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# Effect of Some Mineral Elements on the Yield, Sugar Contents and Improving Resistance to *Cercospora* Leaf Spot of Sugar Beet

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IN THIS study six mineral elements including copper sulfate ($CuSO_4$), potassium sulfate ($K_2SO_4$), micron sulfur (S),boric acid ($H_3BO_3$), copper sulfate+ micron sulfur ($Cu SO_4$ + S) and potassium sulfate + boric acid ($K_2SO_4 + H_3BO_3$) as well as fungicide Montoro 30% were used as foliar spraying. These elements were evaluated against *Cercospora* leaf spot disease on sugar beet under field conditions during two seasons.All foliar applications were significantly reduced the disease severity % compared to untreated treatment during the two seasons. Potassium and combined application of Potassium and Boron treatments recorded the high value of sugar beet leaf dry weight over the control.Sulfur application led to an increase the fresh weight of sugar beet root, while Boron application increased the total soluble solid (TSS), sucrose % and purity %. Results obtained showed significant increasing (%) in catalase (CAT), peroxidase (POX) and polyphenolosxidase (PPO)activities in the case of mineral application and Montoro 30% fungicide compare with untreated treatment. However, the present study showed that foliar application of mineral elements tested in increased yield components of sugar beet, and thus is recommended to increase root yield and quality as well as can reduce disease severity.

**Keywords:** Sugar beet, Leaf spot, Mineral elements, Control.

## Introduction

Sugar beet is considered one of the temperate regions for sugar production. Where, ranked as the second important one after sugar cane for sugar production in Egypt (Eweis et al., 2006 and Amer et al. 2019)

Cercospora leaf spot (CLS), is the foliar disease of sugar beet world-wide (Holtschulte 2000), this disease reduce 42% of sugar yield (Shane and Teng, 1983). The causal pathogen spreads from region to another in the same country, causing losses in root and extractable sucrose yields, and increases impurity concentrations resulting in higher processing losses (Lamey et al., 1987; El-Moghazy et al., 2017; Ghazy, et al., 2017 and Farahat, 2018).

Many investigations reported that, better crop growth led to increased crop yield and crop performance due to micronutrients application. Rehm and Albert (2006) Showed that, higher level of yield was reported for micronutrients application.Foliar spraying is approach for develop plant tolerance to salinity by alleviating Na and Cl damage to plants (Alpaslan et al., 1999 and El-Fouly et al., 2010). Different experiments showed that useful mineral elements can suppress many plant diseases. When the plants under stress such as plant disease the improving effects of mineral elements were achieved. The obtain results reported that nutrients play great role in host defense mechanisms (Omara et al., 2017 and McGovern & Elmer, 2018). Foliar treatments of micronutrients used to reduce the disease severity% of tan spot disease on wheat (Simoglou and Doradas, 2006). Copper compound has the ability to decay the spores and conidia of fungus and suppress spores germination (Agrios, 2005).

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Boron and copper have essential roles in plants, where play important role in functioning as cofactors or activators of enzyme systems. Many enzymes play pivotal roles in disease resistance in the production of defense barriers (Datnoff et al., 2007). Different rice disease incidence was reduced i.e. brown spot (*Drechslera oryzae*) and sheath blight (Rhizoctonia solani) diseases of rice were real reduced after foliar spray with Cu and B. This effect is believed due to the biocidal effect of Cu and the increase of the physiological properties of the plants itself, such as better lignification and fortified cell membrane which is the primary defense mechanism to build up plants resistance (Liew et al., 2012).Many diseases were reduced due to the effects of boron because the role of B on plant metabolism, cell wall structure and plant membranes (Dordas, 2008). Sulfur (S) has been used to control different disease on rose for many time ago i.e. leaf blights such as black spot, powdery mildew and rusts. Also sulfur has essential role in component of proteins. (Haneklaus et al., 2007). Sulfur recorded as the oldest fungicide known (Agrios, 2005). Foliar application of Boron (B) at concentrate 500 increased root weight. Sugar beet quality was significantly increased in case of total soluble solids (TSS), sucrose %, purity % and extractable sucrose% by increasing levels of micronutrients (Masri and Hamza, 2015). Susceptibility of host plants was decreases up to the optimal level for growth and beyond this point there is no further increase in resistance by using Potassium(Dordas 2008).Potassium has many functions in sugar beet growth. It is an essential element for photosynthesis, enzymes activity to metabolize carbohydrates, the manufacture of amino acid and proteins. As well as transported of sugars produced by Photosynthesis to storage in beet roots. Also, increased drought tolerance, increased disease resistance, regulates many metabolic processes, opening and closing of stomates (Stephen, 2003 and Grzebisz et al., 2004). Much larger quantities of K are needed for these physiological functions than for its biochemical role in plant (International Potash Institute, 2013; Reddya et al., 2004 and Roghieh & Arshad, 2009) Potassium is an important nutrient plays important role on sugar beet enzyme activation and in different physiological proses on other plants (Sangakkara et al., 2000 and Cakmak et al., 2005). Foliar application of Potassium fertilizer on sugar beet caused a significant increase foliage and roots weights/plant (Nafei et al., 2010; Kassab et al., 2012; Pechková & Hřivna 2013and Zaki, Nabila et al., 2014).

Biotic stress is the results of the interaction between the pathogen and host to the accumulation of different types of oxygen in the cells, which leads to severe oxidative damage to the pathogen and host. To overcome this damage, both the plant and pathogen developed antioxidant systems to reduce the excess of ROS and maintain the production of ROS and systems scavenging under control (Samsatly et al. 2018). Cells of plants produce antioxidant enzymes such as peroxidase (POX) and catalase (CAT) to protect against oxidative stress (Mittler 2002 and Yildiz & Terzi, 2013).

The aim of the present work was to assessment the efficacy of some mineral elements against cercospora leaf spot disease of sugar beet under open field conditions. Also., to investigate the efficacy of these control methods on some sugar beet yield characters with respect to leaf dry weight, root fresh weight, soluble solid content, sucrose content and purity of sugar.

## Materials and Methods

The present investigation was performed under field conditions at Sakha Agricultural Research Station, Plant Pathology Research Institute Kafrelsheikh governorate Egypt, during 2017/18 and 2018/19 sugar beet growing seasons. Sugar beet (*Betavulgaris* L., Chenopodiaceae) cultivar oscarpoly, as a susceptible sugar beet cultivar to Cercospora leaf spot disease was the target of the evaluation of mineral elements, under study. Cultivar oscarpoly used in this study was obtained from the Field Crops Research Institute, Agricultural Research Center, Giza, Egypt.

*Tested materials*

Seven treatments were used as foliar application, Copper Sulfate, Micron Sulfur, potassium sulfate, boric acid, Copper Sulfate + micron sulfur, potassium sulfate + boric acid and Montoro 30% in addition to control treatment (foliar spray with water) were used at rate of 0.6gm/L., 2.5g/L., 6gmL., and 1.5gm/L., respectively. These elements obtained as technical compounds from Al-Gomhoria Company for Chemical And Glasses, Cairo, Egypt. The fungicide used in this study was Montoro 30% (difencoconazole + propiconazol) at its recommended field rate of 0.5 cm/LTable 1.

**TABLE 1. Chemical formula of mineral elements and fungicide**

| Materials | Chemical composition | Molar mass | Concentration |
|---|---|---|---|
| Copper sulfate | $Cu\ SO_4.5H_2O$ | 240.609 g/mol | 0.6 g $L^{-1}$ |
| Potassium sulfate | $K_2SO_4$ | 174.259 g/mol | 6 g $L^{-1}$ |
| Micron sulfur | S | 32 g·mol١− | 6 g $L^{-1}$ |
| Boric acid | $H_3\ BO_3$ | 61.83 g/mol | 1.5 g $L^{-1}$ |
| Copper sulfate +Micron sulfur | $Cu\ SO_4$+S | 159.609 +32 g·mol١− | 6 g $L^{-1}$ |
| Potassium sulfate + Boric acid | $K_2SO_4$+ $H_3\ BO_3$ | 174.259 + 61.83 g/mol | 6 g $L^{-1}$ |
| Montoro 30% | | | 0.5 cm $L^{-1}$ |

*Evaluation of tested materials against Cercospora leaf spot disease under field conditions*

This study was conducted using randomized complete block design with three replicates. Each replicate was 3 rows with 6.0 m. long and 60 cm width. Each row contained 30 hills with 20cm. apart.All recommended culture practice was performed in the proper time. Plants were sprayed with each of the follow 7 treatments Copper sulfate, Micron Sulfur, potassium sulfate, Boric acid, copper sulfate+micron Sulfur, potassium sulfate+ boric acid and Montoro 30%.Spraying was started when disease symptoms were detected and theuntreated plots were sprayed with water as control.

All treatments applied three times with two-week interval.According to Shane and Teng (1992) disease severity and disease reduction % were recorded.Leaf dry weight,root fresh weight and total soluble solids (T.S.S. %) were estimated in fresh roots of sugar beet after 135 days from planting.Using hand refractometer according to Mc Ginnis (1982), Sucrose % was estimated according to Association of Official Analytical Chemist (A.O.A.C) (1990). Purity percent was determined as described by Carruthers and Oldfield (1962).

*Enzymes activity assay*

Samples were taken after the completion of the third spray of the treatments. Three antioxidant catalase (CAT), peroxidase (POX) and poly phenol oxides (PPO) enzymes activity were determined under this investigation, which the CAT enzyme activity was determined in the homogenates by measuring the decrease in absorption at 240 nm in a 3 ml of reaction mixture containing 0.16 ml of 10% W/V $H_2O_2$ diluted to 100 ml with 0.067 M phosphate buffer and 0.1 ml of enzyme extract, according to Sadasivam and Manickam (1999). POX activity was spectrophotometrically measured using guaiacol/ $H_2O_2$ as substrate according to Lobarzewski et al., (1990). PPO activity was spectrophotometrically measured using catechol as substrate according to Mariey et al. (2016).

*Statistical Analyses*

Statistical software package (MSTAT-C program version 2.10) was used to analyze the obtained data (Anonymous, 1991). Multiple comparisons were first submit to analysis of variance (ANOVA) comparisons among means was performed according to Duncan's multiple range test at ($p = 0.05$) (Duncan, 1995).

## Results

*Efficacy of the tested treatments against cercospora leaf spot disease on sugar beet under field condition*

Disease severity and the relative efficacy are shown in Table 2. Where, disease severity of Cercospora leaf spot % was significantly reduced in all tested treatments comparative to the control in both seasons. Data in this table, indicated thatdisease severity was from 0.7 to 26.3% in the first season and from 06.3%to 23.0% in the second season.

Among the seven treatments under evaluation, the only two foliar application *i.e.* $Cu\ SO_4$ and $CuSO_4$ + S, as well as the Montoro 30% fungicide exhibited the highest antifungal effects activities for controlling Cercospora leaf spot (CLS) in the treated sugar beet plants of the susceptible cultivar; Oscarpoly, during the two growing seasons. Montoro 30%recorded the lowest value (7.0 and 6.3%), in the two growing seasons, respectively. It is occupied the first rank in reducing disease severity in both season, followed by foliar application of $Cu\ SO_4$ (12.0% and 11.3%), and $CuSO_4$ + S (12.3 and 12.0%) during the two seasons of the study, respectively. In contrast, the highest values of disease severity were record with the S application recorded (26.3 and 23.0) in the two growing seasons, respectively, as well as the control treatment, water spray, therefore, it is considered to be a highly disease severity in the two seasons (45 and 35%), respectively.

Regarding the relative efficacy of tested treatments against Cercospora leaf spot, high relative efficacy was recorded for fungicide application; Montoro 30% (83.4%) followed by Cu $SO_4$ (70.20%) as mean valueduring both growing seasons, while thelowest relative efficacy was recorded in the tested foliar application; S (37.95%) in Fig. 1.

*Leaf dry weightof sugar beet plants*

Data in Table 3,showedthe effect of the tested materials on dry weight (g) of 100 g fresh weight of sugar beet leaves in both growing seasons. Leaf dry weight of sugar beet was significantly increased in all the tested treatments relative to the control during the two tested growing seasons. $K_2SO_4 + H_3BO_3$ resulted in the highest increase in leaf dry weight (14.60 % and 15.70 %), followed by S (13.67 % and 14.77 %), Montoro 30% (13.10 %and 14.53 %), and Cu $SO_4$ + S (13.05% and 14.15%) during the two growing seasons of the study, respectively.

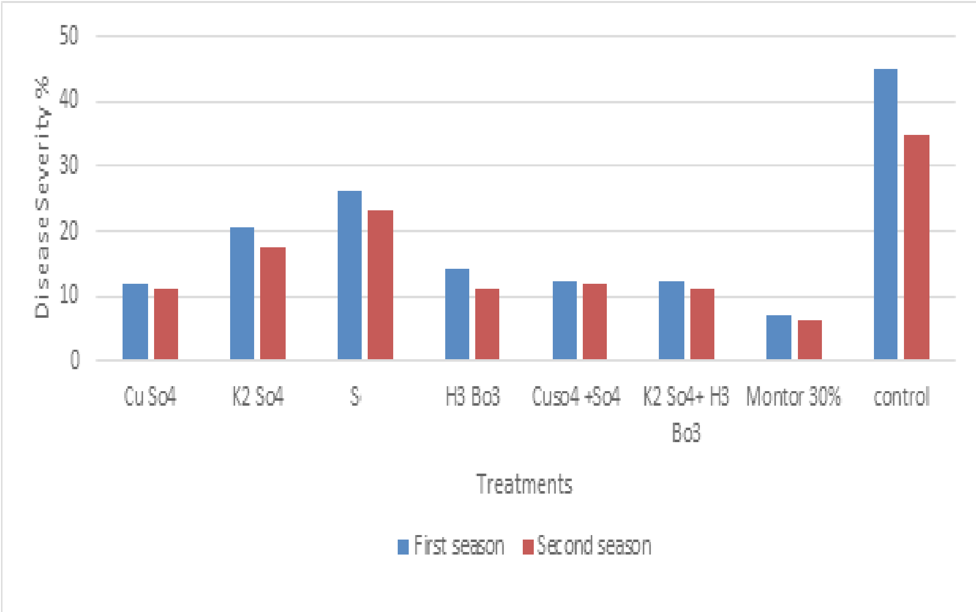


**Fig. 1. Effect of foliar application of six mineral elements as well as one fungicide on disease severity of sugar beet, during 2017/18 and 2018/19**

**TABLE 2. Disease severity and efficacy of different applied treatments against Cercospora leaf spot of sugar beet in the two growing seasons**

| Treatments | Conc.* | Disease severity (%) | | Mean of Efficacy (%) |
|---|---|---|---|---|
| | | 1st season | 2nd season | |
| Cu $SO_4.5H_2O$ | 0.6g/L | 12.0 d | 11.3 d | 70.20 |
| $K_2SO_4$ | 6g/L | 20.7 c | 17.7 c | 51.70 |
| S | 6g/L | 26.3 b | 23.0 b | 37.95 |
| $H_3BO_3$ | 1.5g/L | 14.0 d | 11.0 d | 68.75 |
| Cu $SO_4$ + S | 6g/L | 12.3 d | 12.0 d | 69.50 |
| $K_2SO_4 + H_3BO_3$ | 6g/L | 12.3 d | 11.3 d | 70.20 |
| Montoro 30% | 0.5ml /L | 07.0 e | 06.3 e | 83.20 |
| Control | - | 45 a | 35 a | - |

*Conc. = concentration.

*Root fresh weight*

Data in Table 3, showed the effect of the tested treatments on the fresh weight of sugar beet roots in the two growing season. Root fresh weight of sugar beet was significantly increased due to the effect of the tested treatments compared to untreated control plants.Foliar application treatments had the sequence from the high to low fresh root weight; S (12.25kg and 11.55 kg), $Cu SO_4$ + S (10.80 kg and 10.73kg), $K_2SO_4$(10.50kg and 11.00kg)as well as the Montoro 30% (12.50kg and 11.75kg), followed by, $Cu SO_4$ (10.37kg and 10.87kg), $H_3 BO_3$(09.75kg and 10.25kg) and $K_2SO_4$ + $H_3 BO_3$ (09.50kg and 09.83), during the two growing seasons, respectively.

*Total soluble solid(TSS), sucrose and purity of sugar beet products*

Data in Table 4, showed the relative efficacy of the tested treatments on total soluble solid (TSS %), sucrose % and sugar purity in both seasons. Total soluble solid and purity were significantly increased in all tested treatments as compared to the control treatment in both tested seasons. The highest sucrose and sugar purity % was recorded after spraying sugar beet plants with foliar application of $H_3 BO_3$ (19.0% and 20.0%), (85.2% and 86.6%), in the two growing season, respectively. On the other hand, $K_2SO_4$ exhibited the lowest percentages of sucrose and sugar purity (16.5% and 18.5%), (79.7% and 79.4%),while the control treatment (15.5% and 16.2%) and (78.3% and 79.0%) in the two growing season, respectively.

**TABLE 3. Leaf dry weight % and fresh root weight of sugar beet plants as affected by different applied treatments in both growing seasons.**

| Treatments | Leaf dry weight (%) | | Root fresh weight (kg) | |
|---|---|---|---|---|
| | 1st season | 2nd season | 1st season | 2nd season |
| **$Cu SO_4.5H_2O$** | 11.87d | 12.97de | 10.37bc | 10.87ab |
| **$K_2SO_4$** | 14.47a | 15.57a | 10.50bc | 11.00ab |
| **S** | 13.67b | 14.77ab | 12.25a | 11.55a |
| **$H_3 BO_3$** | 12.90c | 14.00bc | 09.75bc | 10.25bc |
| **$Cu SO_4$ + S** | 13.05bc | 14.15bc | 10.80b | 10.73abc |
| **$K_2SO_4$ + $H_3 BO_3$** | 14.60a | 15.70a | 09.50c | 09.83c |
| **Montoro 30%** | 13.10bc | 14.53cd | 12.50a | 11.75a |
| **Control** | 11.40d | 12.50e | 08.00d | 08.67d |

**TABLE 4. Effect of the tested treatments on total soluble suspended solid (TSS), sucrose and purity of sugar beet products during the two growing seasons 2017/18 and 2018/19**

| Treatments | Conc. | 2017/2018 | | | 2018/2019 | | |
|---|---|---|---|---|---|---|---|
| | | TSS (%) | Sucrose (%) | Purity(%) | TSS (%) | Sucrose (%) | Purity(%) |
| **$CuSO_4.5H_2O$** | 0.6g/L | 21.2d | 16.9bcd | 81.7 | 23.7ab | 19.6ab | 82.3 |
| **$K_2SO_4$** | 6g/L | 20.2g | 16.5cd | 79.7 | 22.3b | 18.5ab | 79.4 |
| **S** | 6g/L | 20.4f | 17.0bcd | 82.9 | 22.9ab | 18.8ab | 82.1 |
| **$H_3 BO_3$** | 1.5g/L | 20.5e | 19.0a | 85.2 | 23.1ab | 20.0a | 86.6 |
| **$Cu SO_4$ + S** | 6g/L | 22.3a | 17.5abcd | 80.6 | 22.1bc | 18.1b | 81.9 |
| **$K_2SO_4$ + $H_3 BO_3$** | 6g/L | 21.7c | 18.5ab | 82.2 | 22.7ab | 18.7ab | 82.0 |
| **Montoro 30%** | 6g/L | 22.0b | 18.0abc | 81.8 | 24.1a | 20.0a | 83.0 |

*Activity of catalase, peroxidase and polyphenol oxidase*

Results obtained indicate that significant increasing (%) in the activities of catalase (CAT), peroxidase (POX) and polyphenol oxidase (PPO) in the case of mineral application and Montoro 30% compare with untreated treatment. Results obtained in Fig.2, showed the activities of anti-oxidant CAT enzymes of the sixmineral elements as well as Montoro application.The responses of CAT activity in Cu $SO_4$ (29.1817%) and Cu $SO_4$ + S(23.158%) were higher than the rest of treatments.The rest of tested foliar applications showed intermediate activities ranged between (11.97 % to 19.77 %). While, both Montoro30% and the untreated treatment recorded the least values (12.019%) and (12.7657%), respectively.

Spraying sugar beet plants with all tested treatments individually increased activity of peroxidase (POX) in sugar beet leaves compared with untreated treatment. The results in Fig. 3 showed that the highest level of peroxidase (POX) activity was obtained from Cu $SO_4$(106.6 %) and $K_2SO_4$ (98.46%) followed by $CuSO_4$ + S (56.888%). On the other hand, the least peroxidase (POX) activity was recorded with $H_3BO_3$(18.59 %) as well as fungicide; Montoro 30% (33.01 %).

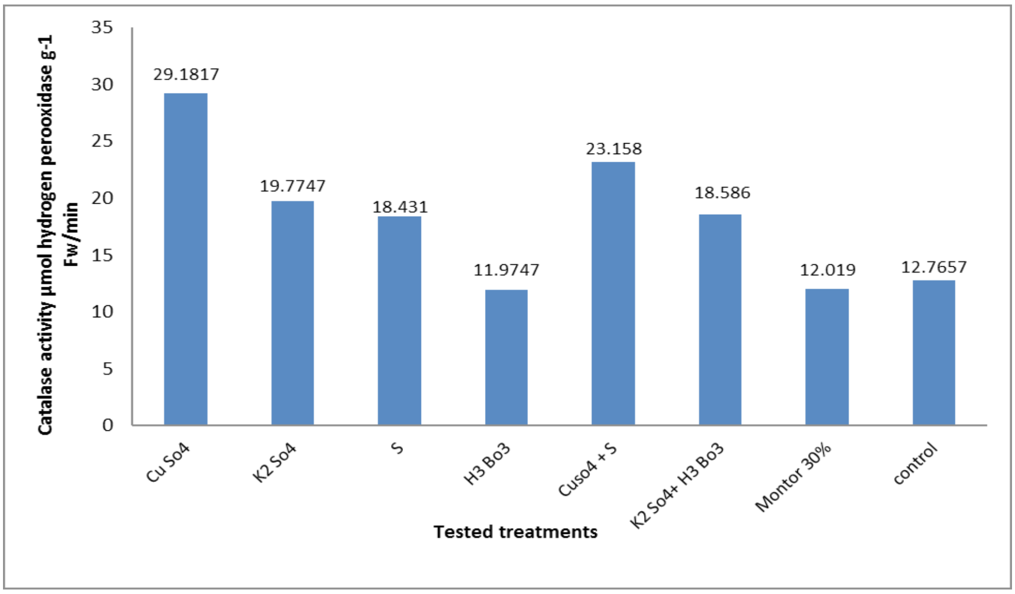


**Fig. 2. Effect of tested treatments on activities of anti-oxidant Catalase enzyme**

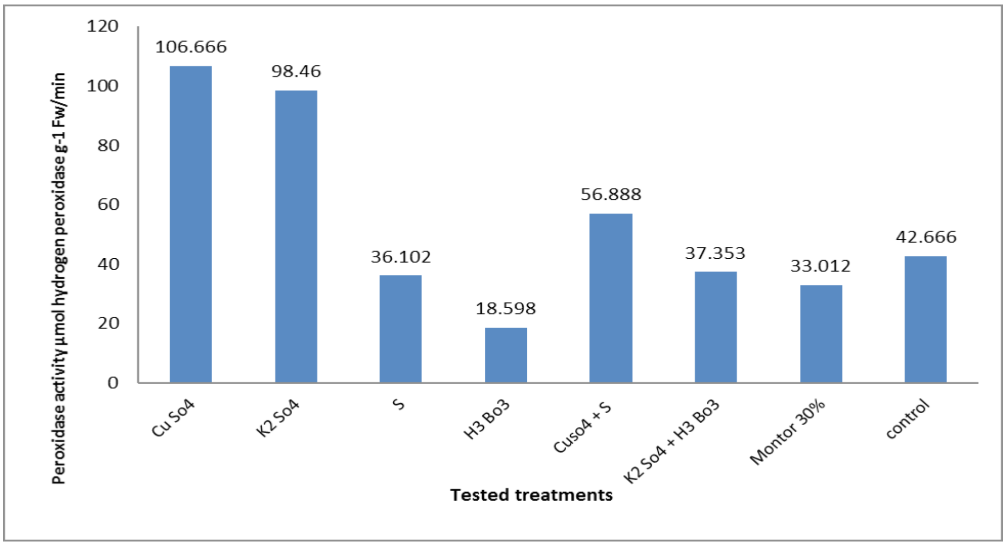


**Fig. 3. Effect of tested treatments onactivities of anti-oxidant peroxidase enzyme**

The results in Fig. 4 showed that the highest activity of PPO enzymes was observed with S (0.212%) and K2 $SO_4$ (0.15733%) followed by Cu $SO_4$ + S (0.11467%) treatments. Whereas, the least enzymatic activity was recorded with Montoro 30% (0.014%).

## Discussion

Sugar beet (*Beta vulgaris* L., Chenopodiaceae) was infected with *Cercospora beticola* fungus causes destructive foliar disease namely Cercospora leaf spot (CLS) where, causes economically yield losses. Sugar beet Crop losses reach 40% (Skaracis et al., 2010) and in absence of fungicide treatments result in complete yield loss (Rossi et al., 2000). The combination between agricultural practices i.e. crop rotation, fungicide application, resistant sugar beet cultivars as well as mineral nutrients could affect the disease tolerance or resistance of plants to pathogens (Shane & Teng, 1992 and Dordas, 2008).

The foliar application of the tested;Cu $SO_4$, $K_2SO_4$, S, $H_3$ $BO_3$, Cu $SO_4$ + S, $K_2SO_4$ + $H_3$ $BO_3$, as well as fungicides Montoro 30% in the present study lead to effective control against Cercospora leaf spot under field condition as indicated by reduction of disease severity. In addition to, all the foliar applications improved of sugar beet yield characters *i.e.*leaf dry weight, root fresh weight, TSS%, sucrose % and purity % achieved the high increase in all the mentioned parameters over the untreated treatment during both growing seasons.

This may be due to Cu $So_4$ extends the plant by two essential (Cu and S), Copper has an important role in lignin formations which affect fungal growth in addition to the toxic effect on the fungi. Sulfur have an important role in protein formation and some amino acids links. Also, K2 So4 extends the plant by two essential elements (S and K). Potassium has an important role in carbohydrate translocation, more enzyme activation and enhances turgor and stomata movement. Boric acid extends the plant by boron, it's has an important role in sugar translocation and storage in addition to its role as insect and fungi tolerate.

The obtained results is in agreement with the finding of Gouda and El-Naggar (2014) they found that difencoconazole + propiconazol applied alone, consistently provided effective Cercospora leaf spot control and resulted in high sucrose yield.

The obtained results in this study revealed, that use or the application of the six mineral elements as well as the fungicide; Montoro 30% enhanced the host resistance in the susceptible sugar beet cultivar; oscarpoly against Cercospora leaf spot infection disease under field conditions in both seasons.Different studies showed that, potassium salts as a induce plant resistance and reduce the susceptibility of plants reached to the optimal level for growth: After this point, there is no moreover increase in resistance which can be carried out by increasing the supply of

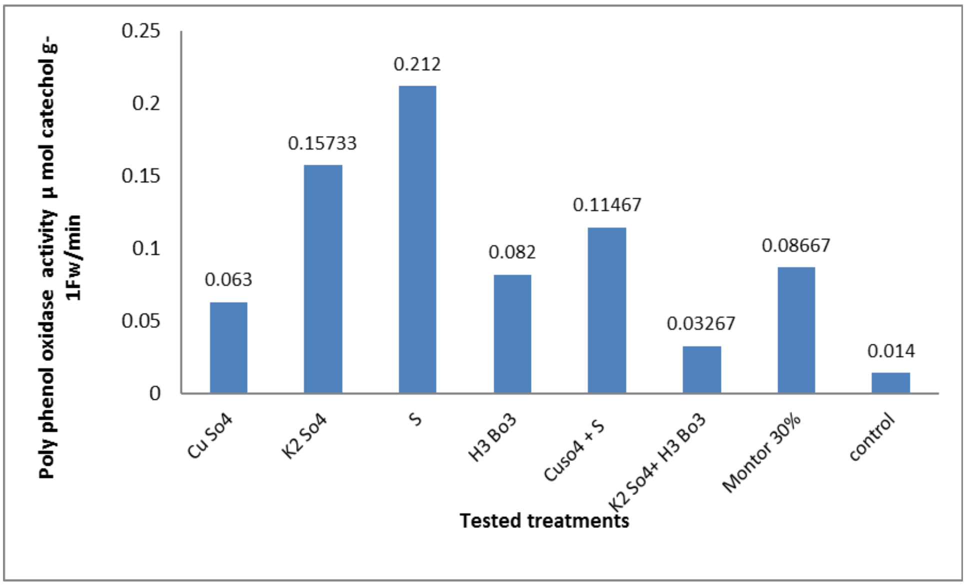


**Fig . 4. Effect of tested treatments on activities of anti-oxidant Poly phenol oxidases enzyme.**

potassium and its contents in plants (Huber and Graham, 1999). Krupinsky and Tanaka (2000) reported that K treatment suppressive the severity of the leaf spot disease. This obtained result also harmonize, with the limited previous findings by Regmi et al. (2002) and Sharma et al. (2004), they showed that K treatment suppressive the severity of plant causal pathogen and increase crop yield

Regarding the effect of the foliar application treatments on quality characters of sugar beet. The results indicated that the Potassium and combined application of Potassium and Boron tested treatments recorded the high value of sugar beet leaf dry weight over the control. While, Sulfur application increased the fresh weight of sugar beet root. Boron application increased the total soluble solid (TSS), sucrose % and purity %.The possible mechanism of copper and Boron against plant pathogens may be due to the ionic components, adversely affecting enzyme activities of the pathogen (Miceli et al., 1999). The efficacy of copper, Boron and potassium against Cercospora leaf spot suggest the ability of using them for leaf spot control either alone or alternative with fungicides to reduce the applied amount of fungicides.

Results obtained showed significant increasing (%) in the activities of catalase (CAT), POX and PPO in the case of mineral application and Montoro 30% compare with untreated treatment. PPO is considered to be the key associated with the plant oxidation of plant phenolic compounds that is why the increase in the activity may give the plant an promote resistance to pathogen invasion by providing increased contents of oxidized quinone derivatives, which delay pathogen progress (Tyagi et al., 2000). Furthermore, Srivastava, 1987mentioned that at sixth day of the infection, resistant plants reported that activities were the highest compared to their susceptible example. In healthy plants very low activity of these enzymes could be registered. Results of this, study showed the possible participation of these oxidative enzymes in the resistance of this disease.More studies emphasized that the increase in activity of defense-regarding enzymes, *i.e.,* peroxidase (PO) or polyphenol oxidase (PPO), has been elicited by strains bio-control agent in different host plants (Govindappa et al., 2010). PO and PPO are thought to support lignification and suberizationof cell walls at the border of infection and moreover limit spread of pathogens (Passardi et al., 2004).

## Conclusion

The tested treatments were potentially useful for enhanced the resistance of *Cercospora* leaf spot disease of sugar beet In addition to, Potassium and combined application of Potassium and Boron tested treatments recorded the high value of sugar beet leaf dry weight over the control. While, Sulfur application increased the fresh weight of sugar beet root. Boron application increased the total soluble solid (TSS), sucrose % and purity %.Also, the obtained results reported that tested treatments led to a significant increase in the activity of the defense enzymes PPO of sugar beet plants infected by *Cercospora* leaf spot.Whereas, that treatments were reduced the environmental dangerous make them ideal spraying for disease control under field conditions and increased root yield and quality.

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