

## MINERAL SALTS IN CONTROLLING POWDERY MILDEW OF SQUASH

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### Abstract

Powdery mildew of squash caused by *Sphaerotheca fuliginea* is more prevalent in Egypt, in Summer and Nili plantations than in the Winter . The effect of spraying different salts on disease incidence was studied. Results revealed that KCl,  $\text{KH}_2\text{PO}_4$  and  $\text{K}_2\text{CO}_3$  were generally effective in powdery mildew control. It was also concluded that potassium salts at concentrations of 750  $\mu\text{g/ml}$  decreased infection, as compared with the fungicide Prochloraz either applied after or before symptom development. Biochemical changes associated with salt application possibly induced systemic resistance. The presence of common protein bands were recognized. Electrophoretic analysis of extracted proteins on polyacrylamide gel showed greater number of protein bands with fungicide treatment as compared with  $\text{K}_2\text{CO}_3$ , KCL,  $\text{KH}_2\text{PO}_4$  and the control.

**key word:** Squash, Powdery mildew, Salt, *Sphaerotheca fuliginea*, Resistante, and Pathogenesis-Proteins.

### INTRODUCTION

Squash (*Cucurbita pepo* L.) is one of the most important vegetable crops in Egypt. It is cultivated mainly at three dates, locally defined as Winter, Nili and Summer plantations . The total area planted in Egypt reached 88372 feddans in 2008, producing approximately 651589 tons of marketable fruit, (Dept.Agric.Stat.,Min.Agric., 2008). Squash plants are subject to several diseases , causing great losses in yield and quality. Disease distribution differs according to the season of plantation ,powdery mildew caused by *Sphaerotheca fuliginea* (Schlechtend:fr). is a widespread and important disease .

Although *Cucurbita* species are susceptible to powdery mildew, symptoms may not be recognized totally on melon cultivars . Plants are known to acquire local and systemic resistance against pathogens in response to primary infection (Sequeira , 1983). The principal effect of the disease on squash may be manifested in decreasing fruit size and fungicidal sprays are a basic aspect required for the disease control (Sherf & MacNab, 1986) . It has been reported that some phosphate salts could induce local and systemic resistance to various plant diseases including powdery mildew of cucumber (Mucharromah & Kuc, 1991). Meanwhile, other inorganic salts

such as sodium bicarbonate (Ziv & Zitter, 1992) and lithium chloride (Abood *et al* 1991) were found to have some inhibitory effects on *Sphaerotheca fuliginia*.

In trials to explain the effect of physiological and chemical changes following salt (s) application. Bournarye *et al* (2005) reported that biochemical analyses showed similar levels of 4-coumarate:CoA ligase (4 CL), protein accumulation for all treatments. However, the results support the idea that induced resistance in cucumber is largely correlated with rapid de novo biosynthesis of flavonoid phytoalexin compounds.

Reuveni *et al* (1995) found that the powdery mildew infection on cucumber was significantly controlled by a single spray of aqueous solutions (25 mM) containing various phosphates and potassium salts which also reduced the production of conidia from colonies.

Nam Jun Kang (2008) found that the powdery mildew infection on cucumber was significantly reduced by foliar application of a mixture of riboflavin and methionine (RM). The effects of fungicidal activity on leaves applied with RM were detected through restriction of progress of colonies and disease severity compared with control plants. Protein analyses revealed that the bands increased after application as compared with the control .

The present work is concerned with evaluating the effect of spraying inorganic salts at different concentrations on powdery mildew disease incidence and severity and to explain their possible modes of action on squash.

## MATERIALS & METHODS

### Greenhouse experiment

Domestic squash cultivar (Eskandarany) susceptible to *Sphaerotheca fuliginia* (Schlecht. Ex Fr. Pol), race 0,1 and 2, (Ahmed *et al* 2000) was used. Five seeds were sown per pot (30 cm diameter) containing a sandy loam soil under greenhouse conditions (20-24 °C), in five replicates .

### Pathogenicity and inoculation

For inoculation, the conidia of *S. fuliginia* were collected from naturally infected leaves of squash plant . Conidial suspensions in sterilized water , were adjusted to  $3 \times 10^4$  conidia /ml, then atomized onto the upper leaf surface of squash plants . The treated plants were separated into two groups :

1) Plants of the first group were inoculated with *S. fuliginia* ( $3 \times 10^4$  conidia/ ml) at the second and third leaf phase, then received the treatment concerned .

2) The second group was prepared in the same way mentioned before, except inoculation with the fungus was carried out 3day after mineral salt or fungicide treatments.

### The treatments included

- 1- Potassium dihydrogen phosphate  $\text{KH}_2\text{PO}_4$
- 2- Potassium chloride  $\text{KCl}$
- 3- Potassium carbonate  $\text{K}_2\text{CO}_3$
- 4- Prochloraz ( Master 25%)
- 5- Control unsprayed plants

Solutions were prepared and used at concentration of 250,500,750 ug/ml and 1 ml/L for the fungicide treatment.

**Disease assessment:** Disease severity was estimated on an arbitrary scale of (0-5) of Descalzo *et al* (1990)., where,

- 0 = no mildew colonies observed
- 1 = 1-25 colonies/ leaf
- 2 = 26-50 colonies/leaf
- 3 = 51-75 colonies/leaf
- 4 = 76-100 colonies/leaf
- 5 = >100 colonies per leaf.

$$\text{Disease severity} = \frac{\sum(\text{disease rating} \times \text{number of leaves of that specific rating})}{\text{Maximum rating of disease severity per test leaf} \times \text{total of test leaves}} \times 100$$

### Protein extraction and electrophoretic analysis

Sampled leaves were collected from different treatments and kept frozen at (-80°C) till use, according to the method of Laemili(1970), however N,N,N,N- Tetra methyl ethylene diamine (TEMED) was reduced to 25ul and Ammonium per sulphate solution (APS) was reduced to 1.3ml. Approximately 3g frozen leaves sample was ground in a mortar and pestle in liquid nitrogen until complete homogenization. The homogenate was transferred to 1ml Eppendorf tube, brought to 200 ul with extraction buffer (50mM Tris-HCl buffer, PH 6.8 , glycerol 10%w/v, ascorbic acid 0.1% ,cysteine hydrochloride 0.1% w/v ) . Centrifugation at 18000 rpm for about 30 min was carried out. Protein in the supernatant was estimated according to the method of Bradford (1976) using bovine serum albumin as a standard. Protein content was adjusted to 2mg/ml, then used for protein analysis on a 12.5% polyacrylamide slab gel in the presence of 0.1% sodium dodocyl sulfata (SDS) as described by Okuno&Furusawa (1979). Gel was fixed with 10% acetic acid in a 45% methanol solution

overnight. Protein was visualized by silver staining. Molecular weight markers used in SDS- PAGE were (116,66,45,35) KD.

## RESULTS

Applications of different salts ( $\text{KH}_2\text{PO}_4$ , KCl,  $\text{K}_2\text{CO}_3$ ) and the fungicide Master 25% to squash plants grown under greenhouse conditions were studied .

### Greenhouse experiment

Table(1) shows the effects of various concentration of different salt treatments either as pre- or post inoculation with *S. fuliginea* on squash leaves. Disease severity decreased on new leaves as compared with untreated control. The most effective treatments were  $\text{K}_2\text{CO}_3$ , KCl, and  $\text{KH}_2\text{PO}_4$ , respectively. The magnitude of reduction was higher in the pre inoculation treatments .

Table (2) shows significant reduction in number and size of colonies per leaf when sprayed with the salts at the high concentration(750 ug/ml). Variable results were observed for different salts at different concentrations, though reductions in disease severity compared with the control . were observed . pre-inoculation applications resulted in reduced number of diameter of colonies. Fungicide application as pre- and post inoculation treatments was more effective in *S. fuliginea* control under greenhouse conditions .

Table 1. Effect spraying squash leaves with different salts on infection with *S. fuliginea*.

Treatment	Concentration Ug/ml	Disease severity %	
		Time	
		After inoculation	Before inoculation
$\text{KH}_2\text{PO}_4$	250	37.3	26.6
	500	32.0	20.0
	750	28.0	14.7
KCL	250	36.0	24.0
	500	30.6	18.6
	750	26.6	13.3
$\text{K}_2\text{CO}_3$	250	33.3	20.0
	500	29.3	18.6
	750	25.3	12.0
Fungicide	Prochloraz 1 ml	10.6	4.5
Control	-----	46.7	46.7
L.S.D 0.05	Treat.= 0.98	Time = 0.72	Time xConc.=1.97
	Conc.= 1.42	Time xTreat. = ns	
	Treat x conc. = ns	Treat. xConc.x Time = ns	

Table 2. Effect spraying squash leaves with different salt on number and colony diameter of *S. fuliginea* in greenhouse.

Treatment	Concentration ug/ml	Av. Number of colony/leaf		Av. Colony diameter (cm)	
		After inoculation	Before inoculation	After inoculation	Before inoculation
KH <sub>2</sub> PO <sub>4</sub>	250	27.4	21.5	0.89	0.61
	500	18.7	12.3	0.78	0.46
	750	13.3	9.9	0.46	0.37
KCl	250	25.2	21.2	0.86	0.86
	500	14.7	11.8	0.78	0.61
	750	11.5	6.9	0.61	0.46
K <sub>2</sub> CO <sub>3</sub>	250	22.9	20.4	0.86	0.78
	500	13.3	9.5	0.78	0.46
	750	9.3	6.2	0.46	0.37
Fungicide	Prochloraz 1 ML	5.9	4.8	0.43	0.34
Control	-----	42.3	42.3	1.2	1.2
L.S.D 0.05	Treat. =	0.85		0.026	
	Conc. =	1.14		0.047	
	Treat.x Conc. =	ns		ns	
	Time =	0.61		0.022	
	Time x Treat. =	ns		ns	
	Time x Conc. =	1.38		0.062	
	Treat. xTime xConc. =	ns		0.088	

### Protein extraction and electrophoretic analysis

Mildewed squash plants at the of 3-4 leaves growth stage were sampled for protein analysis (Table 3). Composite leaves extracts were subjected to electrophoresis for 6 hours. Extracts of non-sprayed mildewed leaves showed 11 bands with molecular weights ranging between (35-109 KD). The fungicide Prochloraz - treated leaves and those sprayed with potassic salts, increased the number of separated bands being more pronounced in the fungicide treatments. The latter revealed 16 bands with molecular weights ranging between 34 and 118 KD, compared to the control(35-109 KD). It is obvious, however, that the fungicide treatment caused a distinct absence of a protein band, with a molecular weight 35 KD, contrary to the rest of treatments and control.

Table 3. Electrophoretic analysis of soluble proteins of different treatment.

KD	Amount %					
	Protein marker	Fungicide	KH <sub>2</sub> PO <sub>4</sub>	KCL	K <sub>2</sub> CO <sub>3</sub>	Control
118		0.17			0.95	
116	20.02			1.12		
112					1.26	
109		0.26				3.34
107			4.65	6.92		
101		0.98				
98		0.24			5.61	
96		0.39			0.63	
94					0.93	
93						4.84
92					0.81	
89		3.64				
87					4.29	4.24
85			8.26	6.47		
70		4.81				9.86
69					8.55	
68			9.25	9.68		
66	41.85					
63						3.19
62			2.35		3.31	
61				2.79		
59				3.34		
58			6.80			17.02
57		15.06				
56			14.56		14.94	
55				16.15		
53		2.78				
52						8.06
51		8.50	9.95	9.56	8.09	
49			4.58			
48				3.60		
47						0.58
45	22.63					
44						7.22
43		7.33	6.70	6.80	9.91	
42		0.83				
41			3.56	2.81		
40		6.20				
38		12.62		17.03	27.42	27.94
37		21.22	16.72			
35	15.50		12.62	13.75	13.29	13.74
34		14.99				

Although the potassic salts showed less effect in increasing the number of protein bands, the highest effect, however, may be considered for potassium carbonate. The latter showed 14 bands, followed by potassium chloride (13 bands) and potassium monobasic phosphate (12 bands). It could be recognized that the salt treatments caused disappearance of bands with molecular weights of 70 and 109 KD compared to the

control. Protein analysis Table (3) showed as well the detected common proteins with molecular weights and protein bands (51 and 43 K.D) in treatment with  $\text{KH}_2\text{PO}_4$  & KCl and  $\text{K}_2\text{CO}_3$ . Treatment with fungicide showed, however, a higher number of protein bands compared with control and different salt, showing unique bands with molecular weight of 101, 89, 57, 53,40 and 34 K.D .

## DISCUSSION

The first systematic enquiry into induced resistance was made by Ross (1961). Induced disease resistance has been adopted as a general term and defined as 'the process of active resistance dependent on the host plant's physical or chemical barriers, activated by biotic or abiotic inducing agents (Kloepper *et al.*, 1992). Resistance to primary infection can result from the presence of preformed defensive barriers (Osbourn, 1996), but often depends on inducible resistance mechanisms, where the infecting pathogen triggers defense responses through the release of elicitors which, in turn, lead to the expression of novel anti-pathogenic activities (Hammond-Kosack & Jones, 1996).

Chemicals, such as salicylic, polyacrylic, and fatty acids, inorganic salts, as well as physical stimuli (wounding, UV, B radiation, osmotic shock, low temperature, water deficit and excess), can be involved in induction of resistance .

In the present study, the control of squash powdery mildew with non traditional methods, such as mineral salts sprays, compared with the recommended fungicides, was considered. The results showed that foliar applications of potassium salts (750ppm) in the form of acid phosphate , chloride and carbonate either before or after leaf inoculation gave promising disease control as compared with the fungicide prochloraz (Master 25%). The treatments in concern gave reassembled disease control , but not as equal as the fungicide, being more pronounced for the K-carbonate followed by K-chloride and K- acid phosphate . Evaluation was based primarily on the number and the size of the fungal colony. These results are in accordance , in part , with those reported by Reuveni (1995). Results obtained on the mode of action of phosphate salts for controlling powdery mildew , has been attributed to the possible increase in the synthesis of host metabolites (Yoshikaw, 1978 ). The phosphate salts were reported to induce systemic resistance to anthracnose in cucumber. This activity may involve the sequestration of calcium ions, which could disrupt the cell wall and cause the release of defence-inducing cell wall fragments (Gottstein & Kuc, 1989). Calcium-binding organic acids such as oxalates also induced resistance to anthracnose in cucumber (Doubrava *et al.*, 1988).

In this regard, the present study showed a remarkable effect of salts on protein metabolism as shown by the different bands separated by electrophoresis. Molecular masses of polypeptides were shown to range in size from 118 to 34 KD on SDS- PAGE. Moreover, the polypeptides accumulation and pattern were changed by treatment . It is observed that the fungicide and  $K_2CO_3$  treatments resulted in larger number of bands than KCl,  $KH_2PO_4$  or the control. The biological functions of such stress induced proteins have been studied extensively and some of them were identified as chitinases and B-1,3 glucanases in many plant species . Christ & Mosinger (1989) attributed induced resistance against *Peronospora tabacina* and *Phytophthora infestans* to both chitinase and B- 1,3 glucanase activities . Meanwhile, the rapid absorption of phosphates by the plant tissues and their extreme mobility within the tissues as well as their characteristic in stimulating plant growth, as shown in previous studies on cucumber and maize (Reuveni *et al* ., 1995). Their low cost, low animal toxicity, comparative environmental safety and nutrient value make them ideal foliar fertilizer, which should be considered for application in the field for disease control.

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## الاملاح المعدنية ومكافحة البياض الدقيقى فى الكوسة

نجلاء عبد الباقي سلام مهنا ، سهام سمير محمد رجب ، جهاد محمد محمد

معهد بحوث أمراض النبات - مركز البحوث الزراعيه - الجيزه

تمت دراسته مكافحة مرض البياض الدقيقى فى الكوسه المسبب عن سفروسىكا فلجينيا باستخدام املاح فوسفات البوتاسيوم وكلووريد البوتاسيوم و كربونات البوتاسيوم مقارنة بأحد المبيدات . تم استخدام تركيبات مختلفه من الاملاح ( ٢٥٠ - ٧٥٠ ) جزء فى المليون وأوضحت الدراسه أن أفضل التركيزات لهذه الاملاح هى ٧٥٠ جزء فى المليون. كما اوضحت أن أفضل الأملاح فى تقليل الإصابة هى كربونات ثم كلوريد ثم فوسفات البوتاسيوم على التوالى وذلك عند معاملة النباتات قبل العدوى بالبياض الدقيقى.

كما أوضحت نتائج الفصل الكهربى للبروتينات وجود إختلاف بين المعاملات فى عدد البروتينات ونسبه تكونها مقارنة بالنبات الغير معاملة حيث يلاحظ أن النباتات المصابه تكسون نسبه البروتين المتكونه أقل من النباتات المعاملة سواء بالأملاح أو المبيد.

ومن هذه الدراسه يمكن القول أن أملاح البوتاسيوم (كربونات البوتاسيوم وكلووريد البوتاسيوم وفوسفات البوتاسيوم) كانت جميعها لها تأثير على تكوين بروتينات لم تتواجد فى النبات المصابه بدون المعاملة بالأملاح وهذه البروتينات ذات الوزن الجزئى يتراوح بين (٥١،٤٣) حيث تعمل هذه الاملاح على حفز النباتات على المقاومه وذلك بتكوين عدد من البروتينات المستحثة وكلما زادت هذه البروتينات زادت مقاومه النباتات للمرض.