

## EFFECT OF FOLIAR SPRAYING WITH MICRONUTRIENTS, ELICITORS, SILICON SALTS AND FERTILIZERS ON POWDERY MILDEW OF SUGAR BEET

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**ABSTRACT:** The capabilities of foliar application by copper sulfate, zinc sulfate, magnesium sulfate and ferrous sulfate as micronutrients, hydrogen peroxides, ascorbic acid and salicylic acid as inducers, potassium silicate and sodium silicate as silicon salts and different combination of NPK, on controlling sugar beet powdery mildew caused by *Erysiphe beta* were studied. Also, yield and sugar purity and changes in oxidative enzymes, comparable with non-treated plants and Eminent (EW12.5% I m/L) fungicide were estimated. In greenhouse under artificial infection all treatments significantly decrease disease severity compared with control. Eminent fungicide was the most effective treatment in reducing disease severity, followed by higher concentration of copper sulfate, zinc sulfate, hydrogen peroxide then salicylic acid and potassium silicate. The lowest treatments were NPK combination levels  $N_2P_1K_1$ ,  $N_1P_1K_1$ ,  $N_2P_2K_1$ , ferrous sulfate 10 ppm and sodium silicate at concentration 4 ppm. Under field condition, all tested treatments significantly reduced disease severity, while yield and sugar purity were increased in both seasons. Eminent, copper sulfate 20 ppm, ascorbic acid 100 ppm, zinc sulfate 20 ppm and Hydrogen peroxide followed by salicylic acid 50 ppm, potassium silicate 15  $cm^3/L$  were significantly higher in decreasing disease severity and increasing root weight, sucrose and purity percentage compared with control treatment. Whereas, the lowest treatments in decreasing severity and yield parameters were magnesium sulfate 30 ppm followed by ferrous sulfate then  $N_1P_2K_2$ . All tested chemicals significantly increased activity of phenylalanine ammonia lyase (PAL), Polyphenol oxidase (PPO) and Peroxidase (PO) activities in sugar beet leaves compared with control. PAL and PPO activity exhibited the highest value with treatments of hydrogen peroxide 1.5%, ascorbic acid 100 ppm, salicylic acid 50 ppm, potassium silicate 15  $cm^3/L$  and copper sulfate 20 ppm followed by zinc sulfate 20 ppm. Meanwhile, PO activity was higher in treatments of hydrogen peroxide 1.5%, potassium silicate 15  $cm^3/L$ , ascorbic acid 100 ppm, salicylic acid 50 ppm, followed by copper sulfate 20 ppm zinc sulfate 20 ppm. On the contrary,  $N_1P_2K_2$  followed by ferrous sulfate 30 ppm, magnesium sulfate 30 ppm and eminent were the lowest treatments in enzymes activity compared with control treatment.

**Key words:** Sugar beet, Powdery mildew, micronutrients, elicitors, silicon salts, ferrous.

### INTRODUCTION

Sugar beet (*Beta vulgaris* L.) is considered as one of the two important sugar cash crops worldwide. In Egypt a total cultivated area reached 8.4 million fedans (Abd El-hady and Bondok 2017). Recently sugar beet surpassed sugar cane in sugar productivity and became the first source of sugar production in

Egypt compared to sugar cane (Hozayn *et al.*, 2013) Powdery mildew of sugar beet is an important disease in several sugar beet growing countries caused by *Erysiphe betae* (Vanha) Weltzien (Kontaxis *et al.*, 1974; Hills *et al.*, 1980). In Egypt, environmental conditions are favorable for the fungus to spread rapidly especially in the late sowings after

September (El-Fahhar, 2008). Disease spread occurs mostly by conidial infections, which dispersed by wind (Kontradowitz and Verreet, 2010). The infection of sugar beet by powdery mildew is economically significant for growers worldwide and can cause sugar yield losses up to 30% (Francis, 2002). Utilizing disease resistance is an important strategy for controlling plant pathogens because it may be relatively easy to use, cost-effective and environmentally more acceptable than reliance on pesticides (Hogenboom, 1993). Dordas (2008) stated that, in recent years the importance of sustainable agriculture has risen to become one of the most important issues in agriculture. In addition, plant diseases continue to play a major limiting role in agricultural production. The control of plant diseases using classical pesticides raises serious concerns about food safety, environmental quality and pesticide resistance, which have dictated the need for alternative pest management techniques. In particular, nutrients, micronutrients, Si and inducers could affect the disease tolerance or resistance of plants to pathogens. Also, it has been reported that some phosphate salts induce systemic resistance against various pathogens including powdery mildew of cucumber (Reuveni *et al.*, 1993 and Reuveni *et al.*, 1995).

The objective of this work was to evaluate the efficacy of some micronutrient, chemical inducers and silicon salts and fertilizer treatments to control powdery mildew disease, enhancement of growth parameters on sugar beet yield and oxidative enzymes activity compare with commercial eminent fungicide and control.

## MATERIAL AND METHODS

### Greenhouse experiment:

The commercial sugar beet variety Hilospoly was sown in pots (40cm diameter) during December and received recommended irrigation and fertilization. Plants were thinned into 3 plants after 30 days of planting. After 12 weeks from cultivation plants were sprayed (Ebrahium, *et al.*, 2016) at 85 days from cultivation with different concentration of N.P.K (N<sub>1</sub>P<sub>1</sub>K<sub>1</sub>, N<sub>1</sub>P<sub>2</sub>K<sub>1</sub>, N<sub>1</sub>P<sub>1</sub>K<sub>2</sub>, N<sub>1</sub>P<sub>2</sub>K<sub>2</sub>, N<sub>2</sub>P<sub>1</sub>K<sub>1</sub>, N<sub>2</sub>P<sub>2</sub>K<sub>1</sub>, N<sub>2</sub>P<sub>1</sub>K<sub>2</sub>, and N<sub>2</sub>P<sub>2</sub>K<sub>2</sub>). Where N<sub>1</sub>= 3ppm, N<sub>2</sub>= 6ppm, P<sub>1</sub>= 2ppm, P<sub>2</sub>= 5ppm, K<sub>1</sub>= 4ppm, K<sub>2</sub>= 6 ppm. Whereas, the source of nitrogen (N) fertilizer was Urea CH<sub>2</sub>N<sub>2</sub>O (N 46%), while, Monocalcium phosphate Ca (H<sub>2</sub>PO<sub>4</sub>). H<sub>2</sub>O. (P 15%) used as the phosphorus source and potassium source was potassium sulfate K<sub>2</sub>SO<sub>4</sub> (K 48%). Spray with micronutrient, solutions of copper sulfate, ferrous sulfate, magnesium sulfate, zinc sulfate at the concentrations of 10, 20 and 40 ppm were done (Radwan, 2017). Antioxidant compounds, ascorbic acid, salicylic acid at the concentrations of 50 ppm, 100 ppm, 200 ppm, hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) at the concentration of 0.5%, 1%, 1.5% were used. Meanwhile Silicon salts, Potassium silicate KSiO<sub>3</sub> content 30% silicon and 10% potassium at 5 cm<sup>3</sup>/L, 10 cm<sup>3</sup>/L, 15 cm<sup>3</sup>/L and Sodium silicate Na<sub>2</sub> SiO<sub>3</sub>.H<sub>2</sub>O at concentration of 4 ppm, 8 ppm, 16 ppm. Eminent 12.5% E.W at rate of 1m/l and control plants sprayed with water as tested treatments.

Inoculation of *Erysiphe betae* enhancement was accomplished by shaking powdery mildew heavily diseased Sugar beet leaves over the treated plants at a height of about 30cm. Inoculated plants were kept in greenhouse until disease assessment was undertaken. Inoculation was done 2 days after foliar application with treatments (Strobel and Kuc, 1995).

### Field experiment:

Field experiment was carried out at farmer's field in Sonoras district El-Fayoium governorate, during 2018/2019 and 2019/2020 growing seasons. The experiment was designed in the randomized complete blocks method in three replications 4 rows/plot and 80 cm apart. Sugar beet seeds, c.v. Hilospoly were planted 4 seeds per hill and thinned after 3 weeks to one plant/hill to spacing of 25 cm. All treatments were fertilized by NPK and watering and all cultural practices were affected as recommended by Ministry of Agriculture. The same procedures used in the greenhouse experiment were used in the field trail with an exception that the plants were left for the natural infection and the significant concentration for each treatment reducing the powdery mildew disease as data obtained from greenhouse experiment were used. Where, NPK treatment (N1P2K2), microelements, solutions of copper sulfate 20ppm, ferrous sulfate 30ppm, magnesium sulfate 30 ppm, zinc sulfate 30 ppm. whereas, inducers ascorbic acid 60 ppm, salicylic acid 50 ppm, hydrogen peroxide 1.5%. Meanwhile, Potassium silicate and Sodium silicate at concentration of (15 cm<sup>3</sup>/L and 8 ppm, respectively). The spraying treatments were applied on sugar beet foliage when the first sign of disease was appeared and all treatments were applied three times in 15 day intervals between each spray.

#### **Disease assessment:**

Treatments were evaluates when foliage was closing the rows. Evaluation was accomplished by examining a recently matured leaf on each of at least 50 plants and rating disease intensity as the extent of leaf area covered by fungus mycelium on a scale of 0 to 4 after one week after spraying of treatments. Both sides of a leaf were examined and an average rating was given. Disease

severity was determined according the scale by Whitney et al., (1983) Scale ranged from 0-4, categories whereas 0= no mildew colonies observed 1=1-25%, 2=26-50%, 3=51-75% and 4=76-100% of matured leaf area covered by mildew and the average disease rating per treatment was calculated. Percentage of disease severity was assessed at greenhouse or field experiments after 10 days from each spraying. The percentage of treatment efficiency in the reduction of powdery mildew severity was calculated using this equation. The efficiency of treatments =  $((\text{control-treatment})/\text{control}) \times 100$ .

Plant roots were harvested after 5 months from planting and 10 roots were taken randomly for determination of root weight and quality traits for each treatment. Quality traits, Total soluble solids (T.S.S.) % was measured in fresh roots using the hand refractometer according to Mc Ginnis (1982), Sucrose % was determined by using succarometer according to Anonymous (1990) and Purity % was calculated by using the formula  $(\text{sucrose \%} / \text{T.S.S \%}) \times (100)$ .

#### **Enzymes assay.**

Effect of application with the compounds related to induce resistance, micronutrients and foliar fertilizing on sugar beet plants defense enzymes were studied from the green house experiment, treated and untreated sugar beet leaves samples were grounded with liquid nitrogen (L-N<sub>2</sub>) as fine powder with a mortar. One gram of the grounded tissues was mixed with one ml of extraction buffer phosphate buffer pH 6.0 according to (Bollage *et. al.*, 1996). Samples were vortexes and centrifuged at 13000 r.p.m. for 15 min. under 4°C to remove cell debris. The clear supernatant (crude enzyme source) was collected and kept at -80°C for further study (Soltis and Soltis 1990).

### **Determination of polyphenol oxidase (PPO).**

Leaf samples were extracted according to the method described by Malik and Singh (1990). The enzyme extract was prepared by grinding 5 g leaves in 0.1 M sodium phosphate buffer pH 7.0 (2 ml / g fresh weight), then centrifuged at 6000 rpm for 30 min. under 4 °C. The clear extract was collected, completed to 15 ml volume using phosphate buffer and used as crude enzyme source. The reaction mixture contained 0.2 ml of crude enzyme source, 1 ml of phosphate buffer pH 7.0, 1 ml of 10<sup>-3</sup> M catechol and complete with distilled water up to 6 ml. The reaction was incubated for 30 min. at 30 °C. One unite of polyphenol oxidase was expressed as the change in absorbance at 420 nm / 30 min. / 1 g fresh weight (Matta and Dimond, 1963).

### **Determination of peroxidase (PO):**

The reaction mixture extraction as described by Malik and Singh (1990) was contained 0.5 ml phosphate buffer pH 7.0, 0.2 ml enzyme source, 0.3 ml of 0.05 M pyrogallol, 0.1 ml of 1%(v/v) H<sub>2</sub>O<sub>2</sub> and distilled water was added up to obtained 3 ml .The reaction mixture was incubated at 30 °C for 5 min. then the reaction stopped by adding 0.5 ml of 5 % (v/v) H<sub>2</sub>O<sub>2</sub> (Kar and Mishra, 1976). One unit of peroxidase activity was expressed as the changes in absorbance at 425 nm/min. / 1 g fresh weight.

### **Phenylalanine ammonia lyase activity:**

Phenylalanine ammonia lyase (PAL) activity, was determined according to the method described by Solecka and Kacperska (2003) with slight modifications by extracting 1g of sugar beet leaves in 2 ml of 50 mM borate buffer (pH 8.8) after 24h of application

using a mortar and pestle at 4°C, centrifuged at 12000 rpm for 10 min at 4°C. The supernatant was used as a source of crude enzyme for assaying PAL activity. Reaction mixture containing 1 ml enzyme extract, 2 ml sodium borate buffer (pH 8.8) and 1 ml of 10<sup>-2</sup> M L-phenylalanine was incubated at 30°C for 1h. The enzyme reaction was stopped by adding 500µl of 6 N HCl. The reaction mixture was centrifuged for 10 min at 12000 rpm to pellet the denatured protein. Enzyme activity was expressed in micromoles of trans-cinnamic acid formed per gram of fresh weight of tissue, measured Unit/hour g/f.w. at 290 nm by using a spectrophotometer model Unico-2100. Enzyme extract was replaced by distilled water it control blank cuvette.

## **RESULTS**

### **Greenhouse experiment:**

Data presented in Table (1) indicated that, all chemical compounds significantly decreased severity of sugar beet powdery mildew under greenhouse conditions compared to the control treatment. The high reduction was achieved by fungicide Eminent 91.9% followed by the higher concentration of copper sulfate 90.2% zinc sulfate 89.6%, Hydrogen peroxide 89.2%, salicylic acid and potassium silicate 88.3%. There are no significant differences between those six treatments at higher concentration, followed by five treatments in higher concentration (ascorbic acid, sodium silicate, magnesium sulfate, ferrous sulfate and N<sub>1</sub>P<sub>2</sub>K<sub>2</sub>) also showing significant differences with control but not in-between particular chemicals and others where, its disease severity ranged between 9 to 11.7 % without no significant differences in between. Also data revealed that, there are any significant differences between second concentration and third concentration of

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copper sulfate, zinc sulfate and salicylic acid.

Data also show that, tested inducers caused significant reduction in disease severity, the highest treatments was obtained by Hydrogen peroxide at concentration 1.5% (89.2% Efficacy) followed by salicylic acid 100 ppm (88.3

% Efficacy) and ascorbic acid 100 ppm (87.9% Efficacy). Whereas, spraying by silicate salts reduced the disease severity at higher concentrations, spraying with potassium silicate at concentration 15 cm<sup>3</sup>/L achieved (88.3 % Efficacy) followed by sodium silicate at 8 ppm (87.9% Efficacy).

**Table 1. Effect of micronutrients, inducers, silicon salts and fertilizers on sugar beet powdery mildew, under greenhouse condition, 2018.**

Treatment	Conc,	Disease severity %	Efficacy%
Copper sulfate	10 ppm	12.7	82.9
	20 ppm	9.0	87.9
	30 ppm	7.3	90.2
Zinc sulfate	10 ppm	13.3	82.1
	20 ppm	10.3	86.2
	30 ppm	7.7	89.6
Magnesium sulfate	10 ppm	16.0	78.5
	20 ppm	14.3	80.8
	30 ppm	9.3	87.5
Ferrous sulfate	10 ppm	20.0	73.2
	20 ppm	16.3	78.1
	30 ppm	10	86.6
Ascorbic acid	25 ppm	15.3	79.5
	50 ppm	12.0	83.9
	100 ppm	9.0	87.9
Salicylic acid <sup>1</sup>	25 ppm	18.0	75.9
	50 ppm	11.3	84.8
	100 ppm	8.7	88.3
Hydrogen peroxide	0.5%	16.0	78.5
	1%	12.0	83.9
	1.5%	8.0	89.2
Sodium silicate	4ppm	20	73.2
	6ppm	16.3	78.1
	8ppm	9	87.9
Potassium silicate	5 cm <sup>3</sup> /l	18.3	75.5
	10 cm <sup>3</sup> /l	13.3	82.1
	15 cm <sup>3</sup> /l	8.7	88.3
N.P.K	N <sub>1</sub> P <sub>1</sub> K <sub>1</sub>	22.3	70.1
	N <sub>1</sub> P <sub>2</sub> K <sub>1</sub>	19.0	74.5
	N <sub>1</sub> P <sub>1</sub> K <sub>2</sub>	14.7	80.3
	N <sub>1</sub> P <sub>2</sub> K <sub>2</sub>	11.7	84.3
	N <sub>2</sub> P <sub>1</sub> K <sub>1</sub>	26.7	64.2
	N <sub>2</sub> P <sub>2</sub> K <sub>1</sub>	20.7	72.2
	N <sub>2</sub> P <sub>1</sub> K <sub>2</sub>	18.7	74.9
N <sub>2</sub> P <sub>2</sub> K <sub>2</sub>	16.0	78.5	

Eminent (EW 12.5%)	1 m/l	6	91.6
Control		74.7	0.0
LSD		2.727	
N1=20 gm, N2 =50 gm, P1=5 gm, P2=10 gm, K1=5 gm, K2=10gm, Bion1= 2mM, Bion2=4 mM, Sal1= 2 mM, Sal2= 4 mM, H2O2 1= 0.5%, H2O2 2=1%			

Results in Table (1) show that treatment by NPK combination were lowest in reducing disease severity except N<sub>1</sub>P<sub>2</sub>K<sub>2</sub>, N<sub>1</sub>P<sub>1</sub>K<sub>2</sub> and N<sub>2</sub>P<sub>2</sub>K<sub>2</sub> levels were the most effective NPK combination levels in controlling the disease where, the reduction (84.4% and 80.3%, respectively) than the other combinations compared with control. Also data revealed that, the lowest treatments were NPK combination levels N<sub>2</sub>P<sub>1</sub>K<sub>1</sub>, N<sub>1</sub>P<sub>1</sub>K<sub>1</sub>, N<sub>2</sub>P<sub>2</sub>K<sub>1</sub>, ferrous sulfate 10 ppm and sodium silicate at concentration 4 ppm where the efficacy achieved were (64.2%, 70.1%, 72.2%, 73.2% and 73.2%, respectively). Generally the reduction in disease severity was significantly increased by increasing concentration of all tested treatments

**Field experiment:**

Efficacy of spraying application on sugar beet c.v. Hilospoly naturally infection under field conditions, by selected efficient concentrations previously screened at greenhouse experiment of micronutrients, antioxidants, silicon salts and NPK fertilization, against *Erysiphe betae* and yield components were carried out in two successful seasons at Sonoras distract farm fields Fayoiium governorate, during 2018/2019 and 2019/2020 growing seasons. The selected treatments were second concentration of copper sulfate 20 ppm, zinc sulfate 20 ppm and salicylic acid 50 ppm and the higher concentration of the rest of chemicals under study and potassium silicate from silicate salts. Data in Table (2) revealed that, all treatments were significantly decreased disease severity and increasing root

weight, sucrose percentage and purity compared with control treatment. The treatments of eminent, copper sulfate 20 ppm, ascorbic acid 100 ppm, zinc sulfate 20 ppm and Hydrogen peroxide followed by salicylic acid 50 ppm, potassium silicate 15 cm<sup>3</sup>/L were significantly higher in decreasing disease severity and increasing root weight, sucrose percentage and purity compared with control treatment. Where, treatment by eminent achieved the lower severity 2.9% obtained 96.5% reduction and 2.6 kg root weight followed by copper sulfate 20 ppm where severity was 7 %, 89.4% reduction and 2.13 kg root weight then ascorbic acid 100 ppm 7.1 % severity, 89.1% reduction and 2 root weight then zinc sulfate 20 ppm where severity was 8.6% reduction 86.9%, root weight was 1.7 kg, where hydrogen peroxide severity was 9.2%, 85.4% redaction and 1.7 kg root weight and Salicylic acid 10% severity and potassium silicate severity was 10.4% where, reduction was 84% and 83.4%, respectively. Meanwhile, sucrose purity was higher in copper sulfate 20ppm treatment than other treatments 98.6% followed by eminent 96.9%, ascorbic acid 100 ppm 96.3%, zinc sulfate 20 ppm 91.6% then hydrogen peroxide 87.1%, followed by salicylic acid 85.4% and potassium silicate 84.1% purity. Whereas, the lowest treatments in decreasing severity and yield parameters were magnesium sulfate 30 ppm 13% severity followed by ferrous sulfate 12.3% severity then combination of NPK (N<sub>1</sub>P<sub>2</sub>K<sub>2</sub>) 12.7% severity.

Results in Table (3), indicated that, Eminent 92.6%, copper sulfate 20

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ppm88.2%, ascorbic acid 100 ppm 87.3%, zinc sulfate 20 ppm86.8%, hydrogen peroxide 84.3% followed by salicylic acid82.9% and potassium silicate 81.9% were superior in controlling disease in second season compared with control

treatment. Also the same trend was obtained like first season in disease reduction, yield parameters and sucrose purity% with slight decreasing in its value and disease reduction percentage.

**Table 2. Effect of micronutrients, inducers, silicon salts and fertilizers on sugar beet powdery mildew, under filed condition, 2019.**

Treat.	Inf%	Reduc. %	Root W.	T.S.S%	Suc.%	Purity%
Copper sulfate 20ppm	7 f	88.8	2.13 b	24. 6 ab	24.3 a	98.6 a
Ferrous sulfate 30 ppm	12.3bcd	80.3	1.4 de	20 cd	18 cd	76.1abcd
Magnesium sulfate 30 ppm	13b	79.2	1.5 de	20. 6 cd	17 def	82. 6 de
Zinc sulfate 20 ppm	8.6 ef	86.2	1.7 cd	25.3 a	22 b	91.6abcde
Salicylic acid 50 ppm	10de	84	1.61cde	26 a	25 a	85.4abc
Ascorbic acid 100 ppm	7.1 f	88.6	2.0 bc	26.3 a	20 bc	96.3e
Hydrogen peroxide 1.5%	9.2 ef	85.3	1.7 bcd	18.3 d	15.6 ef	87.1abcde
Potassium silicate 15 cm <sup>3</sup> /L	10.4 cde	83.4	1.6 cde	19.3 cd	16 ef	84.1 cde
N1P2K2	12.7 bc	79.7	1.73 bcd	18.3 d	15.3 f	83.5bcde
Eminent (EW 12.5%) 1 m/L	2.9 g	95.3	2.6 a	26.3 a	25.5 a	96.9 ab
Control	62.7a	-	1.2 e	22 bc	17. 6 de	80.9 de
LSD	2.55		0.415	2.772	2.157	0.065

**Table 3. Effect of micronutrients, inducers, silicon salts and fertilizers, on sugar beet powdery mildew, under filed condition, 2020.**

Treat.	Inf%	Reduc.%	Root W.	T.S.S%	Suc.%	Purity%
Copper sulfate 20ppm	8 f	88.2	1.93 bc	25 ab	24 ab	96.1a
Ferrous sulfate 30 ppm	12.3 cd	81.9	1.56 d	22.3 bc	17.3 ef	78.5cd
Magnesium sulfate 30 ppm	14.3 c	79	1.46 de	25.3 ab	19.6 d	77.9d
Zinc sulfate 20 ppm	9 ef	86.8	2.2 ab	20.6 cd	18 e	87.3abcd
Salicylic acid 50 ppm	11.6 d	83	1.53 de	19 d	15.3 g	80.8cd
Ascorbic acid 100 ppm	8.6 ef	87.4	1.6 cd	25 ab	22. 6 bc	90.8 abc
Hydrogen peroxide 1.5%	10.6 de	84.4	1.5 de	19. 7 cd	15. 6 g	81.1cd

Potassium silicate 15 cm <sup>3</sup> /L	12 cd	82.4	1.6 cd	25.6 a	21.3 c	83.1bcd
N1P2K2	18 b	73.6	1.58 d	19.0 d	14.8 g	78.1d
Eminent (EW 12.5%) 1 m/l	5 g	92.6	2.4 a	26.6 a	25 a	93.7ab
Control	68.3a	-	1.2 e	21 cd	16.3 fg	77.9 d
LSD	2.645		0.339	3.036	1.620	12.345

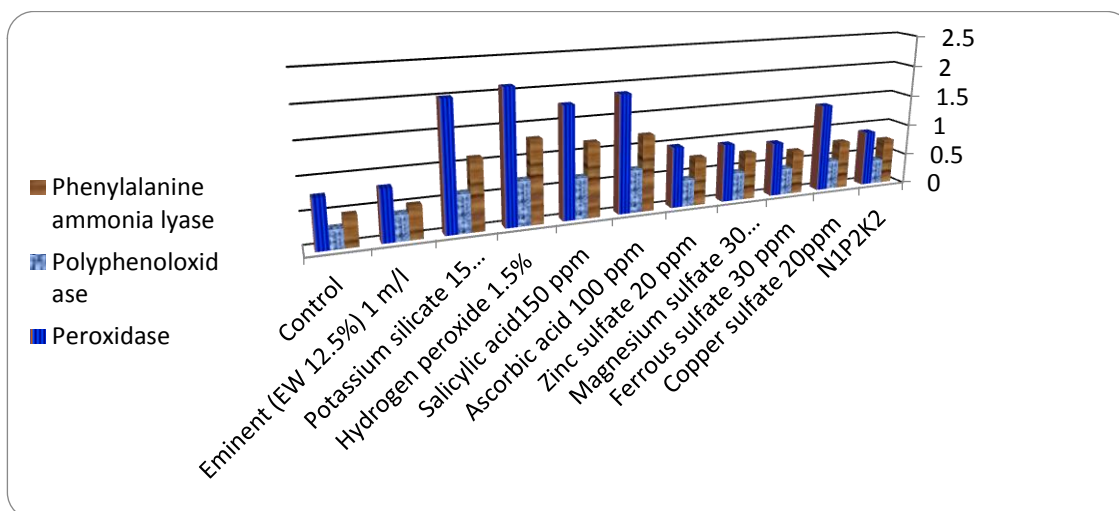
Date in Tables (2, 3) revealed that disease severity in second season was higher than that in first one which slightly decreased the yield parameters (root weight, sucrose percentage and sucrose purity percentage), also treatments differ in root weight where zinc sulfate comes before copper sulfate and ascorbic acid than first ones. Also copper sulfate was the best treatment in sucrose purity in both seasons followed by Eminent then ascorbic acid. It was observed that the increasing in disease severity was decreasing yield parameters percentage and purity. Plants treated by eminent, copper sulfate 20 ppm, ascorbic acid 100 ppm, zinc sulfate 20 ppm and hydrogen peroxide 1.5% were superior in controlling powdery mildew with no significant differences in between in both years.

#### **Biochemical changes in oxidative enzymes activity:**

The effect of selected efficient concentrations of micronutrients, antioxidants, silicon salts and NPK fertilization previously screened at greenhouse experiment as foliar

application on various biochemical changes in sugar beet leaves of treated and untreated plants under field natural infection was studied. Data presented in Fig. (1) indicate that the effect of treatments on phenylalanine ammonia lyase (PAL), Polyphenol oxidase (PPO) and Peroxidase (PO) activities in c.v. Hilospoly sugar beet plants after three times of spraying, all treatments resulted in significant increase in enzymes activity compared to control and fungicide treatments. PAL activity is exhibited the highest value with treatments of hydrogen peroxide 1.5%, ascorbic acid 100 ppm, salicylic acid 50 ppm, potassium silicate 15 cm<sup>3</sup>/L and copper sulfate 20 ppm followed by zinc sulfate 20 ppm (1.3, 1.2, 1.16, 1.1, 0.77 and 0.76 Unit. / hour g /F.W., respectively). The same trend was obtained in PPO activity where, hydrogen peroxide 1.5%, ascorbic acid 100 ppm, salicylic acid 50 ppm, potassium silicate 15 cm<sup>3</sup>/L and copper sulfate 20 ppm followed by zinc sulfate 20 ppm (0.70, 0.68, 0.65, 0.60, 0.47 and 0.43 Unit./30 min. g /F.W., respectively).





**Fig. (1): Effect of foliar treatments of some fertilizers, micronutrients, inducers and silicon salt on enzymes activity of sugar beet.**

Concerning to PO activity it was observed that hydrogen peroxide 1.5%, potassium silicate 15 cm<sup>3</sup>/L, ascorbic acid 100 ppm, salicylic acid 50 ppm, are highest treatments in PO activity at value (2.1, 2, 1.87, 1.77 unit./min. g/F.W., respectively) followed by copper sulfate 20 ppm (1.42) zinc sulfate 20 ppm (0.97) unit./min. g/F.W. On the contrary, N<sub>1</sub>P<sub>2</sub>K<sub>2</sub> followed by ferrous sulfate 30 ppm, magnesium sulfate 30 ppm and eminent were the lowest treatments in enzymes activity compared with control treatment (Fig. 1).

### Discussion

The present study was carried out to investigate the effect of micronutrient, antioxidants, silicon salts and combination levels of NPK fertilization against powdery mildew, plant defense enzymes activity and plant yield component in comparison with Eminent fungicide at recommended concentration and untreated plants (control). *In vitro* screening of micronutrient, antioxidants, silicon salts and combination levels of NPK fertilization against powdery mildew, in greenhouse under artificial inoculation and selected efficient concentration or

chemicals of those treatments from this experiment were applied in the field. The results revealed that, all treatments significantly reduced severity of powdery mildew with slight differences between them comparable with the control. Among the screened thirty five treatments under greenhouse condition results revealed that eleven treatments were significantly superior in reducing powdery mildew. The most efficient treatments were Eminent, copper sulfate zinc sulfate, Hydrogen peroxide, salicylic acid and potassium silicate compared with control treatments followed by ascorbic acid, sodium silicate, magnesium sulfate, ferrous sulfate and N<sub>1</sub>P<sub>2</sub>K<sub>2</sub> which showing significant differences with control but not in-between. Meanwhile, treatments in field experiments show progress in ascorbic acid reduction % than that in greenhouse it comes after Eminent and copper sulfate followed by zinc sulfate, Hydrogen peroxide, salicylic acid and potassium silicate.

Eminent (EW 12.5%) gave the best protection from powdery mildew, this result is in agreement with (Hemmat and Karima 2017, Abd Elhady and Bondok,

2017) they reported that Eminent fungicide was superior in controlling sugar beet powdery mildew and enhancement growth parameters. The highest effect of Eminent fungicide could be attributed to its active ingredients mode of action which are belonged to tetraconazol which concenter as sterol dimethylation inhibiting group (DMI) Lyr (1987) this fungicide group destroying the fungal cell's ability to produce ergosterol causes a disruption of the plasma membrane, thereby resulting in cellular leakage and ultimately the death of the pathogen (Becher and Wirsal, 2012; Karaoglanidis & Karadimos, 2006).

On the other hand, the fungicides resistant races of pathogen have been reported by (Fernández-Aparicio *et al.*, 2009; Weiland and Koch, 2004). As well as the side effects of fungicides on human health were reported (Durmusoglu *et al.*, 1997; Eckert and Ogawa, 1988) and the environment (Garcia, 1993; Horst *et al.*, 1992). Hence, there is a growing trend many years ago to involve other successful methods in disease management program depending on compounds which are useful, nontoxic and safe either on human health or on the environment especially with the risen of sustainable agriculture importance. In particular, nutrients, micronutrients, Si and inducers could affect the disease tolerance or resistance of plants to pathogens (Dordas, 2008). The results obtained from greenhouse and field experiments were in agreement with this aspect, where the foliar application by micronutrients copper sulfate 30 ppm was the highest treatment from micronutrients it comes after eminent treatment followed by zinc sulfate 30 ppm, ferrous sulfate 30 ppm and magnesium sulfate 30ppm were lowest micronutrient in efficacy than other micronutrients. This result agreed with Eliwa *et al.*, (2018) they reported that using those micronutrients in controlling

sugar beet powdery mildew caused significant reduction in disease and increasing micronutrients concentration increasing resistance of sugar beet against powdery mildew disease. Systemic acquired resistance (SAR) may be involved in the suppression of plant diseases by micronutrients. Reduction in disease severity has been reported in other crops after a single foliar application of  $H_3BO_3$ ,  $CuSO_4$ ,  $MnCl_2$  or  $KMnO_4$ ,  $KH_2PO_4$ ,  $K_2HPO_4$  which provided systemic protection against powdery mildew in cucumber plants (Reuveni *et al.*, 1997a, b; Reuveni and Reuveni, 1998; Reuveni *et al.*, 1998 and Quiroga *et al.*, 2000). The same authors also suggested that application of nutrients such as Mn, Cu and B can exchange and therefore release  $Ca^{2+}$  cations from cell walls, which interact with salicylic acid and activate systemic acquired resistance mechanisms. Micronutrients play an important role in plant metabolism by affecting the phenolics and lignin content and also membrane stability (Graham and Webb, 1991). Micronutrients can affect resistance indirectly, as in deficient plants they become more suitable feeding substrate.

Concerning the tested inducers the highest treatment was obtained by hydrogen peroxide at concentration 1.5% followed by ascorbic acid 100 ppm then salicylic acid 100 ppm in greenhouse experiment. In field experiment ascorbic acid 100 ppm was superior in reducing severity followed by hydrogen peroxide 1.5% and salicylic acid 50 ppm. Data are in agreement with those reported by several researchers when they used inducers against several plant diseases caused by various pathogens (Skłodowska *et al.*, 2010; Vimala and Suriachandraselvan, 2009; El-Samawaty & Galal, 2009; Ismail *et al.*, 2006; Shaat & Galal, 2004; Sparla *et al.*, 2004; Galal & Abdou, 1996). The reason for that effectiveness could be explained that

many factors may act on plants to induce high levels of systemic resistance to subsequent pathogen attack. Induction of resistance due to spraying of SA,  $\text{KH}_2\text{PO}_4$ ,  $\text{K}_2\text{HPO}_4$ ,  $\text{H}_2\text{O}_2$  and BTH may be attributed to different factors affecting host-pathogen reaction. Among these factors pathogenesis related proteins, peroxidase,  $\beta$ -1,4-gluconase and chitinase which are actively accumulated in immunized plants (Quiroga *et al.*, 2000). The role of hydrogen peroxide in activation of an array of host defense mechanisms including induced activity of enzymes as peroxidase and chitinase accompanied by a significant increase in the lignin and suberin content (Gusui *et al.*, 1997). Hydrogen peroxide positively influences the local and systemic accumulation of SA that is correlated with the enhancement of peroxidase activity (Martinez *et al.*, 2000). Moreover, hydrogen peroxide inhibits pathogens directly, and /or it may generate other reactive free radicals that are antimicrobial (Peng and Kuc, 1992). On the other hand, Eliwa *et al.* (2018) they stated that the foliar application of inducers significantly reduced severity of disease and the lowest area under powdery mildew progress curve (AUPMOC) value was obtained by Billis fungicide followed by salicylic acid and ascorbic acid.

Regarding to silicon salts treatments whether in the greenhouse or the field results indicate that, a significantly reduction was achieved in the disease infection comparable to the control. Efficiency in decreasing the disease incidence was increased by increasing the compound concentrations potassium silicate was higher than sodium silicate in reducing severity. Results throughout this study led to the hypothesis that Si would act as a modulator influencing the plant defense response as suggested by Fawe *et al.* (2001). They emphasized that Si act as a secondary messenger of

systemic acquired resistance (SAR). It is believed that Si creates a physical barrier which can restrict fungal hyphae penetration, or it may induce accumulation of antifungal compounds such as flavonoid and diterpenoid phytoalexins which can degrade fungal and bacterial cell walls (Alvarez and Datnoff, 2001; Brescht *et al.*, 2004).

Treatments with N.P.K. combinations revealed that, the balance between the three fertilize elements was significantly deference in reducing disease severity compared with control, spraying with low nitrogen level high K and P combination (N1P2K2) was the most efficient ones than other combinations this result are in agreement with several investigation studied the effect of NPK on several diseases they reported that obligate parasites severity increased by increasing N supply (Dordas, 2008; Büschbell and Hoffmann, 1992), the foliar application of K may promote the development of thicker outer walls in epidermal cells, Tissue hardening, influence plant metabolism Marschner (1995). Also, a number of other studies have shown that foliar application of P can induce local and systemic protection against powdery mildew in cucumber, roses, wine grapes, mango and nectarines (Reuveni and Reuveni, 1998; Huber and Graham, 1999; Reuveni *et al.*, 2000).

Regarding to growth parameters data in this study revealed that, all treatments were significantly increasing root weight, sucrose percentage and purity compared with control treatment. The treatments of Eminent, copper sulfate 20 ppm, ascorbic acid 100 ppm, zinc sulfate 20 ppm and Hydrogen peroxide followed by salicylic acid 50 ppm, potassium silicate 15  $\text{cm}^3/\text{L}$  were significantly higher in increasing root weight, sucrose percentage and purity compared with control treatment. These results were in agreement with the

findings of Rossi, *et al.* (2000) they described the effect of disease on yield component as a result of reduction of photosynthetic activity of leaf area firstly. Under severe foliage loss, late season photosynthetic potential is also reduced and vegetative re-growth is stimulated at the expense of root sugar reserves. As a consequence, potential sugar yield (recoverable sugar) of sugar beet crop can be significantly reduced due to the loss of both root weight and sucrose content. Therefore, any suppression of disease development will lead to save yield reduction or increasing yield components than that of untreated plots. Percich *et al.* (1987), Khan and Smith (2005) and Gado (2007), they reported that treated plots of sugar beet by fungicides resulted in increase in yield components, root weight and sucrose%, due to suppressing the causal agent of *Cercospora* leaf spot disease. Concerning the effect of the previously mentioned chemicals on yield components, obtained results showed that, hydrogen peroxide, salicylic acid micronutrients have significantly increased root weight / plant, sucrose and juice quality percentage. Similar results were reported by (Salama, 2006; Zaky *et al.*, 2006 and Gehan *et al.*, 2013). Results revealed that Potassium silicate significantly reduced the disease severity also increased growth parameters and sugar yield and purity this results are in agreement with the findings of Abd El-hady and Bondok (2017) they investigated that foliar application of potassium silicate compared with non treated sugar beet plants and Eminent fungicide was significantly reduced powdery mildew and enhanced growth and productivity and sugar yield. The lowest treatments in decreasing yield parameters and sucrose purity % were

ferrous sulfate followed by magnesium sulfate N1P2K2 treatment.

Concerning to oxidative enzymes activity the obtained results indicated that PAL and PPO activity is exhibited the highest value with treatments of hydrogen peroxide 1.5%, ascorbic acid 100 ppm, salicylic acid 50 ppm, potassium silicate 15 cm<sup>3</sup>/L and copper sulfate 20 ppm followed by zinc sulfate 20 ppm. Meanwhile, N1P2K2 followed by ferrous sulfate 30 ppm, magnesium sulfate 30 ppm and Eminent were the lowest treatments in enzymes activity compared with control treatment. It was observed that inducers achieved higher levels of enzymes activity than micronutrients, results gave evidence that spraying sugar beet plants with previously mentioned chemicals increase activity of oxidative enzymes in plant leaves compared with control treatment which increased reduction to powdery mildew and increased growth parameters and sugar yield and purity percentage. This is in agreement with those reported by Reuveni *et al.* (1997); Abd-El-Kareem (1998) and El-Habbak, (2003). Many plant enzymes are involved in defense reaction against plant pathogen. These included oxidative enzymes such as peroxidase and polyphenoloxidase which catalyses the formation of lignin and other oxidative phenols that contribute to formation of defense barriers for reinforcing the cell structure (Avdiushko *et al.*, 1993; Gusui *et al.* 1997; and Mahmoud *et al.*, 2006). Enzyme activity played an important role in plant disease resistance through increasing plant defense mechanisms that are considered the main tool of varietal resistance (Takuo *et al.*, 1993). Hydrogen peroxide positively influences the local and systemic accumulation of salicylic acid that is correlated with the enhancement of peroxidase activity (Martinez *et al.*,

2000). Moreover, hydrogen peroxide inhibits pathogens directly, and/or it may generate other reactive free radicals that are antimicrobial (Peng and Kuc, 1992). Also results indicated that spraying sugar beet plants with SA increased activity of oxidative enzymes in plant leaves than untreated infected control. This is in agreement with (Zaky *et al.*, 2006; Thabet, 2008; and Hammat *et al.*, 2016) they stated that there was a significant increase in the activity of PO and PPO after spraying wheat, anise and sugar beet plants with salicylic acid. This might be due to the role of salicylic acid in generation of the oxidative burst in incompatible interactions by inducing a rapid transient generation of  $O_2^-$  which is responsible for regulation of peroxidase activity (Rao *et al.*, 1997).

Phenylalanine ammonia lyase (PAL) is the primary entry enzyme that leads to phenylpropanoid pathway resulting in the biosynthesis of a diverse array of plant metabolites, such a cinnamic, coumaric, ferulic and caffeic acids, flavonoids, tannins and lignin (Hahlborck and Sheel, 1989). These products consequently protect plants against various abiotic stresses and pathogenic attacks (Jones, 1984). Plants may accumulate phenolics through the phenylpropanoid pathway on activation of PAL as a means of passive defence (Barry and Manley, 1986) and the magnitude of the accumulation primarily depends on the supply of the primary precursor, L-phenylalanine (Da Cunha, 1987).

The increase of PO and PPO activity was associated with increasing resistance against infection by many diseases (Wang *et al.*, 2000). The role of peroxidase, and other enzymes were also investigated as markers for resistance (Hammerschmidt *et al.*, 1982). peroxidases also may be responsible for

the production of  $H_2O_2$  (Bolwell *et al.*, 2002).  $H_2O_2$  produced by these peroxidases might serve as the substrate for other peroxidases, or act as an antimicrobial agent and signals to trigger self-defence responses like hypersensitive reaction (Wojtaszek, 1997). Peroxidases have roles in both the production and scavenging of reactive oxygen species (Bolwell *et al.*, 2002). Polyphenoloxidases (PPO) generally catalyse the oxidation of phenolic compounds to quinones (antimicrobial compounds) using molecular oxygen as an electron acceptor (Sommer *et al.*, 1994) which are toxic to the invading pathogens and pests (Weir *et al.*, 2004).

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## تأثير الرش الورقي بالمغذيات الصغرى والمستحاثات واملاح السيليكون والاسمده على البياض الدقيقى فى بنجر السكر

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### المخلص العربى

تم دراسة كفاءة العناصر الصغرى وهى كبريتات النحاس وكبريتات الزنك وكبريتات الماغنسيوم وكبريتات الحديد، والمستحاثات التالية فوق اكسيد الهيدروجين وحامض الاسكوربيك وحامض السالسليك، ايضا املاح السيليكون وهى البوتاسيوم سيليكات والصوديوم سيليكات، كما تم ايضا استخدام خليط من NPK بتوليفات مختلفة وذلك لمقاومة مرض البياض الدقيقى فى بنجر السكر المتسبب عن فطر الإبريسيف بيتا. كما تم ايضا دراسة التأثير على وزن المحصول ونسبة نقاء السكر كذلك نشاط انزيمات الاكسده وذلك بالمقارنة بمعاملة المقارنة وهى النباتات الغير معاملة والمعاملة بمبيد الاميننت (12.5% بمعدل 1مل/لتر). وقد اظهرت النتائج خفضا معنويا فى شدة الاصابة بالمقارنه بالكنترول فى الصوية تحت ظروف العدوى الصناعية. وكانت المعامله بمبيد الاميننت افضل المعاملات تأثيرا فى خفض شدة الاصابه 91.9% يلبها التركيزات العالية من كبريتات النحاس، كبريتات الزنك، فوق اكسيد الهيدروجين، وحامض السالسليك وسيليكات البوتاسيوم. بينما كانت اقل المعاملات تأثيرا خليط NPK بمستويات  $N_2P_1K_1$ ،  $N_1P_1K_1$ ،  $N_2P_2K_1$ ، كبريتات الحديد 10 جزء فى المليون وسيليكات الصوديوم بتركيز 4 جزء فى المليون بكفاءة (64.2، 70.1، 72.2، 73.2، و73.2% على الترتيب). وبصفة عامه زاد معدل انخفاض شدة الاصابة معنويا بزيادة التركيز للمعاملات.

بينما تحت ظروف العدوى الطبيعية بالحقل موسمی 2020/2018، ادت جميع المعاملات الى حدوث خفض معنوی في شدة الاصابة وزيادة في المحصول ونقاء السكر في كلا الموسمين. وكانت المعاملات الاميننت، كبريتات النحاس 20 جزء في المليون، حامض الاسكوريك 100 جزء في المليون، كبريتات الزنك 20 جزء في المليون وفوق اكسيد الهيدروجين 1.5% افضل المعاملات تاثيرا في خفض شدة الاصابة وزيادة وزن الجذور ومحصول ونقاء السكر يليها السالسليك 50 جزء في المليون، سيليكات البوتاسيوم 15 سم<sup>3</sup>/لتر بالمقارنه بالكنترول. بينما كانت اقل المعاملات تاثيرا هي كبريتات الماغنيسيوم 30 جزء في المليون يليها كبريتات الحديد ثم خليط NPK بتوليفة N<sub>1</sub>P<sub>2</sub>K<sub>2</sub>.

ادى رش نباتات بنجر السكر بالمواد الكيماويه تحت الاختبار الى زيادة معنويه في نشاط انزيمات الاكسده الفينيلالانين امونيا لاييز PAL والبوليفينيل اوكسيديز PPO والبيروكسيديز في اوراق البنجر بالمقارنه بالنباتات الكنترول. وحقق انزيمي PPO , PAL اعلى نشاط انزيمي مع معاملات فوق اكسيد الهيدروجين 1.5%، حامض الاسكوريك 100 جزء في المليون، وحامض السالسليك 50 جزء في المليون، سيليكات البوتاسيوم 15 سم<sup>3</sup>/لتر وكبريتات النحاس 20 جزء في المليون يليها كبريتات الزنك 20 جزء في المليون. بينما كان نشاط انزيم PO اعلى مع معاملات فوق اكسيد الهيدروجين 1.5%، سيليكات البوتاسيوم 15 سم<sup>3</sup>/لتر، حامض الاسكوريك 100 جزء في المليون و حامض السالسليك 50 جزء في المليون. يليها معاملات وكبريتات النحاس 20 جزء في المليون، كبريتات الزنك 20 جزء في المليون. وفي المقابل كانت معاملة N<sub>1</sub>P<sub>2</sub>K<sub>2</sub> يليها كبريتات الحديد 30 جزء في المليون، كبريتات الماغنيسيوم 30 جزء في المليون و مبيد الاميننت اقل المعاملات تاثيرا على النشاط الانزيمي بالمقارنه بالكنترول.

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