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Efficiency of Some Biocides as Safe Alternatives to Fungicides on Cercospora Leaf Spot Control, their Effect on Biochemical Constituent and Yield of Sugar Beet

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Cercospora leaf spot incited by *Cercospora beticola* Sacc. is a very detrimental fungal disease affecting sugar beet crop production in Egypt as well as sugar yield. The efficacy of four selected commercial biocides *i.e.,* Biobac, Bio-Arc, Bio-Zeid and Plant Guard, was investigated to control Cercospora leaf spot on sugar beet plants in comparison with the recommended fungicide Score as a check difenoconazole fungicide as well as untreated control under field conditions at Sakha Agricultural Research Station, Kafr El-Sheikh Governorate, Egypt. The biocides and fungicide were sprayed at different three spray regimes as two sprays, four sprays or six sprays with 15 days intervals between sprays for such treatment. All tested biocides and Score fungicide led to significant decrease in Cercospora leaf spot disease severity of the treated sugar beet plants in comparison with control treatment. Increasing spraying numbers of the tested biocides or Score fungicide from two times to six sprays per season caused increasing reduction of Cercospora leaf spot severity. Spraying Plant guard, Bio-Zeid and Biobac biocides six times achieved very comparable effectiveness to Score treatment at the same spray regime in the two growing seasons 2015/2016 and 2016/2017, whereas, Bio-Arc was the less effective biocide treatment particularly when sprayed two times per season. All tested biocides at different spraying regimes led to significant increase in values of phenolic compounds, total chlorophyll, (achieved higher quality traits at the end of the season), sucrose (%) and purity (%) and top, root as well as sugar yield quality of sugar beet were significantly increased. The impurities of produced sugar as sodium, potassium and α-amino nitrogen in the juice were significantly decreased in comparison with untreated control during the two growing seasons. Overall results clarified that spraying Score fungicide or Bio- Zeid, Plant guard and Biobac, respectively six times resulted the highest increase of quality traits as sucrose (%) and purity %) and top, root and sugar yields of sugar beet and significant decrease in total amino acid, juice impurities (Na, K and α-a N %), whereas Bio- Arc when applied two times recorded the least effective treatment close to the control during the two growing seasons 2015/2016 and 2016/2017. It was concluded that biocides, Bio-Zeid, Plant guard and Biobac could be sprayed six times/season at 15 days intervals as alternatives to fungicide applications to control Cercospora leaf spot of sugar beet and to produce higher yields of root and sugar with better quality.

Keywords: Alternatives, biocides, Cercospora leaf spot, fungicide and sugar beet.

Sugar beet (*Beta vulgaris* L.) is the second major sugar crop for sugar production in Egypt. Under field conditions, several pathogenic fungi attack growing sugar beet plants causing serious diseases. Cercospora leaf spot (CLS) of sugar beet caused by *Cercospora beticola* is the most destructive foliar disease of sugar beet (El-Mansoub *et al.*, 2010 and Skaracis *et al.*, 2010). Since the fungus damages the leaves, it adversely impacts the photosynthetic capacity of plants and reduces yield. The CLS disease also results in higher impurities in the juice which reduces sucrose extraction. Cercospora leaf spot infection induces changes in the biochemical constituent like amino acids, phenols and sugar which may affect quality and yield (Siddaramaiah and Hegde 1990). Photosynthetic pigments affect the utilization of light energy by plant leaves. The pathogen decreased total chlorophyll content in infected leaves and yield (Shree and Nataraj, 1993). Cercospora leaf spot caused a reduction in growth sugar yield up to 42% (Shane and Teng, 1992). The reduction of root yield and sugar content reached to 30 and 50%, respectively (Wolf *et al*., 1995 and Wolf and Verret, 2002). Since, there is no available resistant varieties for disease, consequently synthetic fungicides have been used with repeated applications in large scale during the last decade and caused harmful effects to environment and human health because of their high toxicity in agriculture led to great disturbance in biological balance and toxic substances in food chain, so biological control offers a logical alternative to synthetic fungicides for the control of different diseases (Jacobsen *et al.*, 2004; Jacobsen, 2010 and Galletti *et al.*, 2008). Repeated application of *Bacillus* spp. reduced CLS symptoms of sugar beet under field conditions due to elicitation of systemic resistance (Bargabus *et al.*, 2002). Different governorates such as Kafr El-Sheikh Governorate showed heavy infection of sugar beet with CLS which had a long bad history of disease incidence and yield losses (Gado, 2007). Several fungal isolates as *Trichoderma* spp. (Harman, 2000; Roberts *et al.*, 2005 and Porras *et al*., 2007), and bacterial isolates as *Bacillus* spp. (Kim *et al.*, 1997) are known for years as potential bio-control agents. They possess mechanisms that allow them to act as bio-control agents against pathogens through mycoparasitism by strain of fungus or bacterium directly attacks and feeds on other fungi (Harman, 2000). Similarly, *Trichoderma* spp. produce antibiotics or enzymes that inhibit the growth or reduce the ability of pathogens to infect plants (Simon *et al*., 1988; Roberts *et al*., 2005). Biocontrol approaches may help to develop an eco-friendly control strategy for managing plant disease (Bharathi *et al.* 2004 and Shahraki *et al*., 2008). Different bioagents *i.e.*, *Trichoderma harzianum*, *T. viride*, *Gliocladium virens*, *Bacillus subtilis* and *Pseudomonas fluorescens* with different doses were effective in reduction of disease incidence and showed highest increasing in the yield compared with control treatment (Srivastava, 2004; Patel and Jasrai, 2012; Ray and Swain, 2013 and Sharma, 2015). *Trichoderma* spp. degraded the cell wall of the pathogen due to the production of lytic enzymes such as chitinases, peroxidase, polyphrnoloxidase and glucan 1-3 β- glucosidases. *B. subtilis* produces a group of enzymes, which dissolve the cell wall of the pathogen, antibiotics such as bacterocin and subtilisin, volatile compounds and phytotoxic substances (Jacobsen *et al.*, 2004; Upadhyay and Mukhopadhyay, 1986, and Muthuvelayudham and Viruthagiri, 2006).

The aim of this study was to evaluate the efficacy of certain commercial bioagents recommended for other diseases on other crops to suppress the Cercospora leaf spot on sugar beet and to determine which application regime could be followed for disease management and high yield.

Materials and Methods

Field trial:

Experiment was carried out at Sakha Research Station, Kafr-El-Sheik Governorate, Egypt. (31-57/ N latitude and 30-57 E longitude) during growing seasons 2015/2016 and 2016/2017, where location is known to have a long history of heavy infection by Cercospora leaf spot disease (CLS) (Gado, 2007). Four commercial biological control formulations *i.e.*, Biobac 50% WP, Bio-Arc 6% WP, Bio-Zeid 25% WP and Plant Guard were tested to control CLS on sugar beet at concentrations of 200g, 250g, 250g and 50 ml/100 L water, respectively, in comparison with the recommended fungicide, Score at 50 ml/100 L water (Table 1). Three application regimes were investigated for such biological or standard chemical control treatments *i.e.*, 2 sprays, 4 sprays and 6 sprays of each with 15 days intervals between sprays in such regime. All treatments were adopted as foliar applications. Untreated plots (spraying with water) served as control.

Table 1. Active ingredients of the four tested biocides and fungicide score

Tested product	Active ingredient	Type	Dose
Biobac 50% WP	*Bacillus subtilis* ($30x10^6$ cell/g)	Biocide	200g/100L water
Bio-ARC 6% WP	*Bacillus megaterium* 6% (w/w)	Biocide	250g/100L water
Bio-Zeid 25% WP	*Trichoderma album* 2.5 % (w/w)	Biocide	250g/100L water
Plant Guard L	*T. harzianum* ($30x10^6$ spore/ml)	Biocide	250ml/100L water
Score 25% EC	Difenconazole	Fungicide	50ml/100L water

The experimental design was split plot with three replications. The main plot was the commercial biocides and the sub plot was the number of sprays. Foliar spraying was started at the first week of January using a hand operated knapsack sprayer. Super film was mixed before spraying with each treatment at the rate of 50 ml/100 L water as a surfactant and sticker material. Three plots were used as replicates for each treatment, each comprised of 5 rows. The seeds of sugar beet cv. Kawamera were sown in plots area 14 m^2 (1/300 fed.). Irrigation and fertilization were practiced as recommended by Sugar Crops Research Institute. The P fertilizer (super phosphate, 15% P_2O_5) was added at the rate of 30 kg P_2O_5/fed before sowing. The N fertilizer (Urea, 46% N) was applied in two equal doses 45 and 75 days after sowing at the rate 60 kg/ N, while K (potassium sulfate) fertilizer at the rate of 48 kg K_2O (48% K_2O) was added with the first dose of N fertilizer. Sugar beet seeds were planted on 15 and 20 October in the two growing seasons 2015/2016 and 2016/2017, respectively.

Assessment of Cercospora leaf spot disease severity:

By the end of the growing season Cercospora leaf spot was counted on 50 plants and disease severity was calculated according to the scale of Shane and Teng (1992). The scale ranged from 0-10 categories where: O; no visual infection; (1) 1-5 spots/leaf (0.1% severity), (2) 6-12 spots (0.35 % severity); (3) 13-25 spots/leaf (0.75% severity); (4) 26-50 spots/leaf (1.5% severity); (5) 51-75 spots/leaf (2.5 % severity); (6) At higher disease incidence, the average affected area per leaf was estimated from standard area diagrams, and categories 6 through 10 represented 3, 6, 12, 25, and 50% disease severity, respectively. Disease severity of each replicate was estimated by dividing the summation of disease severity of each plant contained in the replicate plot by 50. Efficiency % of the tested treatments of biocides and Score fungicide was calculated relative to the untreated control according to the following formula:

$$\% \text{ Efficacy} = \frac{\% \text{ Disease severity in untreated control} - \% \text{ Disease severity in each treatment}}{\% \text{ Disease severity in untreated control}}$$

Assessment of plant chemical contents in sugar beet as affected by Cercospora leaf spot disease:

After 140 days from sowing, the following traits were determined:

1- Total and free phenols compounds were determined in the treated plants with tested biocides using UV/ Vis. Spectrophotometer, Jenway England at wave length 750 nm as described by Singleton *et al.* (1999) using Folin and Ciocalteau phenol reagent. The phenolic compounds contents were expressed at g/100 g fresh weight.

2-Total amino acids were determined by using an amino acids analyzer Beckcman system 7300. The amino acid contents were expressed at g/100 g fresh weight.

3- Total chlorophyll content of leaves was measured by using chlorophyll meter Model (SPAD-502). Chlorophyll contents were expressed at as optical density (OD).

Yield of sugar beet plant tops, roots and sugar (Ton/fed.):

At harvest, yield of such tops and roots (ton fed^{-1}) was determined. Sugar yield (ton fed^{-1}) was calculated by multiplying root yield x sucrose percentage. Quality parameters included Sucrose % and impurities content, (K, Na and Alpha-amino N %) were determined in Delta Sugar Company limited laboratories at El –Hamoul, Kafer El-Sheikh Governorate according to the method described by McGinnus (1971). Juice purity was calculated according the equation described by Devillers (1988).

$$\text{Purity } \% = 99.36 - 14.27(\text{Na} + \text{K} + \alpha\text{-N})/\text{sucrose}\%.$$

Statistical analysis:

All the obtained data during two successive seasons were subjected to statistical analysis and compared according to the least significant difference (L.S.D.) at 5% according to Snedecor and Cochran (1981).

Results and Discussion

Effect of some biocides and Score fungicide at three spraying regimes on Cercospora leaf spot severity (%) on sugar beet plants under field conditions during 2015/2016 and 2016/2017 growing seasons:

Data presented in Table 2 show that all tested biocides and Score fungicide led to significant decrease in Cercospora leaf spot disease severity of sugar beet treated plants in comparison with control treatment. In general, Score fungicide was the most significant effective treatment for reducing Cercospora leaf spot followed by Plant guard and Bio-Zeid which were the most effective biocides followed by Biobac, while, Bio-Arc was the least effective one in this respect during the two successive seasons 2015/2016 and 2016/2017. On the other hand, increasing number of spraying biocides or Score fungicide from two times to six times resulted significant reduction of Cercospora leaf spot severity. Spraying biocides six times recorded the best spray regime followed by four sprays, then two sprays regime during the two seasons. Effectiveness of Score fungicide to control Cercospora leaf spot on sugar beet was also clarified by Gado (2007).

It is worth to put into consideration that Windels *et al.* (1998) reported that while fewer fungicide applications were made when cultivars that considered least susceptible to Cercospora leaf spot are planted, they usually yield lower amounts of recoverable sucrose compared with more susceptible cultivars. Consequently, cultivars with moderate susceptibility to Cercospora leaf spot were preferred, because even with additional fungicide applications, they are more profitable than the less susceptible cultivars. This reporting highlighted the susceptibility of major sugar beet cultivars to Cercospora leaf spot disease and the need to adopt control measures such as fungicides is important.

Table 2. Effect of some biocides and Score fungicide at three spraying regimes on leaf spot (CLS) disease severity (%) on sugar beet plants grown under field conditions during 2015/16 and 2016/17 growing seasons

Biocide	Spray regime	2015/2016		2016/2017	
		CLS severity (%)	*Efficiency (%)	CLS severity (%)	Efficiency (%)
Biobac	Two times	12.27	60.75	13.38	60.81
	Four times	10.56	66.22	11.75	65.10
	Six times	8.48	72.87	9.58	71.55
	Mean	10.44	66.61	11.57	65.82
Bio – Arc	Two times	13.61	56.46	14.48	56.99
	Four times	11.40	63.53	12.36	63.29
	Six times	9.66	69.10	10.54	68.70
	Mean	11.56	63.03	12.46	62.99
Bio- Zeid	Two times	11.87	62.03	12.90	61.69
	Four times	9.52	69.55	10.40	69.11
	Six times	7.87	74.82	8.54	74.64
	Mean	9.75	68.80	10.61	68.48
Plant guard	Two times	10.53	66.32	12.68	62.34
	Four times	8.71	71.14	9.49	71.82
	Six times	6.69	78.60	7.46	77.84
	Mean	8.64	72.02	9.88	70.67
Score (check fungicide)	Two times	5.16	83.49	5.83	82.69
	Four times	4.72	84.90	5.09	84.88
	Six times	3.92	87.46	4.12	87.76
	Mean	4.60	85.28	5.01	85.11
Control	-	31.26	-	33.67	-
Mean of spray regime					
	Two times	14.12	-	15.49	-
	Four times	12.70	-	13.79	-
	Six times	11.31	-	12.32	-
L.S.D at 5% for:					
Biocides (A)		0.39	-	0.56	-
Sprays regime (B)		0.16	-	0.22	-
A x B		NS	-	NS	-

*Efficiency (%): Effectiveness of such treatment to control Cercospora leaf spot relating to the infection of the untreated control.

Regard to efficiency of different treatments for controlling Cercospora leaf spot (CLS) relative to the check control is shown in Table 2, maximum efficiency for reduction of CLS was recorded when Score fungicide and the four tested biocides applied six times during the two growing seasons. Score fungicide treated six times was the most superior treatment in the two seasons 2015/2016 and 2016/2017 (87.46 and 87.76%, respectively), whereas the same spray regime of Plant guard (78.60 and

77.84%, respectively) and Bio-Zeid (74.82 and 74.64%, respectively), came next followed by Biobac, (72.87 and 71.55%%, respectively), whereas, Bio-Arc was the least effective biocide during the two seasons (69.10 and 68.70%, respectively). These findings may be attributed to *Trichoderma* spp. and/or *Bacillus subtilis* inhibitory effect on the fungal growth by competition (for space and nutrients), parasitism (deriving nutrients from the host) and antibiosis (production of inhibitory metabolites or antibiotics) as demonstrated by (Patel and Jasrai, 2012, and Sharma 2015). Similar results were obtained by several authors. In this respect Stefania *et al.* (2008) evaluated several *Trichoderma* isolates as possible biocontrol agents against *Cercospora beticola* in sugar beet and found significant reduction in *C. beticola* sporulation per unit of necrotic area, compared to the untreated control. Metwally *et al*. (2010) revealed that Bio Arc and Bio Zeid led to maximum reduction of chocolate spot disease severity. Khalifa *et al.* (2013) reported that all bioagents such as Bio Zeid and Bio Arc applied as dipping and soil drenching six times and the recommended treatment of Folicur fungicide were the most superior treatments for controlling white rot disease of onion. Mahmoud *et al.* (2013) found that Bio Zeid and Bio Arc recorded the highest efficacy percentage for decreasing downy mildew and purple blotch of onion. Harmful side effects of fungicides were reported on humans and environment (Garcia, 1993). The more effectiveness of the 6 sprays-regime of bioagents than the less sprays regimes could be attributed to the prevalent of favour environmental conditions for CLS infection and development along growing season as well as the susceptibility of sugar beet plants starting 3 months after cultivation to Cercospora infection until the end of growing season (Windels *et al.*, 1998). Thus, the development of nontoxic alternatives to fungicides such as biocides would be useful in reducing these undesirable effects. Biological control through the use of antagonistic microorganisms is a potential non chemical means of controlling plant disease by reducing inoculum levels of pathogens. Such management would help or prevent the pollution and also health hazards (Kumar, 2007).

Effect of some biocides and Score fungicide at three spray regimes on chemical constituents in sugar beet plant and yield, and quality of industrial sugar beet production:

1-Chemical Constitutions in sugar beet plant and yield:

A. Phenolic compounds content in sugar beet leaves (g/100 g fresh weight):

Results in Table 3 show that all different biocides treatments and/or Score fungicide increased amounts of free, conjugated and total phenols in comparison with untreated control at the two growing seasons. Score fungicide treatments gave the maximum amounts of free, conjugated and total phenols in most cases followed by Plant guard and Bio-Zeid, while, Biobac and Bio-Arc showed the least phenolic compounds. Also, increasing spraying numbers of the tested biocides and Score fungicide caused gradual increase in phenolic compounds in fresh sugar beet plants. The amounts of phenolic compounds were higher in plants treated six times with the tested biocides and/or Score fungicide followed by four and two spray times compared to untreated control. Also it could be noticed that the maximum total and free phenols were achieved by spraying biocides and Score fungicide six times followed by Plant guard, Bio-Zeid and Biobac treatments, respectively with few exceptions at the two growing seasons 2015/2016 and 2016/2017. Meantime, the lowest values of total and free phenols were recorded by Bio- Arc when applied two times only. This result may be due to reduction in disease severity (%) occurred after the 3rd spray and also the role of phenolic compound 3-hydroxytriamin present in sugar beet leaves related to resistance to *C. beticola* (Matern and Kneusal, 1988).

B. Total chlorophyll (OD) and Total amino acids content (g /100 g fresh weight) in sugar beet leaves:

Data presented in Table 4 show that the four tested biocides and/or Score fungicide treatments increased total chlorophyll in leaves of sugar beet, while caused reduction of total amino acids in sugar beet roots in the two growing seasons in comparison with untreated control. The highest total chlorophyll content was achieved when plants were sprayed six times with Score fungicide followed by those sprayed with Plant Guard, Bio-Zied and Biobac. Meanwhile, the least effective treatment was Bio- Arc sprayed only twice during 2015/16 and 2016/17 growing seasons, in comparison with control treatment. Increasing number of sprays gradually increased total chlorophyll in leaves of sugar beet plant. This may be due to reduction of disease severity (%) and reducing the loss of photosynthetic leaf area as well as reducing the toxicity from toxins produced by the Cercospora fungus sush as Cercosporin which affect plant vitality and photosynthesis process (Scholes and Rolfe, 2009 and Gary *et al*., 2011). Also, Levall and Bornman (2000) studies showed a decrease of photosynthetic efficiency of young sugar beets due to Cercospora infection on sugar beet leaves. At 16 day after artificial inoculation, leaves that had 3% to 6% infected area referring to disease severity showed significant reduction in chlorophyll fluorescence. Infection of sugar beet with *Cercospora beticola* affects chlorophyll content even before inciting marked spots, where pre-symptomatic detection of the necrotrophic fungal pathogen, *C. beticola* in sugar beet leaves was possible by imaging raw chlorophyll fluorescence (Chaerle *et al.*, 2007).

C. Amino acids contents in sugar beet plant leaves:

The amino acids contents in sugar beet leaves were reduced when sugar beet plants were sprayed with such biocides or Score fungicide (Table 4). The highest amount of amino acids was determined in the untreated control plants in both growing seasons. The lowest amount of amino acids was determined in sugar beet plants sprayed with Score, particularly when sprayed six times. Comparison among tested bioagents revealed that Bio Arc resulted in the highest level of amino acid content. Generally, it was observed that higher CLS disease severity was associated with higher amounts of amino acids contents. It could be concluded that Cercospora leaf spot infections affect the biochemical constituents in sugar beet plants. Also it was noticed that increasing sprays number of the tested biocides exhibited reduction in total amino acid. Spraying six times with such tested treatments gave the lowest amounts of total amino acids in the two seasons tested. This may be attributed to increasing numbers of spray by biocides which caused reduction in disease severity (%) and consequently less content of amino acids which in general enhance susceptibility to fungal diseases.

Table 3. Effect of some biocides and Score fungicide at three spray regimes on phenolic compounds (g/100 g fresh weight) in sugar beet plants grown under field condition during 2015/2016 and 2016/2017 growing seasons

Biocide	Spray regime	2015/2016			2016/2017		
		Total Phenols	Conjug. Phenols	Free phenols	Total Phenols	Conjug. Phenols	Free phenols
Biobac	Two times	5.73	0.53	5.20	5.35	0.52	4.83
	Four times	6.25	0.59	5.66	6.27	0.53	5.74
	Six times	6.64	0.62	6.02	6.78	0.63	6.15
	Mean	6.21	0.58	5.63	6.13	0.56	5.57
Bio- ARC	Two times	5.61	0.51	5.10	5.29	0.56	4.73
	Four times	6.12	0.58	5.54	5.89	0.53	5.36
	Six times	6.53	0.61	5.92	6.47	0.59	5.88
	Mean	6.09	0.57	5.52	5.88	0.56	5.32
Bio-Zeid	Two times	5.82	0.59	5.23	5.86	0.57	5.29
	Four times	6.32	0.60	5.72	6.36	0.55	5.81
	Six times	6.71	0.64	6.07	6.79	0.67	6.12
	Mean	6.28	0.61	5.67	6.34	0.60	5.74
Plant guard	Two times	5.90	0.57	5.33	5.62	0.51	5.11
	Four times	6.19	0.62	5.57	6.18	0.55	5.63
	Six times	6.87	0.71	6.16	6.89	0.68	6.21
	Mean	6.32	0.63	5.69	6.23	0.58	5.65
Score (check fungicide)	Two times	6.36	0.63	5.73	6.25	0.58	5.67
	Four times	6.68	0.69	5.99	6.47	0.64	5.83
	Six times	7.78	0.83	6.95	7.61	0.79	6.82
	Mean	6.94	0.72	6.22	6.78	0.67	6.11
Control	-	4.70	0.49	4.21	4.62	0.43	4.19
Mean of spray regime for:							
	Two times	5.69	0.55	5.13	5.50	0.53	4.97
	Four times	6.04	0.60	5.45	5.97	0.54	5.43
	Six times	6.54	0.65	5.89	6.53	0.63	5.90
L.S.D at 5% for:							
Biocides (A)		0.19	0.06	0.15	0.17	0.07	0.13
Spray regime (B)		0.11	0.02	0.09	0.10	0.03	0.08
A x B		0.31	NS	0.21	0.28	NS	NS

Table 4. Effect of some biocides and Score fungicide at three spray regimes on total chlorophyll optical density (OD) in sugar beet leaves and total amino acids (g/100 g fresh weight) in sugar beet root grown under field conditions during 2015/2016 and 2016/2017 growing seasons

Biocide	Spray regime	Total chlorophyll optical density (OD)		Total amino acids (g/100 g fresh weight)	
		2015/2016	2016/2017	2015/2016	2016/2017
Biobac	Two times	69.40	65.45	1.87	1.82
	Four times	70.44	66.49	1.75	1.65
	Six times	71.58	67.11	1.69	1.58
	Mean	70.47	66.35	1.77	1.68
Bio- ARC	Two times	65.43	62.44	1.90	1.94
	Four times	66.50	63.47	1.82	1.85
	Six times	68.18	64.17	1.71	1.69
	Mean	66.70	63.36	1.81	1.83
Bio-Zeid	Two times	72.36	68.39	1.86	1.80
	Four times	73.48	69.43	1.73	1.67
	Six times	74.78	70.27	1.65	1.61
	Mean	73.54	69.36	1.75	1.69
Plant guard	Two times	75.60	71.63	1.83	1.75
	Four times	76.30	72.51	1.69	1.63
	Six times	77.72	73.43	1.61	1.59
	Mean	76.54	72.52	1.71	1.66
Score (check fungicide)	Two times	76.16	73.22	1.72	1.70
	Four times	78.82	75.39	1.68	1.61
	Six times	79.31	77.11	1.59	1.55
	Mean	78.10	75.24	1.66	1.62
Control	-	64.19	61.81	1.98	2.02
Mean of spray regime for:					
	Two times	70.52	67.16	1.86	1.84
	Four times	71.62	68.18	1.78	1.74
	Six times	72.63	68.98	1.71	1.67
L.S.D at 5% for:					
Biocides (A)		0.35	0.29	0.09	0.06
Spray regimes (B)		0.13	0.18	0.04	0.02
A x B		NS	NS	0.15	0.11

The quantitative increase of certain amino acids in the infected tissues may be due to their synthesis in the host cells, the biodegradation of industrial sugar beet production and sugar yield, or to the contribution of the pathogen, the hyphae of the fungus absorbing and retaining a part of the amino acids for the synthesis of its own proteins (Rosu and Mititiuc 2000). Similar trend was observed by Smith and Martin (1978) who indicated that infection by Cercospora beticola increased amino nitrogen and total nitrogen of sugar beet .

2 -Quality of industrial sugar beet production:

A. On Juice impurities (Na, K and α-amino N %):

Data shown in Table 5 reveal that all tested treatments significantly reduced juice impurities (Na, K and α-amino N %) in comparison with untreated control. Score fungicide treatments recorded the highest decrease of juice impurities (Na, K and α-amino N %) followed by Bio- Zeid, Plant guard and Biobac, meanwhile the least effective treatment was Bio- Arc. Increasing number of sprays with biocides or Score fungicide gradually decreased juice impurities (Na, K and α-amino N %). The most effective treatments that reduced juice impurities were Score fungicide and the tested biocides sprayed six times during the two successive seasons 2015/2016 and 2016/2017.

This may be due to the reduction of disease severity which reflected on decreasing juice impurities, (Na, K and α-amino N %). Martin *et al.* (2001) demonstrated that all the soluble extract components that were not sucrose were considered "impurities." Sodium, potassium, amino N, and betaine were among the most melassigenic compounds in aqueous sugar beet extracts that were not removed appreciably in processing for sucrose recovery. Typically, sodium and potassium salts, amino N compounds, and betaine together represented about 80% of total non-sugars. Shane and Teng (1992) attributed the increase of impurities including sodium, potassium and amino nitrogen to the reduced root size associated with CLS disease which enhanced production of impurities.

B. Sucrose % and Juice purities:

Results illustrated in Table 6 show that all tested treatments exhibited significant increase in sucrose and purity percentage compared to untreated control. Spraying Score fungicide or Bio- Zeid, Plant guard and Biobac six times gave the highest increase in sucrose and purity percentages, whereas Bio- Arc was the least effective treatment when applied two times compared to the untreated control during the two growing seasons 2015/2016 and 2016/2017.

Table 5. Effect of some biocides and Score fungicide at three spray regimes on juice impurities, field experiment during 2015 / 2016 and 2016 / 2017 growing seasons

Biocide	Sprays regime	2015/2016			2016/2017		
		K %)	Na (%)	α-amino N (%)	K (%)	Na (%)	α-amino N (%)
Biobac	Two times	4.59	2.73	1.94	4.77	2.95	2.12
	Four times	4.48	2.63	1.84	4.55	2.83	1.91
	Six times	4.33	2.53	1.74	4.51	2.74	1.85
	Mean	4.47	2.63	1.84	4.61	2.84	1.96
Bio- ARC	Two times	4.80	2.83	2.13	4.93	2.97	2.26
	Four times	4.56	2.73	2.00	4.72	2.91	2.16
	Six times	4.47	2.65	1.95	4.61	2.79	2.09
	Mean	4.61	2.74	2.03	4.75	2.89	2.17
Bio-Zeid	Two times	4.33	2.51	1.74	4.55	2.73	1.84
	Four times	4.25	2.37	1.64	4.39	2.61	1.77
	Six times	4.13	2.30	1.50	4.18	2.52	1.54
	Mean	4.24	2.39	1.63	4.37	2.62	1.72
Plant guard	Two times	4.50	2.61	1.86	4.61	2.83	1.95
	Four times	4.42	2.54	1.74	4.44	2.70	1.86
	Six times	4.29	2.43	1.74	4.38	2.72	1.72
	Mean	4.40	2.53	1.78	4.48	2.75	1.84
Score (check fungicide)	Two times	2.29	1.84	1.75	2.36	1.93	1.82
	Four times	2.17	1.15	1.62	2.24	1.54	1.73
	Six times	1.95	1.02	1.45	2.06	1.19	1.38
	Mean	2.14	1.34	1.61	2.22	1.55	1.64
Control	-	5.47	2.83	2.55	5.54	3.01	2.35
Mean of spray regime for:							
	Two times	4.33	2.56	2.00	4.46	2.74	2.06
	Four times	4.23	2.38	1.90	4.31	2.60	1.96
	Six times	4.11	2.29	1.82	4.21	2.50	1.82
L.S.D at 5% for							
Biocides (A)		0.11	0.09	0.07	0.13	0.12	0.08
Sprays regimes (B)		0.06	0.05	0.03	0.07	0.05	0.05
A x B		0.23	NS	NS	NS	0.21	0.18

Table 6. Effect of some biocides and Score fungicide at three spray regimes on sucrose % and juice purities, field experiment during 2015/2016 and 2016/2017 growing seasons

Biocide	Spray regime	2015/2016		2016/2017	
		Sucrose %	Purities %	Sucrose %	Purities %
Biobac	Two times	16.32	91.25	16.22	90.70
	Four times	16.59	91.66	16.34	91.25
	Six times	16.85	92.08	16.58	91.53
	Mean	16.59	91.66	16.38	91.16
Bio - Arc	Two times	16.16	90.74	16.11	90.37
	Four times	16.47	91.31	16.23	90.76
	Six times	16.73	91.62	16.53	91.17
	Mean	16.45	91.22	16.29	90.77
Bio - Zeid	Two times	16.84	92.09	16.74	91.58
	Four times	17.21	92.51	16.82	91.92
	Six times	17.61	92.93	16.93	92.41
	Mean	17.22	92.51	16.83	91.97
Plant guard	Two times	16.69	91.69	16.64	91.31
	Four times	16.93	92.02	16.74	91.69
	Six times	17.03	92.38	16.83	91.87
	Mean	16.88	92.03	16.74	91.62
Score (check fungicide)	Two times	17.39	92.26	17.27	92.15
	Four times	17.51	92.83	17.44	92.58
	Six times	17.92	93.01	17.63	92.92
	Mean	17.39	92.26	17.27	92.15
Control	-	16.14	89.77	15.95	89.61
Mean of spray regime for:					
	Two times	16.59	91.30	16.49	90.95
	Four times	16.81	91.68	16.59	91.30
	Six times	17.05	91.97	16.74	91.59
L.S.D at 5% for:					
Biocides (A)		0.32	0.17	0.20	0.15
Spray regimes (B)		0.21	0.11	0.13	0.08
A x B		NS	NS	0.67	0.46

Schmittgen (2015) reported that infection of sugar beet with Cercospora leaf spot caused yield loss by reducing photosynthesis within the infected leaves. It was reported also that CLS was widely known to reduce the sugar content of sugar beet. CLS induced morphological changes of taproots, such as volume, cambial ring thickness, ring number and the respective growth rates, which could contribute to disease effects of the canopy affecting the root system clarifying the interrelation of taproot traits with the percentage of sucrose. Similar finding was reported by Stevens (2017) who referred to effectiveness of CLS on sugar yield, and consequently adopting control measures was so recommended. Rossi *et al.* (2000) attributed the effect of CLS disease on yield component not only to the reduction of photosynthetic activity of leaf area which occur at low disease pressure, but also to reduced photosynthesis and stimulated vegetative re-growth at the expense of root sugar reserve under severe foliage loss at late season. Consequence, potential sugar yield or recoverable sugar of the sugar beet crop could be significantly reduced by loss of both root weight and sucrose content. They recommended suppression of disease development to save yield reduction and increase yield components.

3. Root, top and sugar yields:

According to the data shown in Table 7 all tested treatments of Score fungicide and the four tested biocides exhibited significant increase in top, root and sugar yields. Also, increasing spraying numbers of the tested biocides and Score fungicide caused increase in yield of top, root and sugar. The most superior treatments increased the top, root and sugar yields were spraying Score fungicide and Bio- Zeid, Plant guard and Biobac, respectively when applied six times compared to the other treatments. Gouda and El-Naggar (2014) demonstrated the impact of CLS disease on sugar yield and also tested the impact of two Sterol demethylation inhibitors fungicides, tetraconazol and difenoconazole + propiconazole and one multi-site activity fungicide, benalaxyl + copper oxichloride, to control CLS disease and consequently increased sugar beet yield components including root weight (Kg/10 roots), sucrose (%) and gross sucrose (%). It could be noticed from Table (7) that all tested treatments recorded high values of quality traits (sucrose and purity percentages) and top, root and sugar yields of sugar beet and lowest juice impurities (Na, K and α-a N %) as compared with the untreated control under three spraying regimes in the two seasons. This finding may be due to the reduction of disease severity which reflected on root yield and sugar content, and decrease of impurities, *i.e.* sodium, potassium and alpha amino- N contents. Cioni *et al.* (2004) clarified the losses in sugar by infection of sugar beet by Cercospora leaf spot disease, which could be diminished by cultivating resistant varieties or by chemical control with fungicides or using other control treatments. Stefania *et al.* (2008) indicated that *Trichoderma* is able to reduce disease incidence by *Cercospora beticola* and to increase root and sugar yields comparing to the untreated control. It could be concluded from this study that biocides *i.e.*, Bio- Zeid and Plant guard might be useful to be used as alternatives to fungicide treatments when applied six times for disease management of Cercospora leaf spot disease of sugar beet and for increasing root and sugar yield.

Table 7. Effect of some biocides and Score fungicide at three spray regimes on root, top and sugar yields, field experiment during 2015/2016 and 2016/2017 growing seasons

Biocide	Spray regime	2015/2016			2016/2017		
		Root yield (ton fed^{-1})	Top yield (ton fed^{-1})	Sugar yield (ton fed^{-1})	Root yield (ton fed^{-1})	Top yield (ton fed^{-1})	Sugar yield (ton fed^{-1})
Biobac	Two times	21.81	6.81	3.56	21.88	7.84	3.55
	Four times	21.85	6.85	3.63	22.05	7.88	3.60
	Six times	21.94	6.94	3.70	22.41	7.94	3.72
	Mean	21.87	6.87	3.63	22.11	7.89	3.62
Bio-ARC	Two times	21.73	6.73	3.51	20.65	6.65	3.33
	Four times	21.80	6.80	3.59	21.07	7.06	3.42
	Six times	21.84	6.84	3.66	21.58	7.58	3.57
	Mean	21.79	6.79	3.59	21.10	7.10	3.44
Bio-Zeid	Two times	23.26	7.26	3.92	22.88	8.03	3.83
	Four times	23.46	7.46	4.04	23.08	8.13	3.88
	Six times	23.61	7.61	4.16	23.42	8.25	3.97
	Mean	23.44	7.44	4.04	23.13	8.14	3.89
Plant guard	Two times	22.10	7.08	3.69	21.93	7.97	3.65
	Four times	22.42	7.15	3.79	22.24	8.09	3.72
	Six times	22.93	7.22	3.91	22.69	8.19	3.81
	Mean	22.48	7.15	3.80	22.29	8.08	3.73
Score (fungicide)	Two times	26.73	8.75	4.82	25.19	8.78	4.52
	Four times	27.15	9.26	5.17	26.75	9.25	4.67
	Six times	28.52	10.12	5.86	28.32	9.91	5.11
	Mean	27.47	9.38	5.28	26.75	9.31	4.77
Control	-	20.21	8.01	3.26	19.70	8.74	3.14
Mean of spray regime for:							
	Two times	22.64	7.44	3.79	22.04	8.00	3.67
	Four times	22.82	7.59	3.91	22.48	8.19	3.74
	Six times	23.18	7.79	4.09	23.02	8.44	3.89
L.S.D at 5% for:							
Biocides (A)		0.11	0.09	0.07	0.08	0.07	0.08
Spray regimes (B)		0.06	0.05	0.03	0.03	0.02	0.04
A x B		NS	0.16	0.12	0.13	0.12	0.11

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كفاءة بعض المبيدات الحيوية كبدائل آمنة للمبيدات على مقاومة تبقع الأوراق السركسبوري وبعض المكونات الكيميائية والمحصولية لبنجر السكر

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يُعتبر مرض تبقع الاوراق السيركسبوري المتسبب عن الفطر *Cercospora beticola* من أهم الأمراض الفطرية المؤثرة على إنتاج محصول بنجر السكر ومحصول السكر في مصر. تمت دراسة فعالية أربع مبيدات حيوية تجارية هم بايوباك، بايو أرك، بايو زيد و بلانت جارد لمقاومة مرض تبقع الاوراق السيركسبوري في نباتات بنجر السكر بالمقارنة بالمبيد الموصى به (سكور) ومعاملة المقارنة غير المعاملة تحت ظروف الحقل في محطة البحوث الزراعية بسخا محافظة كفر الشيخ- مصر في موسمين متتالين ٢٠١٦/٢٠١٥ و٢٠١٧/٢٠١٦. استخدمت المبيدات الحيوية المختبرة والمبيد الفطري الموصى به رشا بثلاث نظم رش هى ٢، ٤ ، ٦ رشات كل ١٥ يوم بين الرشة والأخري لكل معاملة. أدى استخدام جميع المعاملات المختبرة إلى انخفاض متزايد في شدة الإصابة بمرض تبقع الاوراق السركسبورى على بنجر السكر بالمقارنة بمعاملة المقارنة. زادت فعالية المبيدات الحيوية المختبرة والمبيد الفطري سكور بزيادة عدد الرشات من رشتين إلى ست رشات. أظهر الرش بالمبيدات الحيوية بلانت جارد و بيو زيد وبيو باك على الترتيب فعالية مقاربة جدا لفعالية المبيد الفطري سكور في خفض الإصابة بمرض تبقع الأوراق السكرسبوري بنفس نظام الرش (ستة رشات) في كلا الموسمين المختبرين ٢٠١٦/٢٠١٥ و ٢٠١٧/٢٠١٦. بينما كان المبيد الحيوي بيو أرك هو الأقل فعالية في هذا الصدد خاصة الرش بعدد رشتين في كل موسم. أظهرت جميع المبيدات الحيوية المختبرة و المبيد الفطري سكور تحت أنظمة الرش المختلفة إلى زيادة معنوية في قيم المركبات الفينوليـــة، والكلوروفيل الكلي (بلغت أعلى صفات الجودة في نهاية الموسم) و نسبة السكروز و النقاوة وحاصل العرش والجذور والسكر و أيضا جودة بنجر السكر. انخفضت معنويا الشوائب المقدرة فى عصير بنجر السكر كالصوديوم والبوتاسيوم وحمض الفا أمينو أثناء موسمي النمو. اتضح عموما من الدراسة أن الرش ست مرات بكل من المبيد الفطري سكور والمبيدات الحيوية البيو زيد و البلانت جارد والبيوباك علي الترتيب أدى إلى أعلى زيادة في صفات الجودة لمحصول بنجر السكر مثل زيادة نسبة السكر ونسبة النقاوة وحاصل العرش و الجذر والسكر و انخفاض معنوي في الأحماض الأمينية الكلية والشوائب (% الصوديوم والبوتاسيوم و الفا أمينو نيتروجين) مقارنة بالكنترول في الموسميين المتتاليين ٢٠١٦/٢٠١٥ و ٢٠١٧/٢٠١٦.بينما سجلت معاملة البيو أرك مرتين أقل كفاءة. نستنتج من هذه الدراسة أنه يمكن استخدام المبيدات الحيوية مثل بيو زيد و بلانت جارد والبيوباك رشا ٦ مرات في الموسم الواحد كل ١٥ يوم كبديل للمبيدات الفطرية لمقاومة مرض تبقع الأوراق السركسبوري وإنتاج محصول عال من السكر والجذور بجودة مرتفعة.