
مشروع المكافحة المتكاملة للآفات بالفيوم
F.I.P.M.P
Faculty of Agriculture
Fayoum University
FJARD
The Second Conference
on Farm Integrated Pest Management
16-18 Jan. 2006
Fayoum, Egypt

The 2nd Conf. on Farm Integrated Pest Management 2006, Fayoum, Egypt: 211-222

BIOCHEMICAL CHANGES IN SQUASH LEAVES SPRAYED WITH SOME CHEMICALS FOR INDUCING RESISTANCE 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 evaluate 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 where they caused a 100% systemic protection on the upper leaves. Among the tested agents, six of them 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 where it was higher than the control. In addition, it was found that most of the tested compounds caused significant increase in the total soluble protein of the 4th 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 Egypt, where it 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 could be a consider the 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. Also, **Reuveni *et al.*, (1995 and 1997)** tested solutions of K_2HPO_4, KH_2PO_4, $CuSO_4$, $MnCl_2$ and boric acid on cucumber. **Gamil (1995)** and **Ahmed (2005)** used foliar sprays of $CoSO_4$ and K_2HPO_4 on squash and cucumber plants to inducers of systemic resistance against powdery mildew.

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.

Gottstein and Kuć (1989) proved that systemic accumulation of defence-related enzyme peroxidase can be induced in leaves treatmed with chemicals for inducing resistance to diseases in cucumber. **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)** detected the high 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)** and **Ahmed (2005)** found an increase in the activities of peroxidase and polyphenoloxidase in all plant parts of cucumber treated with phosphate for the induction of systemic acquired resistance against powdery mildew.

This study aimed to examine the efficacy of certain chemical agents in inducing systemic protection against squash powdery mildew. Also, assaying some biochemical changes, which expressed the systemic acquired resistance resulting from treating squash leaves with these chemical agents.

MATERIALS AND METHODS

Sampling and propagules of mildew inoculum:

Heavily infected squash plants with powdery mildew, *Sphaerotheca fuliginea* (Schltdl.) Pollacci which collected during September, 2001 were used as a source of powdery mildew inoculum. Then, the collected spores were shaked gently over healthy squash plants two weeks age grown in glasshouse. The newly mildewed squash plants were used in this case as a source of powdery mildew propagules for further experiments.

Growing squash plants:

Pots (15 cm ϕ) filled with 2 kg clay soil were sown with squash seeds (cv. Eskandarani) at rate one seed per each pot, then, irrigated and left in the glasshouse until sprouting the seeds. Three pots were used for each treatment. The growing squash seedling was transferred to a separate part in the glasshouse and surrounded by squash plants heavily infected with powdery mildew.

Application of induced resistance chemicals:

Squash seedlings (14 days age) were sprayed onto the upper surface of the first two true leaves with one of the tested aqueous solutions 2 days before inoculation by conidia of the powdery mildew fungus **(Strobel and Kuć, 1995)**. In this respect, salicylic acid (SA), ascorbic acid (AA), 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 was used at 50, 100 and 200mM.

Control treatments were sprayed firstly with the powdery mildew 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 secondly, sprayed with tap water only. Sprayed and inoculated squash plants were incubated on glasshouse benches until appearance of powdery mildew symptoms.

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 post inoculation, powdery mildew disease 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 assaying protein and enzyme activities was 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 and rest of distilled water (final volume of 3.0 ml). 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 **Tanеja 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 Δ430/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 the absorbance was measured at 595 nm after 2 min as well as before one hour. Protein concentration was calculated as $mg.g^{-1}$ fresh weight from a standard curve of bovine serum albumin.

RESULTS AND DISCUSSION

1- Effect of the tested foliar treatments on powdery mildew infection:

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

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. Meanwhile, the fungicide Penconazole was the most effective followed by SA, OA, AA, $CuSO_4$, KH_2PO_4, BA, $MnSO_4$, $CoCl_2$ and $CaCl_2$, respectively. Only $CaCl_2$ had no clear significant effect in decreasing number of colonies compared with control treatment. These results are in agreement with **Mosa (1997)** who 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) 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.

2. Biochemical changes in the upper leaves:

2.1. Sugars and phenols contents:

Results presented in **Table (2)** indicate that all tested chemical substances as resistance inducer affected significantly sugars content. In this respect, copper sulphate, Penconazole, potassium dihydrogen phosphate, oxalic acid, manganese sulphate, calcium chloride and cobalt chloride increased the reducing sugars comparing to the control treatment. Meanwhile, boric acid, SA, and ascorbic acid decreased it compared with the control. Also, increasing concentration of tested substances increased consequently reducing sugars. As for 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 AA 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, AA, $CuSO_4$ and OA increased it compared with control case 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 AA, 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 fungicide.

Data in **Table (3)** indicate 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 off 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.

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

Table (1): Number of powdery mildew 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			
L.S.D. at 5% Concentration	3.53				1.09				0.06				0.96			
L.S.D. at 5% 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

phenols were significantly decreased by few interactions. Also, the total phenols were decreased significantly by all tested interactions compared with the control. Applying AA at 20mM caused the highest decrease 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 $MnSO_4$ at 20mM. While, AA 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-induced plants, the plants induced by either biotic or abiotic inducers contained higher levels of sugars **(Liu *et al.*, 2000)** and phenols **(Meena *et al.*, 2001)**. **Ahmed (2005)** found that, phosphate salt (K_2HPO_4) increased sugars and phenols content in cucumber leaves after treated to induce resistance against powdery mildew.

2.2. The activities of peroxidase and polyphenol oxidase enzymes:

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 of 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, data in **Table (4)** declare 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.* $CaCl_2$, AA, 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)** who stated that foliar spraying of squash plants with cobalt sulfate 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 induced plants **(Mosa, 1997; Reuveni *et al.*, 1997; Orober *et al.*, 1998; Mosa, 2002 and Ahmed, 2005).**

2.3. The total soluble protein content:

Data in **Table (4)** indicate 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 (AA), cobalt chloride ($CoCl_2$), potassium di-hydrogen phosphate (KH_2PO_4), salicylic acid (SA), manganese sulfate ($MnSO_4$) and calcium chloride ($CaCl_2$) significantly increased the protein content. The obtained results could be supported by **Mills and Wood**

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 polyphenoloxidase 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.

(1984) who 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 24h. **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 leaf and also in the upper 2nd leaf provided challenge inoculation was made 3-6 days but not 1-24h 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, boric acid (BA) and oxalic acid (OA) at 5, 10 and 20mM, $CaCl_2$ 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

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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 لقطر "سفيروثيكا فوليجينيا" 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 ٢٥ 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 ١٠٠% على الأوراق العليا مثله في ذلك مثل المعاملة بكبريتات المنجنيز ٢٠ ملي 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