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EVALUATION THE EFFECTIVENESS OF SOME NATURAL COMPOUNDS IN CONTROLLING POWDERY MILDEW [*Uncinula necator* (Schw.)] AND GRAPE CHARACTERISTICS

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ABSTRACT: *The efficacies of KH_2PO_4; $NaHCO_3$; Na_2SiO_3; $CaCl_2$ salicylic acid (SA) and biofungicide AQ10 against powdery mildew were studied on grapevine cv. Thompson seedless. The investigation was carried out during 2005 and 2006 seasons in El-Khatatba region, Cairo –Alexandria desert road under natural infection conditions. All the tested compounds provided protection at different level against powdery mildew on both leaves and bunch as compared with Punch and AQ10 which record the highest effect. KH_2PO_4; $NaHCO_3$ and Na_2SiO_3 + sulphur were more effective on leaves and bunch as compared with used in a single treatment. The test chemicals did not cause any toxic effect but phytotoxicity only occurred when high concentration (8mM) of salicylic acid when applied in the first season as coarse droplets on berries. No injury was noted when SA was applied at 4mM in the second season. All the application programs showed positive effects on cluster weight and other physical traits. Similar effects were found on chemical properties of grapes where, it leads to increase sugar contents and other traits in addition to reducing of total acidity ratio.*

Key words: *Grapevine, Uncinula necator, Powdery mildew, Potassium di-hydrogen phosphate, Baking powder, Water glass, Calcium chloride, Salicylic acid and AQ10.*

INTRODUCTION

Grapevine (*Vitis vinifera* L.) is one of the most important fruits grown in Egypt.It ranked the second after Citrus. The cultivated areas reached 159929 feddans (138499 feddan as fruit vines) with a production of 1275288 tons (According to annual statistical of the Ministry of Agricultural in 2005). This number is increasing rapidly as more desert areas are being planted every year for local market consumer and exportation.

Grapevine is subjected to the infection of powdery mildew caused by *Uncinula necator* (Schw.) Burr. [teleomorph of *Oidium tuckeri* Berk. (recently renamed *Erysiphe necator* Schw. and placed into the section Uncinula of the genus) Braun and Takamatsu, 2000. Powdery mildew is a worldwide economically important fungal disease in the grapevine farms and the most enduring and widespread problem. It costs millions dollars annually to vine growers, due to crops losses and an intensive usage of fungicides for its control (Miazzi *et al.* 2003).

Use of synthetic fungicides has been the traditional option of control plant diseases but, the extensive and prolonged use of synthetic fungicides has resulted in the development of resistance in the fungus. Furthermore the residual effects on the crop and environmental pollution are other problems associated with the use of these chemicals. Therefore, the use of pre- and post harvest chemical treatments is increasingly limited due to consumer concerns. Present focuses on the development of alternative means of controlling fungal in the field that are safe to human and environment have been initiated (Blaich and Wind, 1989; Renolds *et al.* 1996 and Yildirim and Onogur, 2001). An alternative to fungicidal application, it may be possible to utilize a scheme of inducible plant defenses which may provide protection against a broad spectrum of disease-causing organisms (Yildirim *et al.* 2002). Systemic acquired resistance proved its efficiency in controlling diseases and increasing yield (Kue and Hammerschmidt, 1995). During the last few years, several reports have been published on use of fungal and bacterial preparations, dairy by-products, sodium and potassium salts, silicate compounds and vegetable and mineral oils ...etc for induction of resistance (Aly *et al.* 1988; Aly and Afifi, 1989; Reuveni *et al.* 1996 and Yildirim *et al.* 2002). Some formulations of sodium and potassium bicarbonate have also proven successful in controlling powdery mildew on grapes (Reh and Schlosser, 1995 and Zerbetto *et al.* 2002). A new biofungicide, AQ10, which contains the fungal parasitic agent *Ampelomyces quisqualis*, is labeled for control of powdery mildew on grapes (Falk *et al.* 1995 and Mahrous, 2001). Also, using of these compounds to control powdery mildew in vineyards was followed by improving productivity (Winkler, 1953; 1965; Winkler *et al.* 1974; Weaver, 1976, Ware, 1983 and Abd Elghani *et al* 2007)

So, this study aims to evaluate the effectiveness of potassium di-hydro phosphate (KH_2PO_4) Baking powder ($NaHCO_3$) Water glass (Na_2SiO_3) Calcium chloride ($CaCl_2$), salicylic acid (SA) and abiofungicide AQ10 compared to fungicide punch to manage powdery mildew and recording their effects on grapes characteristics.

MATERIALS AND METHODS

Plant materials:

The experiments was performed in vineyard at El-Khatatba region , Menofyia governorate during two successive seasons of 2005 and 2006 on ten years old , Thompson seedless cultivar. The vines were planted in a light calcareous sandy soil and placed at 1.5 m (between vines in the row) x 2.75 m (between rows). The vines were trained to the modified cane training and supported by telefon trellis system. Five canes 14 buds each and approximately five renewably spurs, two buds each, were retained on each vine at winter pruning in the middle of January in both seasons according to Fawzi *et al.* 1984. The vines were drip irrigated and fertilized with macro and micro nutrient through fertigation. All vines received the same agricultural practices already applied in the vineyard.

Vineyard trials:

One hundred and seventy six vines were chosen. Several weeks prior to anthesis on the basis of uniformity of foliage and cluster development. All vines were adjusted to the same cluster number (i.e. 25 cluster) prior to anthesis. The vineyard experiments were arranged in a randomized complete block design with four replications for each variant, four vines for each replicate. The treatment programmes were applied at 15 day intervals during the growing season starting at 10-15 cm shoot length. Tested compounds (Table 1) were sprayed with a Knapsack sprayer. In order to provide a homogeneous spread on the leaf and bunch surface, surfactant triton (B 1956) was added at the dose of 50 ml / 100 L water. Control vines were sprayed with water.

Disease assessment:

For powdery mildew assessment, the evaluations on leaves and bunches were made 15 day after the last application. Leaf infection was evaluated based on the scale of 0-3 , on 150 leaves for each replication (Delen *et al.* 1987).Bunch infection was evaluated according to Delen *et al.* 1987 on the scale of 0-4 , on 60 bunches for each replication. The efficiency of chemical and fungicidal treatments was calculated by the following formula: Efficacy of treatment = ((control-treatment)/control) 100,(Mousa *et al.* 2006).

Table (1): Application programs and test chemicals.

Pro. No.	Chemical trade name	Active ingredient	Formulation	Usage dosage/100 L	
				2005	2006
P1	Potassium di-hydrogen phosphate	KH_2PO_4	w.p	500 g	500 g
P2	Baking powder	$NaHCO_3$	w.p	500 g	500 g
P3	Water glass (Na_2SiO_3)	$Na_2O_3 + SiO_2$	liquid	500 ml	500 ml
P4	Calcium chloride	$CaCl_2$	w.p	250 g	250 g
P5	Salicylic acid (SA)	2-Hydroxybenzoic acid ($C_7H_6O_3$)	w.p	296g (8mM)	150g (4mM)
P6	AQ10	Ampelomyces quisqualis		150 g	150 g
P7	Punch	1-[[Bis(4-fluorophenyl) methylsilyl] methyl]-1H-1,2,4-triazole	40% EC	3 ml	3 ml
P8	P1 + Sulphur	KH_2PO_4 + Sulphur	w.p	500+250g	250+250g
P9	P2 + Sulphur	$NaHCO_3$ + Sulphur	w.p	500+250g	250+250g
P10	P3 + Sulphur	($Na_2O_3 + SiO_2$) + Sulphur	w.p	500+250g	250+250g
P11	Water	H_2O	--	--	--

Physical and chemical characteristics of grapes:

Efficacies of the application programmes on some physical and chemical characteristics were determined after 15 days from last treatment at harvest data (middle July) in each season. For each treatment, the yield / vine were recorded in terms of weight (kg) and the average weight of cluster (g) was calculated. Six bunches of each replicate were randomly chosen and the following were determined: berry weight (g), size (cm), length (L/cm), width (w/cm) and berry shape index (L/W) was calculated. Juice total Soluble Solids (TSS), acidity and Total sugars % were determined according to the Association of official Agricultural chemist, AOAC, (1985) and TSS / acidity ratio was calculated.

All treatments were observed to determine any phytotoxic effects or disorders occurred on leaves and bunch. When necessary, the obtained data were subjected to statistical analysis according to Snedecor and Cochran (1984) using L.S.D test to differentiate the differences between various treatments.

RESULTS

1. Efficacies of application programs:

1.1. On disease incidence:

The efficacies of tested chemical under natural conditions were determined in two seasons.. In vineyards , the alternatives substances provided protection at an important level against powdery mildew infection on both leaves and bunches as compared with fungicide Punch or biofungicide(AQ10) which recorded the highest effect(Table, 2,3). Programmes number 8 (KH2PO4 + Sulphur), 5 (Salicylic acid) and 9 ($NaHCO_3$ + Sulphur) proved to be the most effective. Other programs showed effectiveness ranged from 51.4 % (P4) to 63.8 % (P2) on leaves and from 55.8% (P1) to 60.4 % (P4) on bunches in season 2005. Low infection risk on both leaves and bunches were found in P8 (KH_2PO_4 + Sulphur, P10 ((Na_2O_3 + SiO_2) + Sulphur) and recorded more efficiency as compared with those used in a single treatment in P1 (KH_2PO_4) , P2 ($NaHCO_3$) and P3(Na_2O_3 + SiO_2). (Tables 2, 3)

Another important finding was that programs Nos.P8, P9 and P10 were found to be more effective than the other programs in season 2006. In general, similar efficacies were found among the application programs in both seasons. Calcium chloride showed relatively low effectively on leaves and bunches in both seasons. The substances containing silicate (Na_2O_3 + SiO_2) showed relatively low efficacies in season 2005 as 2006 season. Also, a phytotoxic effect was observed as coarse droplets on berries when used Salicylic acid at the rate of 296g / 100 L (8 mM) in season 2005 as compared when used at 150 g /100 L (4 mM) in 2006 season.

Table (2): Efficiency of application programs against powdery mildew Thompson seedless grapevine, season 2005.

Program No.	Application program	Season 2005			
		On the leaves (%)		On the bunches (%)	
		Disease severity	Efficacy	Disease severity	Efficacy
P1	KH_2PO_4	17.3	53.2	19.0	55.8
P2	$NaHCO_3$	13.4	63.8	18.1	57.9
P3	$Na_2O_3 + SiO_2$	16.5	55.1	17.0	60.4
P4	$CaCl_2$	18.0	51.4	17.5	59.3
P5	Salicylic acid (SA)	10.2	72.4	13.0	69.7
P6	*Ampelomyces quisqualis*	6.0	83.8	7.0	83.7
P7	Punch	4.0	89.2	5.5	87.2
P8	KH_2PO_4 + Sulphur	9.0	75.7	12.1	71.8
P9	$NaHCO_3$ + Sulphur	11.3	69.5	14.5	66.3
P10	($Na_2O_3 + SiO_2$) + Sulphur	14.4	61.1	17.1	60.2
P11	Water (control)	37.0	--	43.0	--

L.S.D. at 0.05 level for: Leaves 1.95 Bunches 2.09

Table (3). Efficacies of test chemicals and biocontrol agent against powdery mildew in Thompson seedless grapevine, season 2006.

Program No.	Application program	Season 2006			
		On the leaves (%)		On the bunches (%)	
		Disease severity	Efficacy	Disease severity	Efficacy
P1	KH_2PO_4	19.5	52.4	22.5	54.1
P2	$NaHCO_3$	16.5	59.7	19.0	61.2
P3	$Na_2O_3 + SiO_2$	18.0	56.1	17.0	65.0
P4	$CaCl_2$	20.0	51.2	19.5	60.2
P5	Salicylic acid (SA)	14.0	65.8	16.0	67.3
P6	*Ampelomyces quisqualis*	7.0	82.9	8.5	82.6
P7	Punch	6.5	84.5	7.5	84.7
P8	KH_2PO_4 + Sulphur	13.0	68.3	12.5	74.4
P9	$NaHCO_3$ + Sulphur	11.5	71.9	13.0	73.4
P10	($Na_2O_3 + SiO_2$) + Sulphur	12.0	70.7	16.5	66.3
P11	Water (control)	41.0	--	49.0	--

L.S.D. at 0.05 level for: Leaves 1.75 Bunches 1.98

1.2. Physical and chemical characteristics of grapes:

The efficacy of these application programs on some physical and chemical properties indicate that remarkable increase in cluster weight (g) ranged from 475.0 to 636.0 g in the first season and from 480.0 to 650.0 g in the second season respectively as compared with 396.0 and 400.0 g in control treatment in both seasons respectively (Tables 4and5). The highest cluster weight resulted from P1 program (KH_2PO_4) being 636.0 g in2005 season which recorded the same level of cluster weight in season 2006 (being 650.0 g). Furthermore, programs employed have a positive effect on the physical characteristics (*i.e.* weight (g) , size (ml) Length (L.cm) , width (w.cm) and berry shape index (L/W)) as compared with control treatment in both seasons (Tables 4and 5).

Table (4): Effect of application programs on some physical and chemical characteristics of Thompson seedless grapes in season2005.

Application programmes	Physical characteristics							Chemical characteristics			
	Yield/vine (kg)	Bunch weight(g)	Average Berry				Berry shape index	TSS %	Total acidity	TSS/acid ratio	Total sugars %
			Weight (g)	Size(ml)	Length (cm)	Width (cm)					
P1	15.900	636.0	2.86	2.60	1.80	1.54	1.17	24.0	0.645	37.21	22.00
P2	15.575	623.0	2.25	2.00	1.72	1.62	1.06	17.0	0.694	24.50	15.80
P3	15.000	600.0	2.15	2.00	1.80	1.42	1.29	18.5	0.686	26.97	17.20
P4	15.825	633.0	2.53	2.00	1.64	1.48	1.11	21.0	0.678	30.97	19.50
P5	11.875	475.0	1.87	1.90	1.60	1.26	1.27	18.0	0.690	26.09	16.80
P6	12.700	508.0	2.14	1.80	1.68	1.36	1.24	17.0	0.692	24.57	15.90
P7	11.100	444.0	1.85	1.78	1.66	1.36	1.22	19.0	0.688	27.62	17.70
P8	15.150	606.0	2.05	1.40	1.64	1.32	1.24	32.0	0.660	34.85	21.30
P9	14.150	566.0	1.67	1.58	1.54	1.26	1.22	20.0	0.680	29.41	18.60
P10	15.600	624.0	2.30	1.80	1.62	1.30	1.25	20.0	0.675	29.46	18.70
P11	9.900	396.0	1.64	1.80	1.52	1.16	1.31	22.0	0.662	33.23	20.40
L.S.D 0.05	2.10	14.5	0.41	0.36	0.39	0.22	0.12	3.20	0.019	3.40	3.21

Table (5): Effect of application treatments on some physical and chemical characteristics of Thompson seedless grapes in season2006.

Application programmes	Physical characteristics							Chemical characteristics			
			Average Berry								
	Yield/vine (kg)	Bunch weight(g)	Weight (g)	Size(ml)	Length(L(cm)	Width(w,(cm)	Berry shape index	TSS %	Total acidity	TSS/acid ratio	Total sugars %
P1	16.250	650.0	2.96	1.50	1.50	1.20	1.25	20.0	0.680	29.41	18.60
P2	16.000	640.0	2.74	2.20	1.94	1.26	1.54	18.0	0.692	26.01	16.80
P3	15.250	610.0	2.28	2.10	1.58	1.36	1.16	19.0	0.688	27.62	17.70
P4	16.250	650.0	2.86	2.80	1.64	1.46	1.12	16.0	0.698	22.92	14.90
P5	12.000	480.0	2.38	2.40	1.70	1.36	1.25	18.0	0.692	26.01	16.70
P6	14.750	590.0	2.08	2.00	1.66	1.26	1.32	17.0	0.693	24.53	15.80
P7	12.500	500.0	2.12	2.00	1.74	1.24	1.40	19.0	0.688	27.62	17.60
P8	15.625	625.0	2.42	2.80	1.80	1.40	1.29	20.0	0.682	29.33	18.50
P9	15.250	610.0	2.40	2.80	1.80	1.34	1.34	18.0	0.690	26.09	16.80
P10	15.875	635.0	2.80	2.20	1.62	1.24	1.31	17.0	0.695	24.46	15.90
P11	10.000	400.0	1.60	1.30	1.50	1.24	1.21	16.0	0.698	24.92	15.00
L.S.D 0.05	2.40	16.1	0.38	0.32	0.29	0.36	0.21	2.40	0.021	2.90	3.10

Besides the effect of application programs on physical properties, there were significant effects of these programs on chemical characteristics of berries in both seasons (Table 4&5). In the first season the obtained data revealed that all application programs lead to significant decrease of TSS %, total sugar contents %, TSS / acid ratio and increase of total acidity as compared with control treatment except of P1 and P8 programs for TSS % and P1for TSS % acid ratio.

In contrast, in the second season a positive correlation between application programs and increase of total sugars contents (%), TSS % , and TSS / acid ratio in addition to significant decrease of total acidity as compared to control. As in the first season P1 (KH2PO4) and P8 (KH2PO4 + sulphur) resulted in significant highest increase in total sugars, TSS% and TSS% acid ratio.

DISCUSSION

Using synthetic chemicals to control plant diseases has many restrictions nowadays. The added costs for controlling diseases make losses even more important economically. Development of resistance in the pathogens, residual effects, and environmental pollution along of the cost of controlling diseases are problems associated with use of these chemicals. Recently, search for substances alternative have been initiated.

In vineyard trails all application programs used, in addition to traditional treatments with Punch and biofungicide AQ10, proved to be effective in reducing the risk of infection. These findings and previous reports (Reuveni *et al.* 1996; Abd-El-Kareem, 1998 and Yildirim *et al.* 2002) support the assumption that these chemicals could be used as alternatives to registered fungicides. Salicylic acid (SA) and $NaHCO_3$ was found to have the highest efficacy in both seasons when used in a single treatment. Bicarbonates, which are widely used in the food industry, have also been evaluated for their ability to control plant pathogens. Sodium, potassium and ammonium bicarbonates were found to suppress various fungal diseases on greenhouse-grown cucumbers (Horst *et al.* 1992; Fallik *et al.* 1997 and Ziv and Zitter, 1992). $NaHCO_3$ causes the collapse of hyphae of the fungal pathogen and shrinkage of their conidia and conidiophores (Ziv and Zitter, 1992). Salicylic acid (SA) may act as transmissible signal for induction of resistance (Metraux *et al.* 1990 and Vernooij *et al.* 1994). It plays its role by stimulation biosynthesis of different P-R proteins (Raskin, 1992) and β- 1, 3-glucanase (Schneider and Ullrich, 1994). Decrease of effectiveness of SA in 2006 season may be as a result of reducing the used dosage to avoid problems with phytotoxicity on berries in the first season.

For the protective efficacy of silicate (Na_2O_3+SiO_2), moderate effect on reduce the infection ratio on leaves and bunches in both seasons. However, the efficiency was increased when used with sulphur in a mixture. This finding might ascribed to its role in formation of thick and wide cells as a result of silica accumulation in the epidermal cells (Blaieh and Wind, 1989 and Kue and Harmmerschmidt, 1995). In addition to their protective efficacies, silicates also showed post infection inhibitive efficacy and it can inhibit conidial germination by increasing the PH of the medium (PH=10) (Yildirim *et al.* 2002).

Mixing sulphur with KH_2PO_4 and (Na_2O_3+SiO_2), increased their efficiency than used alone. The efficacy of these substances is expected to be higher if used in mixture with sulphur (Yildirim *et al.* 2002). All the application compounds have a positive effect on the physical traits (*i.e.* cluster weight; berry weight; size Length; width and berry shape index). Also, similar effect on chemical characteristics where, it lead to increase of total sugar contents, TSS % and TSS / acidity ratio in addition to decrease of total acidity. These effects might be ascribed to their positive action of application compounds on reduction of disease incidence (Abd-El-Kareem, 1998 and Mahrous, 2003) in addition to the improve fruit characteristics caused by these chemicals (Abd-El-Kareem *et al.* 2001). Some of these application compounds contain nutrient elements have a positive action for improving the yield and quality of grapes (Ahmed *et al.* 1991; El-Garhy, 1990, Kelany *et al.* 2000 and Abd-Elghany *et al.* 2007). On the other hand, using of these compounds to controlling powdery mildew were accompanied with protecting clusters from fruit rots which reflected in decreasing fruit dropping and succeeded in gaining heaviest clusters and high yield (Winkler, 1953 and Winkler *et al.* 1974).

The data obtained from this study indicated that these application compounds could be successfully used alone or in a mixture with sulphur without any additional synthetic fungicides. Also, these substances can be used instead of systemic fungicides or biofungicide (AQ10) to avoid the risks of resistance development.

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تقييم فعالية بعض المركبات الطبيعية على الإصابة بالبياض الدقيقى ومواصفات الثمار فى العنب

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الملخص العربى

قيمت فعالية فوسفات البوتاسيوم الثنائية القاعدية وبيكربونات الصوديوم وسيليكات الصوديوم وكلوريد الكالسيوم وحمض الساليسيليك فى مقاومة البياض الدقيقى فى العنب مقارنة بالمبيد الجهازى بانش والمبيد الحيوى **AQ10** . أجريت التجارب تحت ظروف الإصابة الطبيعية بإحدى مزارع العنب بمنطقة الخطاطبة بطريق مصر الأسكندرية الصحراوى خلال موسمى ٢٠٠٥ و ٢٠٠٦. أعطت كل المركبات المستخدمة وقاية من البياض الدقيقى على كل من الأوراق والثمار بنسب متباينة عند مقارنتها بمبيد البانش والمبيد الحيوى **AQ10** واللذان كانا الأكثر فعالية فى المقاومة. أوضحت التجارب أن إستخدام فوسفات البوتاسيوم الثنائية القاعدية وبيكربونات الصوديوم وسيليكات الصوديوم مختلطة مع الكبريت الميكرونى كانت أكثر فعالية منة عند إستخدامها فى معاملات منفردة. لم تظهر أى من هذه المعاملات المستخدمة تأثيرات سامة على أى من الأوراق والثمار فيما عدا عند إستخدام حامض الساليسيليك بتركيز عالى (٨ مللى مول) فى الموسم الأول حيث أدى لظهور بقع صغيرة خشنة على حبات العنب والتى إختفت فى الموسم الثانى عند خفض التركيز المستخدم الى ٤ مللى مول. كل المعاملات المستخدمة أدت لزيادة فى وزن العنقود وكان لها تأثير إيجابى على باقى الخصائص الطبيعية للثمار. نفس التأثير بالنسبة للخصائص الكيماوية للثمار حيث أدت المعاملات الى زيادة محتوى الثمار من السكريات بالإضافة الى خفض النسبة المئوية للحموضة الكلية.