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EFFECT OF SOME CHEMICAL COMPOUNDS AND BIOCIDES ON *Cercospora* LEAF SPOT DISEASE OF SUGAR BEET

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ABSTRACT

Cercospora leaf spot of sugar beet plants (*Beta vulgaris* L.) caused by *Cercospora beticola* Sacc. is one of the most serious foliar disease of sugar beet worldwide. Efficacy of five chemical compounds, *i.e.* Boric acid, Zink sulphate, Calcium chelate, Potassium bicarbonate and Salicylic acid, two biocides (Bio - ARC and Blite stop) and score fungicide beside untreated treatment (control) were tested in the fields of two locations, New Nubariya County and Kafr El-Sheikh County under natural infection. Three spraying programs were utilized. For the first program, plants were sprayed twice, at 60 and 90 days after planting. In the second program, plants were sprayed twice, at 90 and at120 days after planting, while plants of the third program were sprayed three times at 60, 90 and at 120 days after planting. The results showed that all the tested materials exhibited a different degree at efficiency for the disease severity percentage and sucrose percentage, extractability percentage, root yield and sugar yield in comparison with the control treatment. Score fungicide gave the best results compared to all the tested materials ، biocide Bio-ARC recorded disease severity (8.58%) followed by Blite stop (as a new Egyptian commercial bio-product) (12.27%). Salicylic acid was the best tested chemical inducer (9.36%) followed by Potassium bicarbonate (10.35 %); Calcium chelate (11.43 %); Boric acid (13.56%) and Zink sulphate (16.62%) as compared with (23.4 %) for control treatment. Spraying at 60,90 and 120 days after planting gave the best results of all the measurements, where sugar yield recorded (2.97 ton/fed) while the spraying at 90 and 120 days gave (2.47 ton/fed). New Nubariya plants were the best of the disease tolerance, root yield and sugar yield while sucrose (%) and extractability (%) was not affected by the location. We found an increase of leaf content of free, conjugated and total phenols as a result of spraying with the chemical compounds and the biocides, also we found an increase in the activity of peroxidase and polyphenoloxidase.

Key Words: *Sugar beet (Beta vulgaris L.), Cercospora leaf spot disease, Cercospora beticola, plant resistance inducers.*

1. INTRODUCTION

Sugar beet (*Beta vulgaris* L.) is one of the most important crops grown for sugar production in temperate regions. In Egypt, it ranked second after sugar cane for sugar production (Eweis *et al*., 2006). *Cercospora beticola* is the causative agent of Cercospora leaf spot disease (Felipini *et al.*, 2015; and El-Sayed and El-Sherbeni, 2017). Cercospora leaf spot infection can cause severe yield loss in sugar beet susceptible varieties ranging from 10-50 % in Australia, and from 15-40 % in France (Kaiser and Varrelmann, 2009).

There are many products composed of living organisms, primarily bacteria and fungi, that increase plant health (Bloom *et al*., 2003).

Some chemical compounds enhance the defense system in the plant against diseases, e.g. Salicylic acid has been shown to induce resistance in plants (Mandal *et al.*, 2009). This could be due to the increase of phenols and activation of many enzymes such as polyphenoloxidase and peroxidase (Hussein, 2011; Abd El-Al *et al*., 2012). The role of calcium in reducing disease disorders is mentioned by Conway *et al.* (1992).

The mechanism of calcium salts in reducing disease severity percentage depends on the Ca^{+2} status of the membrane (Xing *et al.*, 1996). Boron has a direct function in cell wall structure and stability and has a beneficial effect on reducing disease severity. However, in several diseases, the function of boron in disease resistance or tolerance is the least understood of all the essential micronutrients in plants (Blevins and Lukaszewski, 1998). The relationship between boron and sugar beet infection percentage with cucumber mosaic virus (CMV) was reported by Awad (2005). He found that the infection percentage with CMV decreased in sugar beet plants treated with boron in addition to decreasing the effect of CMV on leaf content of chlorophyll and root content of total carbohydrates and sucrose. Plant spraying with potassium bicarbonate solution provided an effective protection against some diseases (Smilanick *et al*., 2006). It could be suggested that using potassium bicarbonate may be useful for controlling sugar beet leaf spot disease under field conditions (Hussien *et al.*, 2009). Zink element is involved in membrane protection against oxidative damage through the detoxification of superoxide radicals (Cakmak, 2000).

The traditional means to control this destructive disease is applying harmful fungicide. However, using biological agents is encouraged at the time being. Many of previous investigations succeeded in reducing infection with leaf spot diseases of different crops by using some bioagents (El-Assiuty *et al.*, 2009).

The main objective of the present research was studying the effect of some chemical compounds and biocides against Cercospora leaf spot disease on sugar beet caused by *Cercospora beticola*.

2. MATERIALS AND METHODS

In the current trial, some control materials were tested for their potentialities to decrease the disease severity percentage under field conditions at two Egyptian locations of sugar beet cultivation (New Nubariya County, Beheira Governorate and Kafr El-Sheikh County, Kafr El-Sheikh Governorate) during 2015-2016 season using three spraying programs. During the present study, application of the control materials Table (1) was carried through 3 spraying programs depending on plant age: In the first, sugar beet plants were sprayed twice, *i.e.* at 60 and at 90 days after planting; for the second, plants were sprayed twice with the tested materials at 90 and 120 days after planting and for the third program, plants were sprayed three times at 60, 90 and 120 days after planting. The following data were recorded:

1- Disease severity (%) was estimated according to Shane and Teng (1992), after 30 days from the last spray in each program.

2- Root yield (ton/fed).

3- Sugar yield (ton/fed).

4- Sucrose percentage which was estimated using the method adopted by Le- Docte (1927).

5- Extractability percentage = Sugar extraction (%) / sucrose (%)

where sugar extraction (%) = Sucrose (%) – Sugar loss to molasses (%) – 0.6 according to Dexter *et al*. (1967).

6 - Phenolic content:

Phenolic components were determined in the ethanolic extraction of sugar beet leaves using Folin-Ciocalteu's reagent described by Bray and Thrope, (1954). The ethanolic extracts of sugar beet leaves were made by shaking 100 ml ethanol containing 5 g sugar beet leaves for 24 hours and then left at the room temperature until ethanol evaporates, then completed to 100 ml with distilled water.

To determine the free phenols (mg/g fresh weight), 1 ml of the ethanolic extract was mixed with 1mI of Folin-Ciocatalteu's reagent and 1 ml sodium carbonate (14%). The reaction mixture was completed to 5 ml with distilled water, thoroughly mixed, heated in a boiling water bath for 10 min., then cooled and the developing colour was measured at 650 nm against reagent blank using the Spectrophotometer. A standard curve from caticol was used to calculate the amount of phenols in different samples.

To determine the total phenols (mg/g fresh weight), 1 ml of the ethanolic extract was mixed with 0.5 mI of Folin-Ciocatalteu's reagent and incubated at room temperature for 3 minutes, then 2 ml of 20% sodium carbonate were added, mixed well and incubated in boiling water bath for 1 minute then cooled rapidly and read absorbance at 650 nm against reagent blank using the Spectrophotometer. A standard curve from caticol was used to calculate the amount of phenols in different samples.

The conjugated phenols (mg /g fresh weight) were calculated by subtracting free phenols from the total phenols.

Standard curve from caticol was prepared by making different concentrations of caticol and read each of them at 650 nm using the Spectrophotometer and then using microsoft office excel program to elicitation linear relationship.

7 - Activities of antioxidant enzymes:

Fresh leaf samples were grind in a mortar with 0.1 M sodium phosphate buffer (pH 7.1) at rate (2 ml buffer/ 1 g fresh leaf) according to (Goldschmidt *et al.*, 1968). The mixture was filtrated through several layers of cheesecloth, then centrifuged at 1500 rpm for 20 min. at 6°C. The obtained supernatant fluids were used for determination the activities of oxidative enzymes (polyphenoloxidase and peroxidase) as follows:

a - Polyphenoloxidase assay: Polyphenoloxidase was determined according to (Malta and Dimond, 1963). The reaction mixture contained 1.0 ml of enzyme extract, 1.0 ml of 0.2 M sodium phosphate buffer at pH 7, 1.0 ml of 10^{-3}M catechol and completed with distilled water to 6.0 ml. The activity of polyphenoloxidase was expressed as the change in absorbence per 30 second for 5 minute procedures at 495 nm wave length.

b - Peroxidase assay: Determination of peroxidase activity was based on oxidation of pyrogallol to purpurogallin in the presence of H_2O_2 according to the method described by Allam and Hollis (1972). The reaction mixture contained 0.5 ml of 0.1 M sodium phosphate buffer at pH 7, 0.3 ml of enzyme extract, 0.3ml of 0.05M pyrogallol, 0.1 ml of 1.0% H_2O_2 and completed with distilled water to 3.0 ml. The activity of peroxidase was expressed as the change in absorbency per 1 minute for 10 minute procedures at 425 nm wave length.

Statistical analysis: The collected data were statistically analyzed according to the method described by Snedecor and Cochran (1981). The collected data were subjected to the proper statistical analysis of Complete Block Design Combined over Locations.

Table (1): Control materials tested against *Cercospora* leaf spot disease of sugar beet grown in New Nubariya County and Kafr El-Sheikh County.

Locations	Spraying program	Control materials, concentrations	
Nubariya county Kafr El Sheikh county	The first program: Two times, at 60 and at 90 days after planting The second program: Two times, at 90 and 120 days after planting The third program: Three times, at 60, at 90 and at 120 days after planting	Chemical compound	Boric acid 1 g/l Zink sulphate 5 g/l Calcium chelate 6 g/l Potassium bicarbonate 2 g/l Salicylic acid 4 g/l
		Biocide	Bio ARC(*Bacillus megaterium*) 2.5 g /l Blite stop* (<u>*Trichoderma harzianum*</u> + *B. subtilis*) 6.6 ml /l
		Fungicide	Score 250 EC 0.5 ml/l

*** New Egyptian commercial bio-product.**

3. RESULTS AND DISCUSSION

The data illustrated in Table (2) show that all control materials used significantly decreased the disease severity (%) compared with the control treatment. Score treatment gave the highest decrease where disease severity recorded 2.73% followed by Bio-ARC 8.58%, then Salicylic acid 9.36% while Zink sulphate exhibited the lowest decrease 16.62% followed by Boric acid 13.56%, then Blite stop12.27%. Spraying the plants at 60,90 and 120 days after planting achieved the highest decrease where disease severity recorded 10.59% while spraying at 90 and 120 days decreased the disease severity to 13.92%. With respect to locations, New Nubariya was the best where disease severity recorded 10.2% compared with Kafr El-Sheihk which recorded 13.8%. This result is in agreement with those reported by Jung and Kim (2005) and Kildea *et al.* (2008) who reported that Bacillus megaterium (the active organism of Bio- ARC) showed potential for the biocontrol of Rhizoctonia-root rot of soybean, Phytophthora-blight of red-pepper and Septoria blotch of wheat. Many Bacillus species have provn to be effective against a broad range of plant pathogens (Kumar *et al.*, 2011 and Shafi *et al.*, 2017). Also, Galletti *et al.* (2008) reported that in-depth screening among different Trichoderma isolates can lead to the selection of isolates effective in increasing the defensive response of sugar beet plants to *C. beticola* under field conditions. The mechanism seems to be linked more to induce resistance than to direct competition. Many researchers reported that chemical inducers of plant resistance possess quite different modes of action as compared to synthetic biocides as they have no direct toxicity to pathogens, plants and animals; no negative effects on plant growth, development and yield, broad spectrum of defence, low loading amount, long lasting protection, low economical cost for farmers and good profit for producers (Ganesan and Thomas, 2001; Li and Zhang, 2012 and Takatsuji, 2014). This result also is in agreement with the findings of (Hussien, 2006) who pointed a significant reduction of Cercospora leaf spot disease severity on sugar beet treated with by Salicylic acid. Also, Banerjee *et al*. (2010) reported the induction of plant defense using chemical inducers results in increased ability of the plant to reinforce the plant cell wall by altering its composition, primarily with lignins, pectins, amide I structure, and lipids. Also, Hussien (2010) and Hussein (2011) reported that disease severity of sugar beet *Cercospora leaf* spot was reduced by application of potassium bicarbonate. Regarding boron action in reducing disease susceptibility, Brown *et al*. (2002) stated that this function may be due to: (1) its function in cell wall structure, (2) its function in cell membrane permeability, stability. In respect of calcium compounds action in reducing disease susceptibility, El-Kholi and Esh (2011) mentioned that *Cercospora leaf* spot severity on sugar beet plants treated with the two tested calcium compounds (calcium chelate and

Table (2): Effect of spraying some control materials on disease severity (%) of *Cercospora* leaf spot on Sugar beet in fields at two locations, 2015/2016 growing season.

	New Nubariya			Mean	Kafr El-Sheikh			Mean	Overall mean			Mean
	60-90	90-120	60-90-120		60-90	90-120	60-90-120		60-90	90-120	60-90-120	
Boric acid	10.74	13.65	10.11	**11.49**	14.31	17.70	14.79	**15.60**	**12.51**	**15.69**	**12.45**	**13.56**
Zink sulphate	12.81	14.55	12.24	**13.20**	17.70	26.91	15.60	**20.07**	**15.24**	**20.73**	**13.92**	**16.62**
Calcium chelate	9.57	10.80	8.16	**9.51**	12.54	14.85	12.60	**13.32**	**11.07**	**12.84**	**10.38**	**11.43**
Potassium bicarbonate	8.88	10.14	7.44	**8.82**	11.85	12.60	11.19	**11.88**	**10.35**	**11.37**	**9.33**	**10.35**
Salicylic acid	7.71	9.24	7.14	**8.04**	10.86	11.01	10.29	**10.71**	**9.30**	**10.14**	**8.70**	**9.36**
Bio- ARC	6.63	8.85	6.75	**7.41**	10.11	10.05	9.21	**9.78**	**8.37**	**9.45**	**7.98**	**8.58**
Blite stop	10.44	11.85	9.24	**10.50**	13.35	15.30	13.38	**14.01**	**11.91**	**13.59**	**11.31**	**12.27**
Score	1.23	2.28	1.20	**1.56**	3.51	3.75	4.41	**3.87**	**2.37**	**3.00**	**2.82**	**2.73**
Control (untreated)	20.34	27.30	15.90	**21.18**	23.91	29.61	21.15	**24.87**	**22.14**	**28.44**	**18.54**	**23.04**
Mean	**9.81**	**12.09**	**8.67**	**10.20**	**13.11**	**15.75**	**12.51**	**13.80**	**11.46**	**13.92**	**10.59**	

L.S.D at (0.5%):Location (L)= 0.31 Spraying time (S)= 0.38 Control materials (C)= 0.65
(L x S)= n.s (L x C)= n.s (S x C)= 1.13 (L x S x C)= n.s

calcium chloride) was significantly decreased as compared to untreated plants.

The data illustrated in Table (3) show that all control materials significantly increased the root yield compared with the untreated control. Score treatment showed the highest increase where root yield recorded 23.53 ton/fed followed by Bio ARC 20.95 ton/fed, then Salicylic acid 19.22 ton/fed and potassium bicarbonate 18.33 ton/fed while Zink sulphate showed the lowest increase where root yield recorded 14.37 ton/fed. Other treatments, i.e. Boric acid gave 15.38 ton/fed and Blite stop 16.46 ton/fed. Spraying at 60, 90 and 120 days after planting achieved the highest increase where root yield recorded 18.59 ton/fed while spraying at 90 and 120 days gave the lowest increase where root yield recorded 16.41 ton/fed. With respect to locations, New Nubariya was the best where root yield recorded 18.21 ton/fed compared with Kafr El-Sheikh which correlates between disease severity (%) and root yield:-0.633 recorded 17.02 ton/fed. It is worth mentioning that the correlation between disease severity and root yield was medium negative which recorded (-0.633). Hussien (2010) and Hussein (2011) reported that the highest sugar beet root yield was recorded by application of potassium bicarbonate.

Data illustrated in Table (4) show that all control materials significantly increased sucrose (%) compared with the untreated control. Score treatment showed the highest increase where sucrose (%) recorded 17.23% followed by Bio ARC 16.75% then Salicylic acid 16.45% while Zink sulphate showed the lowest increase where sucrose (%) recorded 13.93%. Other treatments, *i.e*. Boric acid gave 14.56% and Blite stop 15.12%. Spraying at 60,90and 120 days after planting achieved the highest increase where sucrose (%) recorded 15.82% while spraying at 90 and 120 days gave the lowest increase where sucrose (%) recorded 14.79%. With respect to locations, New Nubariya was the best where sucrose (%) recorded 15.46% compared with Kafr El-Sheikh which recorded 15.33%. It is worth mentioning that the correlation between disease severity and sucrose (%) was high negative which recorded (-0.789).

Table (3): Effect of spraying the control materials on rootyield (ton/fed) of sugar beet under field conditions at two locations.

	New Nubariya				**Kafr El-Sheikh**				**Overall mean**			
	60-90	**90-120**	**60-90-120**	**Mean**	**60-90**	**90-120**	**60-90-120**	**Mean**	**60-90**	**90-120**	**60-90-120**	**Mean**
Boric acid	**15.87**	**15.03**	**16.37**	**15.76**	**14.97**	**14.27**	**15.77**	**15.00**	**15.42**	**14.65**	**16.07**	**15.38**
Zink sulphate	**14.88**	**14.44**	**16.09**	**15.13**	**12.77**	**12.85**	**15.19**	**13.60**	**13.82**	**13.64**	**15.64**	**14.37**
Calcium chelate	**17.89**	**17.40**	**19.34**	**18.21**	**16.98**	**15.90**	**17.85**	**16.91**	**17.44**	**16.65**	**18.60**	**17.56**
Potassium bicarbonate	**18.81**	**17.87**	**20.19**	**18.96**	**18.03**	**16.13**	**18.94**	**17.70**	**18.42**	**17.00**	**19.57**	**18.33**
Salicylic acid	**19.34**	**18.36**	**21.12**	**19.60**	**19.69**	**17.02**	**19.78**	**18.83**	**19.51**	**17.69**	**20.45**	**19.22**
Bio ARC	**22.17**	**19.88**	**22.84**	**21.63**	**22.48**	**17.38**	**20.94**	**20.27**	**22.33**	**18.63**	**21.89**	**20.95**
Blite stop	**16.55**	**16.71**	**17.02**	**16.76**	**16.46**	**15.16**	**16.83**	**16.15**	**16.51**	**15.93**	**16.92**	**16.46**
Score	**25.36**	**22.61**	**25.53**	**24.50**	**23.11**	**22.39**	**22.20**	**22.56**	**24.23**	**22.50**	**23.86**	**23.53**
Control (untreated)	**13.67**	**10.62**	**15.60**	**13.30**	**12.13**	**11.43**	**12.98**	**12.18**	**12.90**	**11.03**	**14.29**	**12.74**
Mean	**18.28**	**16.99**	**19.34**	**18.21**	**17.40**	**15.84**	**17.83**	**17.02**	**17.84**	**16.41**	**18.59**	

L.S.D at (0.5%):Location (L)=0.35 Spraying time (S)=0.43 Control materials (C)=0.74
(L x S)=n.s (L x C)= n.s (S x C)= 1.28 (L x S x C)= n.s

Hussienl (2010) and Hussein (2011) reported that the highest sugar beet sucrose percentage was recorded by application of potassium bicarbonate.

The data illustrated in Table (5) show that all control materials significantly increased the sugar yield compared with the untreated control. Score treatment showed the highest increase where sugar yield recorded 4.05 ton/fed followed by Bio-ARC

Table (4): Effect of spraying control materials on sucrose (%) of sugar beet grown under field conditions at two locations.

	Nubariya			Mean	Kafr-El-Sheikh			Mean	Overall mean			Mean
	60-90	90-120	60-90-120		60-90	90-120	60-90-120		60-90	90-120	60-90-120	
Boric acid	15.20	13.37	15.50	14.69	15.03	13.17	15.07	14.42	15.12	13.27	15.28	14.56
Zink sulphate	14.80	12.93	15.00	14.24	12.97	12.90	14.97	13.61	13.88	12.92	14.98	13.93
Calcium chelate	15.93	14.90	16.13	15.66	16.00	15.47	15.60	15.69	15.97	15.18	15.87	15.67
Potassium bicarbonate	16.10	15.57	16.73	16.13	16.57	15.50	16.13	16.07	16.33	15.53	16.43	16.10
Salicylic acid	16.53	15.87	16.93	16.44	16.83	16.10	16.43	16.46	16.68	15.98	16.68	16.45
Bio ARC	16.67	16.53	17.30	16.83	16.90	16.53	16.57	16.67	16.78	16.53	16.93	16.75
Blite stop	15.47	13.87	15.77	15.03	15.73	14.60	15.27	15.20	15.60	14.23	15.52	15.12
Score	17.07	17.07	17.68	17.27	17.27	17.43	16.87	17.19	17.17	17.25	17.27	17.23
Control (untreated)	13.13	12.33	13.07	12.84	12.27	12.17	13.70	12.71	12.70	12.25	13.38	12.78
Mean	15.66	14.71	16.01	15.46	15.51	14.87	15.62	15.33	15.58	14.79	15.82	

L.S.D at (0.5%):Location (L)=n.s Spraying time (S)=0.22 Control materials (C)=0.38
(L x S)= 0.31 (L x C)= n.s (S x C)= 0.65 (L x S x C)= n.s

Table (5): Effect of spraying some control materials on sugaryield (ton/fed) of sugar beet under field conditions at two locations.

	New Nubariya			Mean	Kafr El-Sheikh			Mean	Overall mean			Mean
	60-90	90-120	60-90-120		60-90	90-120	60-90-120		60-90	90-120	60-90-120	
Boric acid	2.37	1.98	2.47	2.27	2.28	1.91	2.44	2.21	2.32	1.94	2.45	2.24
Zink sulphate	1.93	1.86	2.41	2.07	1.89	1.66	2.27	1.94	1.91	1.76	2.34	2.00
Calcium chelate	2.87	2.69	3.02	2.86	2.70	2.37	2.89	2.65	2.78	2.53	2.95	2.75
Potassium bicarbonate	3.12	2.76	3.26	3.05	2.90	2.51	3.17	2.86	3.01	2.64	3.22	2.95
Salicylic acid	3.26	2.94	3.48	3.22	3.26	2.70	3.35	3.10	3.26	2.82	3.41	3.16
Bio ARC	3.76	3.27	3.79	3.60	3.75	2.87	3.62	3.41	3.75	3.07	3.71	3.51
Blite stop	2.60	2.44	2.60	2.55	2.54	2.10	2.65	2.43	2.57	2.27	2.62	2.49
Score	4.38	3.94	4.31	4.21	3.94	3.82	3.93	3.90	4.16	3.88	4.12	4.05
Control (untreated)	1.68	1.27	2.13	1.69	1.59	1.41	1.70	1.57	1.63	1.34	1.91	1.63
Mean	2.88	2.57	3.05	2.84	2.76	2.37	2.89	2.68	2.82	2.47	2.97	

L.S.D at (0.5%):Location(L)=0.06S Spraying time(S)=0.07 Control materials(C)=0.13
(L x S)= n.s (L x C)= n.s (S x C)= 0.22 (L x S x C)= n.s

3.51 ton/fed, then Salicylic acid 3.16 ton/fed while Zink sulphate gave the lowest increase where sugar yield recorded 2 ton/fed. Other treatments, *i.e.* Boric acid recorded 2.24 ton/fed and Blite stop 2.49 ton/fed. Spraying at 60,90 and 120 days after planting achieved the highest increase where sugar yield recorded 2.97 ton/fed while spraying at 90 and 120 days gave the lowest increase where sugar yield recorded 2.47 ton/fed. In respect to locations, New Nubariya showed the best, where sugar yield recorded 2.84 ton/fed compared with Kafr El-Sheikh which recorded 2.68 ton/fed. It is worth mentioning that the correlation between disease severity and sugar yield was high negative which recorded (-0.714). In the same respect, Kristek *et al*. (2007) reported that the highest value of sugar yield was obtained by inoculation sugar beet roots with *Pseudomonas fluorescens*. Also, Soudi and El- Guibali (2008) reported that application of Zn increased yield and quality of sugar beet.

The data illustrated in Table (6) show that the tested control materials significantly increased phenol contents compared with the untreated control. Salicylic acid treatment showed the highest increase where free phenols recorded 92.15 mg/g fresh weight, conjugated phenols recorded 102.81 mg/g fresh weight and total phenols recorded 194.96 mg/g fresh weight followed by Potassium bicarbonate where free phenols recorded 84.17 mg/g fresh weight, conjugated phenols recorded 99.57 mg/g fresh weight and total phenols recorded 183.74 mg/g fresh weight, then Calcium chelate where free phenols recorded 74.81 mg/g fresh weight, conjugated phenols recorded 96.38 mg/g fresh weight and total phenols recorded 171.19 mg/g fresh weight while zink sulphate caused the lowest increase where free phenols recorded 36.72 mg/g fresh weight, conjugated phenols recorded 80.65 mg/g fresh weight and total phenols recorded 117.38 mg/g fresh weight. Other treatments such as Boric acid gave free phenols 44.04 mg/g fresh weight, conjugated phenols recorded 87.63 mg/g fresh weight and total phenols131.67 mg/g fresh weight, and Blite stop where free phenols recorded 54.6 mg/g fresh weight, conjugated phenols 91.07 mg/g fresh weight and total phenols (145.68 mg/g fresh weight). In respect to locations, New Nubariya showed the highest values for conjugated phenols, being 92.79 mg/g fresh weight and total phenols 150.83 mg/g fresh weight compared to Kafr El-Sheikh where conjugated phenols recorded 85.72 mg/g fresh weight and total phenols recorded148.15 mg/g fresh weight while Kafr El-Sheikh was the best for free phenols, being 62.43 mg/g fresh weight compared with New Nubariya free phenols recorded 58.04 mg/g fresh weight. It is worth mentioning that the correlation between disease severity and free phenols was medium negative which recorded (-0.586), the correlation between disease severity and conjugated phenols was high negative which recorded (-0.714) and the correlation between disease severity and total phenols was high negative which recorded (-0.706). In the same respect, Hammerschmidt and Smith-Becker (1999) reported that Salicylic acid (SA) is a phenolic compound that affects a variety of biochemical and molecular events associated with induction of disease resistance. Salicylic acid has been shown to play an important role in expression of both local resistance controlled by major genes and systemic induced resistance developed after an initial pathogen attack. Also, Hussien (2010) and Hussein (2011) reported that all phenols free, conjugated and total were higher in plants treated by potassium bicarbonate. In regards to of boron action in reducing disease susceptibility, Brown *et al*. (2002)) showed its role in metabolism of phenolics or lignin.

The data illustrated in Table (7) show that the tested control materials significantly increased Polyphenoloxidase and peroxidase activity compared to the untreated control. Salicylic acid treatment caused the highest increase where polyphenoloxidase recorded 0.562 and peroxidase recorded 0.974 followed by Potassium bicarbonate where polyphenoloxidase recorded 0.510 and peroxidase recorded 0.843 while Bio ARC treatment caused the lowest increase where polyphenoloxidase recorded 0.331 and peroxidase recorded 0.615 followed by calcium chelate polyphenoloxidase recorded 0.435 and peroxidase recorded 0.763. With respect to locations, New Nubariya was the best where peroxidase recorded 0.785 compared with Kafr El-Sheikh where it recorded 0.765 while Kafr El-Sheikh was the best in polyphenoloxidase which recorded 0.443 compared with New Nubariya which recorded 0.440. It is worth mentioning that the correlation

Table (6): Effect of some control materials on phenol contents (free, conjugated and total phenols) (mg/g fresh weight) in sugar beet under field conditions at two locations.

	Nubariya			Kafr El-Sheikh			Overall mean		
	Free phenols	Conjugated phenols	Total phenols	Free phenols	Conjugated phenols	Total phenols	Free phenols	Conjugated phenols	Total phenols
Boric acid	43.64	88.29	131.93	44.44	86.97	131.41	44.04	87.63	131.67
Zink sulphate	36.06	78.42	114.48	37.38	82.89	120.27	36.72	80.65	117.38
Calcium chelate	72.23	101.09	173.32	77.39	91.68	169.07	74.81	96.38	171.19
Potassium bicarbonate	81.24	105.34	186.58	87.09	93.80	180.89	84.17	99.57	183.74
Salicylic acid	90.83	108.50	199.32	93.47	97.13	190.60	92.15	102.81	194.96
Bio ARC	62.75	100.92	163.67	69.07	86.28	155.35	65.91	93.60	159.51
Blite stop	50.18	95.58	145.76	59.02	86.57	145.59	54.60	91.07	145.68
Contro (untreated)	27.39	64.18	91.57	31.58	60.45	92.03	29.49	62.31	91.80
Mean	58.04	92.79	150.83	62.43	85.72	148.15			

L.S.D at (0.5%):

Locations (L) =	n.s	n.s	n.s
Cont.materials.(C) =	6.11	5.93	6.58
(L x C) =	n.s	n.s	n.s

Table (7): Effect of some control materials (chemicals, biocides) on polyphenoloxidase and peroxidase (optical density/minute /gram fresh weight) of sugar beet plants grown under natural infection with *Cercospora beticola* **under field conditions at two locations.**

	Nubariya		Kafr El-Sheikh		Overall mean	
	Polyphenol oxidase	Peroxidase	Polyphenol oxidase	Peroxidase	Polyphenol oxidase	Peroxidase
Calcium chelate	0.435	0.763	0.459	0.748	0.447	0.755
Potassium bicarbonate	0.510	0.843	0.510	0.818	0.510	0.830
Salicylic acid	0.562	0.974	0.560	0.948	0.561	0.961
Bio ARC	0.362	0.730	0.368	0.698	0.365	0.714
Control (untreared)	0.331	0.615	0.317	0.616	0.324	0.615
Mean	0.440	0.785	0.443	0.765		

L.S.D at (0.5%):

Locations (L) =	n.s	0.011
Cont.materials (C) =	0.039	0.024
(L x C) =	n.s	n.s

between disease severity and Polyphenoloxidase was medium negative which recorded (-0.554) and the correlation between disease severity and Peroxidase was medium negative which recorded (-0.574). These results are in agreement with the findings of Mahmoud *et al.* (2006) who found a significant increase in the total peroxidase activity in peanut plants treated with Salicylic acid; Daw *et al*. (2008) recorded an increase of peroxidase activity after rice foliar spray with 8 mM SA. Kamal *et al*. (2009) stated that onion plants treated by SA and inoculated with the pathogen exhibited higher peroxidase, polyphenoloxidase activities and phenolic content at all time intervals than inoculated water-treated plants. Thus, application of SA on onion plants led to significant reduction in disease severity compared to the control plants. Also, Ibrahim *et al.* (2013) found an increase of oxidative enzymes activity and content of phenol compounds after peanut leaf treated with Salicylic acid. Hammerschmidt and Smith-Becker (2000) reported that among the most commonly chemical elicitors, Salicylic acid has been shown to play an important role in expression of both local resistance, controlled by major genes, and systemic induced resistance developed after an initial pathogen attack. Thanh *et al*. (2017) reported that foliar sprays with Salicylic acid (SA) provided an increase production of O_2^-. The O_2^- not only has direct toxicity to pathogens, but it is also the central component of the plant defence signal transduction pathways. Also, they observed enhancement of polyphenoloxidase activity and production of plant phytoalexins.

In general, New Nubariya plants were the best, as they showed disease tolerance, root yield and sugar yield more than Kafr El-Sheikh plants. This may be due to different soil types in the two locations.

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تأثير بعض المركبات الكيمائية والمبيدات الحيوية علي مرض تبقع الأوراق *Cercospora* في بنجر السكر

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ملخص

يعد مرض تبقع الأوراق السركسبوري علي نباتات بنجر السكر المتسبب عن الفطر سركسبورا بيتيكولا أخطر أمراض بنجر السكر علي مستوي العالم. وقد إختبرت فعالية خمسة مركبات كيميائية هي حمض البوريك، كبريتات الزنك، مخلبيات الكالسيوم، بيكربونات البوتاسيوم وحمض السلسليك ومبيدان حيويان هما بيو- آرك وبليت ستوب و المبيد الفطري سكور ضد الفطر سركسبورا بيتيكولا المسبب المرض بالإضافة للمعاملة القياسية (الكنترول) في حقول موقعين مختلفين لزراعة البنجر

في مصر، هما مركز النوبارية الجديدة (محافظة البحيرة) ومركز كفر الشيخ (محافظة كفر الشيخ) تحت ظروف العدوي الطبيعية. وتم اختبار تأثير 3 برامج لرش النباتات. البرنامج الأول الرش مرتين (بعد 60 يوم وبعد 90 يوماً من الزراعة). البرنامج الثاني يشمل الرش مرتين (بعد 90 وبعد 120 يوماً من الزراعة). البرنامج الثالث يشمل الرش ثلاث مرات (بعد 60 وبعد 90 وبعد 120 يوماً من الزراعة). أظهرت جميع المركبات المختبرة كفاءة مختلفة الدرجة في نسبة شدة الإصابة و نسبة السكر ونسبة الاستخلاص ومحصول جذور البنجر ومحصول السكر في الفدان مقارنة بالمعاملة القياسية (الكنترول).

أعطي المبيد الفطري (سكور) أفضل النتائج مقارنة بكل المواد المختبرة ، سجل المبيد الحيوي بيو- آرك شدة إصابة 8.58 % يليه بليت – سوب (مبيد الحيوي مصري تجاري جديد) 12.27 %. كان حمض السلسليك افضل المستحثات الكيماوية المختبرة 9.36 % يليه بيكربونات البوتاسيوم 10.35 % ثم مخلبيات الكالسيوم 11.43 % ثم حمض البوريك 13.56% وأخيراً كبريتات الزنك 16.62 % في مقابل 23.40 % لمعاملة المقارنة. أعطي الرش عند 60 و 90 و 120 يوم بعد الزراعة أفضل النتائج في كل القياسات حيث سجل محصول السكر 2.97 طن للفدان بينما أعطي الرش عند 90 و 120 يوم أقل النتائج 2.97 طن للفدان.

كانت نباتات النوبارية الجديدة الأفضل في تحمل المرض و محصول الجذور و محصول السكر بينما لم تتأثر نسبة السكروز و الإستخلاص بالموقع. وجدنا زيادة في محتوي الأوراق من الفينولات الحرة والمرتبطة و الكلية كنتيجة للرش بالمركبات الكيماوية و المبيدات الحيوية ، وجدنا أيضا زيادة في النشاط الإنزيمي للبيروكسيديز و البولي فينول أكسيديز.

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