0.2 ml of enzyme extract and rest of 0.05 M sodium phosphate buffer pH 6.8 in a final volume of 4 ml. Enzyme activity was expressed as Δ_{430}/min/g.

Soluble protein assay:

Protein content was determined according to the method of **Bradford (1976)** using crystalline bovine serum albumin (BSA) as a standard. Five ml of the Bradford dye (reagent) were added to 100 µL of protein extract, vortexed and absorbance was measured at 595 nm after 2 min and before one hour. Protein concentration was calculated as mg/g^{-1} fresh weight from a standard curve of bovine serum albumin.

Results and Discussion

Effect of the tested foliar treatments on:

1. Number of the powdery mildew colonies on the upper leaves:

Results presented in **Table (1)** indicate that, most tested treatments inducing systemic protection against the natural infection with powdery mildew and this was greatly varied on the three upper leaves that expanded after foliar application as follow.

Average numbers of colonies on the upper 3 leaves revealed that all tested foliar spray treatments were significantly effective in this respect compared with control plants that sprayed with water. Comparing tested compounds, the fungicide Penconazole was the most effective followed by SA, OA, AsA, $CuSO_4$, KH_2PO_4, BA, $MnSO_4$, $CoCl_2$ and $CaCl_2$, respectively. Only $CaCl_2$ at 5mM had no clear significant effect in decreasing number of colonies when used at 10mM and 20mM compared with control treatment. These results are in agreement with **Mosa (1997)** he reported that the most effective

treatments were K_2HPO_4 and K_3PO_4 showing both protective and curative effects against *S. fuliginea* infection. The systemic fungicide Penconazole at 25 ppm provided complete protection as it reduced averages of number of mildewed colonies and disease severity by 100.0% on the upper leaves. Several investigators, in fact, proved the efficiency of systemic fungicides in controlling powdery mildew diseases **(Reuveni *et al.*, 1998)**. Also, **Ahmed** (**2005**) stated that, the induction of cucumber resistance to powdery mildew by phosphate salt (K_2HPO_4) exhibited that significantly reduced the percentage of powdery mildew incidence and severity. The high reduction was induced by Topas-100 at concentration 50 cm^3/100L and phosphate salt (K_2HPO_4) at concentration 100 mM/L.

2. Biochemical changes in the upper leaves:

2.1.Sugars and phenols contents:

Results presented in **Table (2)** indicate that sugars content was significantly affected by the tested treatments. Concerning with the tested chemical compounds and regardless concentration, copper sulphate, Penconazole, potassiun dihydrogen sulphate, oxalic asid, manganese sulphate, calcium chloride and cobalt chloride increased the reducing sugars over control treatment. While boric acid, SA, and ascorbic acid decreased it compared with the control. Reducing sugars were increased as concentration increased. Concerning interaction the same results proved that $CuSO_4$ used at 20 and 10mM induced the highest increase in the reducing sugars followed by Penconazole and OA at 20mM., while, AsA at 5 and 10mM, BA at 5mM, OA at 5mM, SA at 5 and 10mM decreased it. As for the content of non-reducing sugars, Penconazole, AsA, $CuSO_4$ and OA increased it by over control while, $MnSO_4$, BA, SA, $CaCl_2$, $CoCl_2$ and KH_2PO_4 decreased it comparing to the control treatment. With few exceptions, increasing tested concentration increased the non-reducing sugars also. The non-reducing sugars were significantly decreased by most tested treatments. The highest decrease was induced by KH_2PO_4 at 5 & 10mM. Concerning the total sugars, Penconazole, $CuSO_4$, OA, $MnSO_4$, KH_2PO_4, $CaCl_2$ and $CoCl_2$ increased it over control while AsA, BA and SA decreased it compared with control treatment. The total sugars were increased, in general, by increasing concentration of the tested chemicals. The highest increase in the total sugars was induced by Penconazole used at 25 ppm.

The data in **Table (3)** found that, the free, conjugated and total phenols were affected significantly by the tested treatments. Compared with control, all tested chemical compounds, except SA, increased the free phenols. The highest increase in the free phenols was induced by Penconazol. The observed increase in the free phenols occurred mainly on account of the reduction in both total and conjugated phenols. All tested chemical compounds caused significant decrease in both conjugated and total phenols. Percentage of reduction particularly in total phenols was proportionally increased, in most cases, as the tested concentration increased.

Table (1): Number of powdery mildewy colonies on the upper three leaves as affected by the tested foliar spray treatments.

Concentration / Chemical compound		Number of powdery mildewed colonies															
		3rd leaf				4th leaf				5th leaf				Average on the upper leaves			
		5mM	10mM	20mM	Mean	5mM	10mM	20mM	Mean	5mM	10mM	20mM	Mean	5mM	10mM	20mM	Mean
Ascorbic acid		43.0	22.5	21.0	28.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	14.3	7.5	7.0	9.6
Boric acid		87.0	41.0	25.0	51.0	7.0	0.0	0.0	2.3	0.0	0.0	0.0	0.0	31.3	13.7	8.3	17.8
Calcium chloride		177.0	162.0	76.0	138.3	67.0	60.0	50.0	59.0	1.5	0.0	0.0	0.5	81.8	74.0	42.0	65.9
Cobalt chloride		96.5	66.0	54.0	72.2	16.5	8.5	0.0	8.3	0.0	0.0	0.0	0.0	37.7	24.8	18.0	26.8
Copper sulfate		33.0	30.0	28.0	30.3	1.0	0.5	0.0	0.5	0.0	0.0	0.0	0.0	11.3	10.2	9.3	10.3
Potassium dihydrogen phosphate*		53.5	30.0	11.5	31.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	17.8	10.0	3.8	10.6
Manganese sulphate		85.0	80.0	0.0	55.0	4.0	2.5	0.0	2.2	0.0	0.0	0.0	0.0	29.7	27.5	0.0	19.1
Oxalic acid		22.5	18.0	8.5	16.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	7.5	6.0	2.8	5.4
Salicylic acid		5.0	0.0	1.5	2.2	9.5	6.0	0.0	5.2	0.0	0.0	0.0	0.0	4.8	2.0	0.5	2.4
Penconazole (25ppm)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Control		180.0	180.0	180.0	180.0	68.0	68.0	68.0	68.0	1.5	1.5	1.5	1.50	83.2	83.2	83.2	83.2
Mean		71.14	57.23	36.86		15.73	13.23	10.73		0.23	0.14	0.14		29.0	23.5	15.9	
L.S.D. at 5%	Compound	6.76				2.07				0.11				1.83			
	Concentration	3.53				1.09				0.06				0.96			
	Interaction	11.70				3.615				0.18				3.18			

*** Concentrations of KH_2PO_4 were 50, 100 & 200mM**

Table (2): Sugars contents in squash leaf-4 as affected by the tested foliar spray treatments.

Concentration / Chemical compound		Sugars contents (mg/g fresh weight)											
		Reducing sugars				Non-reducing sugars				Total sugars			
		5mM	10mM	20mM	Mean	5mM	10mM	20mM	Mean	5mM	10mM	20mM	Mean
Ascorbic acid		0.84	1.93	4.34	2.37	4.46	4.87	4.33	4.553	5.30	6.26	8.67	6.74
Boric acid		2.40	2.89	3.86	3.05	1.94	1.92	3.37	2.410	4.34	4.81	7.23	5.46
Calcium chloride		5.78	6.26	7.23	6.42	1.93	1.93	1.44	1.767	7.71	8.19	8.67	8.19
Cobalt chloride		4.82	6.74	7.71	6.42	1.44	0.97	0.89	1.100	6.26	7.71	8.60	7.52
Copper sulfate		8.67	12.08	13.98	11.58	4.82	2.37	1.92	3.037	13.49	14.45	15.90	14.61
Potassium dihydrogen phosphate*		7.23	8.19	8.67	8.03	0.48	0.48	1.93	0.963	7.71	8.67	10.60	8.99
Manganese sulphate		6.26	6.75	7.23	6.75	1.39	3.37	3.37	2.710	8.19	10.12	10.60	9.64
Oxalic acid		4.33	8.67	9.64	7.55	3.86	1.93	2.89	2.893	8.19	10.60	12.53	10.44
Salicylic acid		0.84	2.41	5.30	2.85	2.05	2.40	0.96	1.803	2.89	4.81	6.26	4.65
Penconazole (25ppm)		10.12	10.12	10.12	10.12	6.27	6.27	6.27	6.270	16.39	16.39	16.39	16.39
Control		4.34	4.34	4.34	4.34	2.88	2.88	2.88	2.880	7.22	7.22	7.22	7.22
Mean		5.06	6.40	7.49		2.87	2.67	2.75		7.97	9.02	10.24	
LSD at 5%	Compound	0.38				0.16				0.35			
	Concentration	0.20				0.08				0.18			
	Interaction	0.6504				0.2680				0.6015			

*** Concentrations of KH_2PO_4 were 50, 100 & 200mM**

Table (3): Phenols contents in squash leaf-4 as affected by the tested foliar spray treatments.

Concentration / Chemical compound		Phenols contents (mg/g fresh weight)											
		Free phenols				Conjugated phenols				Total phenols			
		5mM	10mM	20mM	Mean	5mM	10mM	20mM	Mean	5mM	10mM	20mM	Mean
Ascorbic acid		12.8	4.0	2.6	6.47	8.0	21.0	3.4	10.80	20.8	25.0	6.0	17.27
Boric acid		11.4	0.5	7.7	6.53	12.8	18.8	5.0	12.20	24.2	19.3	12.7	18.73
Calcium chloride		13.2	11.3	8.9	11.13	13.2	9.5	6.4	9.70	26.4	20.8	15.3	20.83
Cobalt chloride		17.7	13.9	9.2	13.60	11.4	13.8	12.9	12.70	29.1	27.7	22.1	26.30
Copper sulfate		18.8	5.1	5.4	9.77	4.6	5.3	2.7	4.20	23.4	10.4	8.1	13.97
Potassium dihydrogen phosphate*		11.0	19.3	11.4	13.90	17.5	8.5	14.8	13.60	28.5	27.8	26.2	27.50
Manganese sulphate		12.0	9.7	10.4	10.70	13.5	6.4	1.8	7.23	25.5	16.1	12.2	17.93
Oxalic acid		12.8	7.7	11.3	10.60	16.3	18.4	8.5	14.40	29.1	26.1	19.8	25.00
Salicylic acid		11.5	2.2	1.0	4.90	10.8	11.6	6.2	9.53	22.3	13.8	7.2	14.43
Penconazole (25ppm)		19.7	19.7	19.7	19.70	6.5	6.5	6.5	6.50	26.2	26.2	26.2	26.20
Control		5.4	5.4	5.4	5.40	25.3	25.3	25.3	25.30	30.7	30.7	30.7	30.70
Mean		13.30	8.982	8.455		12.72	13.191	8.50		26.02	22.17	16.95	
LSD at 5%	Compound	0.53				0.62				0.69			
	Concentration	0.28				0.33				0.36			
	Interaction	0.914				1.081				1.194			

*** Concentrations of KH_2PO_4 were 50, 100 & 200mM**

Table (4): Activity of peroxidase and polyphenol oxidase enzymes* and protein content in the 5th leaf as affected by the tested foliar spray treatments.

Concentration / Chemical compound		Peroxidase activity				Polyphenol oxidase activity				Protein content			
		5mM	10mM	20mM	Mean	5mM	10mM	20mM	Mean	5mM	10mM	20mM	Mean
Ascorbic acid		0.075	0.086	0.080	0.0803	0.137	0.146	0.322	0.2017	1.69	3.12	1.76	2.19
Boric acid		0.068	0.144	0.106	0.1060	0.296	0.245	0.057	0.1993	0.03	0.09	0.11	0.078
Calcium chloride		0.061	0.082	0.054	0.0657	0.215	0.300	0.163	0.2260	0.24	0.59	0.51	0.45
Cobalt chloride		0.080	0.079	0.149	0.1027	0.122	0.183	0.281	0.1953	0.03	2.12	2.01	1.39
Copper sulfate		0.073	0.076	0.055	0.0680	0.164	0.094	0.156	0.1380	2.16	3.05	2.81	2.67
Potassium dihydrogen phosphate*		0.079	0.096	0.133	0.1027	0.481	0.413	0.515	0.4697	0.82	1.39	1.59	1.27
Manganese sulphate		0.152	0.202	0.210	0.1880	0.132	0.445	0.541	0.3727	0.86	1.08	1.01	0.98
Oxalic acid		0.098	0.101	0.131	0.1100	0.373	0.600	0.432	0.4683	0.15	0.07	0.18	0.13
Salicylic acid		0.084	0.188	0.153	0.1417	0.248	0.173	0.178	0.1997	1.26	0.81	1.09	1.05
Penconazole (25ppm)		0.075	0.075	0.075	0.0750	0.176	0.176	0.176	0.1760	0.15	0.15	0.15	0.15
Control		0.078	0.078	0.078	0.0780	0.216	0.216	0.216	0.2160	0.11	0.11	0.11	0.11
Mean		0.084	0.110	0.111		0.233	0.272	0.276		0.682	1.144	1.030	
LSD at 5%	Compound	0.03				0.03				0.14			
	Concentration	0.02				0.01				0.08			
	Interaction	0.04893				0.04614				0.24			

*** Activities expressed as change in absorbance/ 5 min./g fresh weight**

****BVA = Bovine Serum Albumin.**

***** Concentrations of KH_2PO_4 were 50, 100 & 200mM.**

As for the interaction between compound and concentration, the same data proved that free phenols content was increased significantly by most interactions. The highest increase was induced by the fungicide Penconazole at 25 ppm. On the contrary, the free phenols were significantly decreased by few interactions. Also, the total phenols were decreased significantly by all tested interactions compared with the control. Applying AsA at 20mM caused the highest decreases in the total phenols while, $CoCl_2$ and OA used at 5mM caused the lowest significant decreases in the total phenols. The conjugated phenols content was affected similarly as in the total phenols. The highest reduction was induced by MnSO4 at 20mM. While, AsA at 10mM induced the lowest decrease in the conjugated phenols.

It is well known that plant phenols, particularly the free phenols – which are toxic substances - play a significant role in controlling pathogenic microorganisms attacking variety of plants. Unlike situation in the non-inducted plants, the plants inducted by either biotic or abiotic inducers contained higher levels of sugars **(Liu *et al.*, 2000)** and phenols **(Meena *et al.*, 2001)**. On the contrary, fractions of both reducing and total sugars and phenols contents were significantly decreased by applying AsA, BA and SA at all tested concentrations (with very few exceptions) compared with control. **Ahmed** (**2005**) found that, phosphate salt (K_2HPO_4) increased of sugars and phenols content in cucumber leaves after treated to induction resistance against powdery mildew.

2.2. The activities of peroxidase and polyphenol oxidase enzymes:

The data in **Table (4)** showed that the peroxidase activity expressed as change in absorbance/ 5 min./g fresh weight was affected differently by the tested treatments. Most tested chemical compounds caused significant increase in the peroxidase activity compared with control. Applying $MnSO_4$ induced the highest increase in peroxidase activity followed by SA, OA respectively. However, both $CuSO_4$ and $CaCl_2$ did not affect peroxidase activity compared with the control treatment. The peroxidase activity was increased, in general, as the concentration of the tested compound increased. Among all tested treatments, peroxidase activity was significantly increased by $MnSO_4$ at 20mM.

Concerning with activity of polyphenol oxidase enzyme, the data in **Table (4)** declared that KH_2PO_4, OA and $MnSO_4$ caused significant increase in the PPO activity. However, Penconazole and $CuSO_4$ significantly decreased its activity. The other tested chemical compounds *i.e.* $CaCl_2$, AsA, SA, BA and $CoCl_2$ did not affect PPO activity compared with control.

The highest significant increase in the PPO activity was induced by the middle and higher concentration compared with the low one. These results are in agreement with those finding by **Gamil (1995)** stated that spraying foliar of squash plant with Cobalt sulfate treatment reduced peroxidase and polyphenol oxidase activity in detached squash leaves after inoculation. Potassium phosphate decreased polyphenol oxidase activity but increased peroxidase in detached leaves 48 h after inoculation. **Orober *et al.* (1998)** recorded that the

foliar application of phosphate induced systemic acquired resistance (SAR) in cucumber against powdery mildew (*Sphaerotheca fuliginea*). As a further consequence of phosphate application, activities of typical defense-related enzymes like peroxidase and polyphenoloxidase increased in all parts of the induced plants. Similar increases in the oxidative enzymes activities were observed also by several investigators in the inducted plants **(Mosa, 1997; Reuveni *et al.*, 1997; Orober *et al.*, 1998**; **Mosa, 2002** and **Ahmed (2005)**.

2.3. The total soluble protein content:

The data in **Table (4)** stated that, the soluble protein content in the 5th leaf of squash plants was responded differently against the tested treatments. Copper sulfate ($CuSO_4$), ascorbic acid (ASA), cobalt chloride ($CoCl_2$), potassium di-hydrogen phosphate $(KH_2PO)_4$, salicylic acid (SA), magnesium sulfate ($MnSO_4$) and calcium chloride ($CaCl_2$) significantly increased the protein content. The obtained results could be supported by **Mills and Wood (1984)** they reported that injection of cucumber cotyledons with salicylic acid (SA) and other phenolic acids induced resistance to inoculations with *Colletotrichum lagenarium* when inoculation followed injection by 96 h but not 24 h. **Okuno *et al.* (1991)** recorded that spraying cucumber leaves with salicylic acid (SA) reduced the diseased area caused by *Pseudoperonospora cubensis* by >50% in the sprayed 1st leaves and also in the upper 2nd leaves provided challenge inoculation was made 3-6 days but not 1-24 h after treatment. Electrophoretic analysis of extracted proteins on polyacrylamide gel showed that both the SA treatment and localized infection with *P. cubensis* induced several novel acid soluble proteins in the treated and the upper untreated leaves in correlation with induced resistance. **Feussner *et al.* (1997)** investigated changes in lipoxygenase protein pattern and/or activity in relation to acquired resistance of cucumber leaves against 2 powdery mildews (*Sphaerotheca fuliginea* and *Erysiphe cichoracearum*).

On the contrary, the fungicide Penconazole (at 25 ppm), boric acid (BA) and oxalic acid (OA) at 5, 10 and 20mM and $CaCl_2$ and $CoCl_2$ (at 5mM), however, did not affect the total soluble protein content in tissues of the upper 4th squash leaf compared with control. It is well known that a variety of chemicals have been shown to induce systemic resistance and their action often involves signaling steps that are also required for the expression of systemic acquired resistance **(Ward *et al.*, 1991)**.

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التغيرات البيوكيميائية في أوراق الكوسة المرشوشة ببعض الكيماويات لاستحثاث المقاومة للبياض الدقيقي.

نوال عبد المنعم عيسى ، عبد المنعم إبراهيم الفقي ، فتحي جاد محمد ، و محمد حامد الهبّاق
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في هذه الدراسة تم اختبار الرش الورقي بتسع مركبات كيميائية هي حامض الأسكوربيك، حامض البوريك، كلوريد الكالسيوم، كلوريد الكوبلت، كبريتات النحاس، كبريتات المنجنيز، حامض الأوكساليك، فوسفات البوتاسيوم ثنائية الهيدروجين، و حامض السالسيليك بالنسبة لقدرتها على استحثاث المقاومة لفطر "سفيروثيكا فيوليجينيا" مسبب البياض الدقيقي على نباتات الكوسة المنزرعة تحت الصوبة الزجاجية.

تم رش بادرات الكوسة في عمر أول ورقتين حقيقيتين بالمركبات المختبرة (باستخدام ثلاثة تركيزات متتابعة من كل منها) مع الرش بالمطهر الفطري "بنكونازول" الفعال في مقاومة المرض بتركيز 25 جزء في المليون على سبيل المقارنة. تم تقدير شدة المرض في عمر الورقة الحقيقية الخامسة.

أوضحت النتائج أن جميع المركبات المختبرة – فيما عدا كلوريد الكالسيوم – كانت فعالة في استحثاث الوقاية الجهازية للبياض الدقيقي، إلا أن معظمها كان أقل فعالية في مقاومة المرض عن المطهر الفطري "بنكونازول" الذي وفر وقاية جهازية قدرها 100% على الأوراق العليا مثله في ذلك مثل المعاملة بكبريتات المنجنيز 20 مللي مولر.

تم دراسة بعض التغيرات البيوكيميائية في الأوراق الناتجة بعد المعاملة في إطار تحديد جزء من الميكانيكيات التي من خلالها تقوم عملية الاستحثاث بتوفير الحماية من مرض البياض الدقيقي. وجد أن ستة من المركبات المختبرة سببت زيادة معنوية في محتوى السكريات الكلية في الأوراق بينما قللت جميعها من محتوى الفينولات الكلية مقارنة بالكنترول. أظهرت المعاملة بكل من كبريتات المنجنيز، وحامض السالسيليك، وحامض الأوكساليك، وحامض البوريك زيادة واضحة في نشاط إنزيم البيروكسيديز. بينما تأثر نشاط إنزيم البولي فينول أوكسيديز بمعاملات كبريتات المنجنيز، وفوسفات البوتاسيوم الأحادية تأثيرا إيجابيا عاليا مقارنة بالكنترول. إضافة إلى ذلك وجد أن أغلب المركبات المختبرة سببت زيادة معنوية في محتوى الأوراق من البروتين الذائب الكلي.