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Safely Application of some Fungicides to Control Cercospora Leaf Spot Disease of Sugar Beet

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Laboratory study and field trials were conducted to verify the effectiveness of some recommended fungicides in controlling Cercospora leaf spot (CLS) of sugar beet. These fungicides are: Score, Eminent, Montro, Opus, Foliogold and Amistar. All fungicides at 10 ppm conc. significantly reduced the radial growth of *C. beticola* in the Lab. Score, Eminent, Montoro and Opus provided high levels of efficacy. Amistar (Azoxystrobin) and Foliogold (chlorothatonol) gave the lowest effect in reducing the growth rate compared with Trizole fungicides. The same trend was found in the field where Score and Eminent were the most effective fungicides in controlling CLS disease. Fungicides treatment caused significant increase in yield and quality traits (root weight, total soluble solids (T.S.S.) %, sucrose % and purity%) comparing with control plots. Also, T.S.S%, sucrose% and purity% were increased in root samples stored 15 days after harvest. Treating with Score and Eminent were the most efficient in this respect. Determination of residues in the produced beet roots proved the degradation and safety of using these two fungicides, which belong to tetraconazole and difenoconazole in the sugar beet roots. The results are coincided with the allowed limits of the maximum residual limit (MRL) according to FAO and WHO and EU database.

Keywords: Cercospora leaf spot, score, eminent, montoro and opus, amistar (azoxystrobin) and foliogold (chlorothatonol)

Sugar beet (*Beta vulgaris* L.) is one of the most important sugar crops in the world, produces about 30% of the world's sugar (Cooke and Scott, 1993). In Egypt, it ranks the second crop after sugar cane for sugar production. Cercospora leaf spot (CLS), caused by *Cercospora beticola* Sacc. is one of the most destructive foliar diseases of sugar beet worldwide, especially in warm and humid areas (Holtschulte, 2000 and Weiland and Koch, 2004). The disease reduces the photosynthetic capacity of plants as a consequence of necrotic leaf lesions, which results in reduced root yield and sugar content along with an increase in the concentration of impurities, leading to considerable economic losses (Shane and Teng, 1992 and Khan and Smith, 2005). Losses due to CLS disease have gone as high as a 42% reduction in gross sugar and 32% reduction in root weight (Smith and Martin, 1978).

Disease control is achieved mainly through a combination of cultural practices, the use of resistant varieties and repeated applications of fungicides. These methods are relatively effective in disease control, but breeding for resistance is unfavored by the crop producers because of their lower agronomic properties and the long period and much effort needed for getting the resistant varieties (Weiland and Koch, 2004).

Under favorable environmental conditions for disease progress, applications of fungicides are indispensable (Weiland and Koch 2004). Due to concerns of expense, exposure risks, fungicide residues, disease resistance and other health and environmental hazards, the Benzimidazol derivatives were the first group of systemic fungicides that became available for *C. beticola* control (Georgopoulos and Dovas, 1973). Also, Tetraconazole resulted in significant Cercospora leaf spot control, root yield, and recoverable sucrose compared to Fenbuconazole with an adjuvant (Khan and Smith, 2005). Because of the degradation of fungicides within the end plant tissues is of great importance for man health, persistence and dissipation behavior of fungicides were studied intensively by some investigators (Ellen *et al.*, 1997; Banerjee *et al*, 2008 and Guo *et al.*, 2010).

This study aimed to evaluate the safest fungicide(s) belonging to the aforementioned chemical groups which could be used safely on the crop without hazardous effect to manhood.

Materials and Methods

Experiments were conducted in two consecutive seasons; 2015/2016 and 2016/2017 in the Lab. and field of Gemmeiza Agricultural Research Station, Agricultural Research Center (A.R.C.).

Source of plant cultivar and pathogen:

Seeds of sugar beet (*Beta vulgaris* L.) c.v. Pleno cultivar were provided by Sugar Crops Research Institute, A.R.C., Giza, Egypt and used in the present study as a sensitive variety for CLS disease. The pathogen, *i.e. C. beticola* was recovered from foliage of infected sugar beet plants grown at the field of Gemmeiza.

Laboratory tests:

1. Fungicidal evaluation against C. beticola:

Six fungicides were tested *in vitro* to assess their inhibitory effect against *C. beticola*. Inhibitory effect of the tested fungicides was determined as percentage of the fungal growth inhibition. Trade and common names along with the recommended rates of application and formulations used in the present study are shown in Table (1).

Table 1. Specifications of fungicides used:

Trade name	Common name	Formulation	Rate of applic./L
Foliogold	Chlorothatonol	SC 37.5%	2m
Amistar	Azoxystrobin	SL 25%	1m
Opus	Epoxiconaxole	SC 12.5%	2m
Eminent	Tetraconaeole	EW 12.5%	1m
Score	Difenoconazol	SC 25%	0.5m
Montoro	Difenoconazol/*Propiconazol*	EC 30%	0.5m

Each fungicide was tested against the target pathogen at the concentrations of 0.01, 0.1, 1, 10 and100 ppm. Each concentration of fungicides was added to PDA to give the required concentration after autoclaving. After cooling to about 40-45°C, each concentration of fungicides was added to PDA in three Petri dishes (9 cm diam.). By the aid of 5 mm diam. cork-borer, a disc of 7-day old culture of *C. beticola* was transferred each plate and incubated at 26ºC. Fungicide free plates acted as control. At the full growth of plates of any treatment, radial growth of the pathogen was measured for each treatment and results were recorded as the average reduction percent in colony diameter for each concentration.

Field experiment:

A field trial was carried out to study the efficacy of fungicides under study in controlling Cercospora leaf spot in the field at Gemmeiza under natural infestation in two successive seasons: 2015/2016 and 2016/2017. The experiment was designed in the randomized complete blocks method in three replications with 21 m^2 plots, 4 rows/plot and 80 cm apart. Sugar beet seeds, cv. Pleno were planted 4 seeds per hill and thinned after 3 weeks to one plant/hill to spacing of 25 cm. All treatments were fertilized by N.P.K. and watering and all cultural practices were affected as usual.

Plants were sprayed with the experimented fungicides and recommended doses of application as mentioned in Table 1; three times at 2-week intervals, starting just at the onset of the disease symptoms. Meanwhile, control plants were sprayed with water.

Disease severity was recorded as percentage of infection of plant foliage three times every 15 days after two weeks from last spray. Plant roots were harvested after 5 months from planting and 5 roots were taken randomly for determination of root weight and quality traits for each treatment. Quality traits, viz. total soluble solids (T.S.S.) % were measured in fresh roots using the hand refrectometer according to Mc Ginnis (1982). Sucrose% was determined by using succarometer according to Anon. (1990). Purity% was calculated by using the formula (Sucrose%/T.S.S %)× (100).

2. Assessment of disease severity (CLS):

Cercospora leaf spot CLS was determined on 10 plants, 5 leaves, each was selected randomly taken from the center of two rows of each micro plot (Shane and Teng, 1992). Average percent of CLS was recorded three times every 15 days starting two weeks after the last spray. Disease severity was calculated according to a scale of 1-10 as described by Windels *et al.* (1997) and formulated as follows:

$$\text{Disease severity \%} = \frac{\Sigma\text{ (Each category x number of leaves in each category)}}{\text{The total leaf number X the highest degree of category}} \text{ x 100}$$

The Efficacy of each treatment in reducing Cercospora leaf spot severity% was calculated as follows:

$$\text{Efficacy \%} = \frac{\text{DSC - DST}}{\text{DSC}} \times 100$$

Where:
DSC: Disease severity under control.
DST: Disease severity under treatment.

Residual effect of fungicides used:

Effect of treatment with fungicides belongs to the groups of tetraconazole and difenoconazole in the resulting roots was determined at harvest time and 15 days after harvest as well. Ten grams of fresh weight of the obtained roots were taken randomly from each treatment and the fungicide residues were estimated by gas chromatography (GC). Methodology adopted in this analysis was made and equipment conditions followed as described by Central Lab of Residual Analysis of Pesticides and Heavy Metals in Food (QCAP). Agilent 6890 GC equipped with an Ni^{63} electron capture detector was used and GC conditions: HP-5 (J&W Scientific) capillary column (30m length x 0.32mm internal diameter (I.D.) x 0.25 um film thickness), carrier gas: N_2 at a flow rate of 4 ml/min; injector and detector temperatures were 300^{o}C and 320^{o}C, respectively. The initial column temperature was initial oven temperature, 200^{o}C for 2 min, raised at 10^{o}C/min and then held at 270^{o}C for 2 min. The maximum residue limit (MRL) was compared with Codex according to Anon. (2008a) and European database on Pesticide Residues web page Anon. (2008b), as mg/kg in sugar beet roots.

Statistical analysis:

The obtained data were subjected to analysis of variance (Steel and Torrie, 1960). Least significant differences (L.S.D) and Duncan's multiple range tests (DMRT) were applied to comparing means under study (Duncan, 1955).

Results

Effect of fungicides on radial growth of C. beticola in vitro:

A laboratory experiment was conducted to determine the efficacy of the six fungicides under study, five concentrations each against *C. beticola*. Each concentration was added in PDA after cooling in three replicates. Results in Table 2 and Fig. 1 show that all tested fungicides affected the radial growth of *C. beticola,* in general, except Foliogold, the least concentration used (0.01ppm) reduced the fungal growth comparable with the control (8.9 cm). It is clear that score caused the highest effect (0.4 and 0.2 cm) at 0.01 & 0.1 ppm, respectively compared with the other fungicides.

The effect of fungicides on the fungal growth was gradually increased by the increase of fungicide concentration. Most of fungicides used caused complete inhibition to the fungal growth at the 10 ppm. concentration Amistar, however caused 100% reduction to the mycelial growth at 100 ppm. Whereas, Foliogold failed to cause complete reduction to the mycelial growth at 100 ppm.

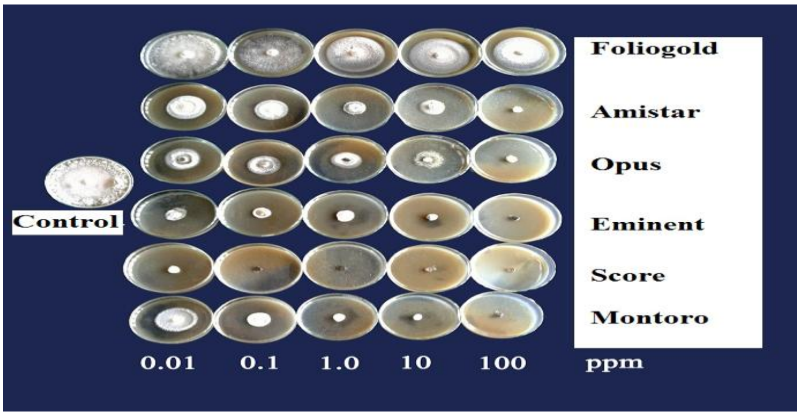

Fig. 1. Influence of different concentrations of fungicides under study on radial growth of *C. beticola* on PDA.

Table 2. Effect of fungicides under different concentrations on radial growth of *C. beticola* on PDA medium

Fungicide	Growth rate (cm) at /ppm					Mean	Efficacy %					Mean
	0.01	0.1	1	10	100		0.01	0.1	1	10	100	
Foliogold	8.7	6.2	5.2	4.1	3.5	5.5	2.4	30.3	41.6	53.9	60.7	37.8
Amistar	4.4	3.9	2.6	1.3	0	2.4	50.6	56.2	70.8	85.4	100	72.6
Opus	7.6	6.0	4.1	0	0	2.1	14.6	32.6	53.9	100	100	60.2
Eminent	4.3	4.3	1.3	0	0	1.9	51.7	51.7	85.4	100	100	77.8
Score	0.4	0.2	0	0	0	0.1	95.5	97.7	100	100	100	98.6
Montoro	4.9	3.7	2.6	0	0	2.2	50.0	58.4	70.8	100	100	75.8
Control			8.9									

Field experiment:

A field trial was conducted under natural infestation with *C. beticola* in the Experimental field of Gemmeiza to study the effect of spraying with tested fungicides on controlling Cercospora leaf spot for two seasons 2016-2017. Efficacy of treating with these fungicides on root yield along with the quality traits and residual effect were also analyzed.

Efficacy of fungicides against Cercospora leaf spot disease:

Data presented in Table 3 indicated that all fungicidal treatments caused significant effect on the disease severity compared to the control in both seasons of experimentation. Significant differences in reducing the disease severity were found among treatments. Eminent and Score achieved the first rank and gave the highest effect on CLS compared with the other fungicides (Table 3). However, Opus and Montoro achived the second rank. Whereas, Amistar and Foliogold caused the least effect on the disease.

Table 3. Effect of different fungicides on Cercospora leaf spot under natural infestation, Gemmeiza, 2015/2016 and 2016/2017 growing seasons

Fungicide	Disease severity %		Efficacy%		Average of two seasons	
	2015/2016	2016/2017	2015/2016	2016/2017	Disease severity%	Efficacy %
Foliogold	31.48[c]	28.04[b]	41.7	46.6	29.76	44.15
Amistar	39.14[b]	27.33[b]	27.5	47.9	33.23	37.7
Opus	18.54[d]	16.41[c]	65.7	68.9	17.47	67.3
Eminent	11.00[e]	9.66[d]	79.5	81.6	10.33	80.6
Score	8.92[e]	7.42[d]	83.5	85.6	8.17	84.55
Montoro	21.33[d]	16.66[c]	60.5	68.3	19.00	64.4
Control	54.00[a]	52.5[a]	-	-	53.25	-
L.S.D 0.05	3.2	4.135				

Effect of fungicide treatment on root yield and yield components:

Results of root weight of sugar beet in Tables 4 & 5 show that most fungicides caused significant increase comparable to the control in general. However, Folliogold caused controversial results whether at harvest or after harvest in both seasons of experimentation. The same trend was found on percentage of sucrose content, purity and T.S.S. Score gave the best effect in both seasons compared to the other fungicides whether at harvest or after harvest time (Tables 4&5).

Table 4. Effect of fungicide treatments on weight and sugar content of sugar beet root, 2015 /2016 growing seasons

Fungicide	Root weight (Kg)		T.S.S%		Sucrose%		Purity%	
	At harvest	After 15days	At harvest	After 15days	At harvest	After 15days	At harvest	After 15days
Foliogold	1.46[bc]	1.23[c]	21.33[de]	22.69[bc]	16.23[de]	19.16[bc]	76.07[cd]	84.45[b]
Amistar	1.30[c]	1.4[bc]	21.50[cd]	23.05[b]	16.86[d]	18.76[c]	78.55[c]	81.42[bc]
Opus	2.06[a]	1.6[ab]	23.00[b]	23.58[b]	19.76[c]	20.66[b]	85.99[b]	87.63[ab]
Eminent	1.70[b]	1.8[a]	24.66[a]	25.33[a]	21.83[b]	23.83[a]	88.43[ab]	94.06[a]
Score	2.18[a]	1.7[ab]	25.70[a]	26.10[a]	23.43[a]	25.10[a]	91.20[a]	96.18[a]
Montoro	1.53[bc]	1.6[ab]	22.61[bc]	23.61[b]	19.80[c]	20.76[b]	87.53[ab]	88.06[ab]
Control	1.30[c]	1.13[c]	20.33[e]	21.71[c]	14.86[e]	16.20[d]	73.10[d]	74.76[c]
L.S.D 0.05	0.358	0.341	1.141	1.015	1.457	1.866	4.905	8.623

Table 5. Effect of fungicide treatments on weight and sugar content of sugar beet root, 2016/2017 growing seasons

Fungicide	Root weight (Kg)		T.S.S%		Sucrose%		Purity%	
	At harvest	After 15days	At harvest	After 15days	At harvest	After 15days	At harvest	After 15days
Foliogold	1.50[b]	1.37[bc]	23.83[c]	25.23[d]	18.73[d]	20.50[c]	78.61[cd]	81.25[c]
Amistar	1.53[b]	2.05[a]	23.83[c]	26.66[bc]	19.03[d]	21.50[bc]	80.09[c]	80.66[c]
Opus	1.8[ab]	1.48[bc]	25.33[b]	25.66[cd]	21.60[c]	22.66[b]	85.27[bc]	88.33[b]
Eminent	2.21[a]	1.56[bc]	26.84[a]	27.50[ab]	24.16[b]	26.13[a]	90.00[ab]	95.04[ab]
Score	2.23[a]	1.53[bc]	27.86[a]	28.33[a]	25.83[a]	27.10[a]	92.70[a]	95.65[a]
Montoro	1.53[b]	1.76[ab]	24.76[bc]	24.94[d]	21.63[c]	22.00[b]	87.33[ab]	88.31[b]
Control	1.36[b]	1.26[c]	22.33[d]	23.04[e]	16.03[e]	16.02[d]	71.78[d]	70.32[d]
L.S.D 0.05	0.486	0.457	1.116	1.082	1.639	1.407	7.184	6.914

Detection for residues Difenoconazole and Tetraconazole in sugar beet roots:

Results presented in Tables 3, 4 & 5 indicate that fungicides used throughout the present study were differed in their effectiveness in reducing CLS in sugar beet and increasing the plant parameters and yield components as well. Score and Eminent (belong to the Difenoconazole and Tetraconazole, respectively) showed the highest efficiency in controlling CLS and increasing the plant growth parameters and yield components, so recovery of these active ingredients was estimated, following GC detection as described under Materials and Methods two times, at harvest and two weeks after harvest.

Data presented in Table 6 show the allowed residues as MRL (mg/kg) according to Anon. (2008a) and Anon. (2008b). As regards to the maximum residue limits shown in Table 6, the residues of Difenoconazole (Score fungicide) registered lesser levels than the allowed limit (0.2 mg/kg) whether at harvest or after 15 days of harvest. Whereas, the residues of Tetraconazole were found to be high at harvest, but detected equally to the allowed MRL after two weeks of harvest.

Table 6. Determination of residues of Difenoconazole and Tetraconazole in sugar beet root samples at harvest and after 15 days

Fungicide	Group	Time of analysis	Residues (mg/Kg)	MRL (mg/Kg)
Score	Difenoconazole	At harvest	0.086	0.2*
		After 15 days	ND	
Eminent	Tetraconazole	At harvest	0.135	0.05**
		After 15 days	0.050	

ND: Not detected MRL: Maxim Residue Level

* MRL for Difenoconazole according to FAO and WHO database (2008)

** MRL for Tetraconazole according to EU pesticides database (2008)

Discussion

As fungicidal treatment is considered as one of the most methods used to control Cercospora leaf spot (CLS) of sugar beet, the current study dealt with the re-evaluation of some of the recommended fungicides belong to the following groups: Chlorothatonol, Azoxystrobin, Epoxiconaxole, Tetraconazole, Difenoconazole and Difenoconazole + Propiconazole. Assay of the existence of the toxic constituents in each of the evaluated fungicides were, also, done. Except the Chlorothatonol and Azoxystrobin groups, all fungicides belong to the other groups that included the Sterol Dimethylation inhibiting group (DMI) were highly recommended. The DMI fungicides inhibit one specific enzyme C14-dimethylase that plays a role in sterol production as reported by Lyr (1987). Sterols such as Ergosterol are needed for membrane structure and function; thus they are essential for the development of functional cell walls. Therefore, these fungicides result in abnormal fungal growth and death.

In vitro screening of fungicides under study revealed that all of them significantly reduced the radial growth of *Cercospora beticola* with slight differences between them comparable with the control. These results are consistent with those obtained by Jiang (2016) who stated that these fungicides in DMI class varied in their efficacy *in vitro* according to EC_{50} values.

Field trial carried out to control CLS showed that the disease could be reduced by spray application with fungicides under study. The most effective fungicides were Score, Eminent, Opus and Montoro. Whereas, Amistar and Foliogold had the lowest effect on the disease. These effective systemic fungicides could be absorbed into the leaf surface after spraying, which in turn gives them persistence against the environmental factors like solar radiation and water wash-off as stated by Lyr (1987) and Lyr (1995). The variation in the level of activity of each fungicide against the pathogen could be explained by the DMI as emphasized by work on cercospora leaf spot of sugar beet by Jiang (2016). Also, Karaoglanidis *et al.* (2002) stated that the actual development of resistance to DMI fungicides is slow and quantitative. They reported also, that the loss of fungicidal activity of *C. beticola* is incomplete.

The disease reduction by using fungicides can improve the plant growth parameters as well as the yield components of the produced roots. Logically any suppression of disease development will lead to increasing the yield components. This statement supports the findings obtained throughout the present study and also in agreement with the findings of Rossi, *et al*. (2000) who described the effect of disease on yield component as a result of reduction of photosynthetic activity of leaf area firstly, while under severe foliage loss, late season photosynthetic potential is also reduced and vegetative re-growth is stimulated at the expense of root sugar reserves. As a consequence, potential sugar yield (recoverable sugar) of sugar beet crop can be significantly reduced due to the loss of both root weight and sucrose content. Therefore, any suppression of disease development will lead to save yield reduction or increasing yield components than that of untreated plots. Our results are consistent with those of Percich *et al*. (1987), Khan and Smith (2005) and Gado

(2007), who reported that treated plots of sugar beet by fungicides resulted in increase in yield components, root weight and sucrose %, due to suppressing the causal agent of Cercospora leaf spot disease.

Success of the fungicidal application obtained throughout the current work posed us to identify whether there is hazardous effect on consumers or not. It is necessary to emphasize the necessity of the maximum residue level (MRL) determination in the obtained beet roots. Eminent and score, the most effective fungicides in reducing the disease and increasing yield components as shown in our study were used to determine the pesticidal residues. Both fungicides were shown to be bellow the MRL levels according to Anon. (2008a) and Anon. (2008b). in the produced roots after 15 days of harvest. These results are in agreement with the findings of Radmila *et al.* (2006) who reported that fungicides belonging to this group are safely to use in sugar beet where they agree with the principles of good agricultural practice. Also, the current results are consistent with those found by Kandu *et al.* (2014) in their work on the Tetraconazole on water melon.

Therefore, we highly recommend the use of these fungicides to control Cercospora leaf spot of sugar beet safely under the field conditions of Egypt.

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الاستخدام الآمن لبعض المبيدات الفطرية المستخدمة في مكافحة مرض التبقع السركوسبوري في أوراق بنجر السكر

عبد الناصر بدوى السيد – أمل عزت عبد الغنى الشهاوى – السعيد محمد الشبراوى – أسماء محمود القللى
مركز البحوث الزراعية – معهد بحوث أمراض النباتات

في دراسة معملية وحقلية لتقييم كفاءة المبيدات الفطرية الموصى بها لمكافحة مرض تبقع الأوراق السركسبوري فى بنجر السكر. وهي: سكور، إمييننت، مونترو، اوبس، فوليوجولد وأميستار كانت جميعها لها القدرة على تثبيط نمو الفطر سيركوسبورا بيتيكولا بالمعمل عند تركيز ١٠ جزء فى المليون. وكانت أعلاها كفاءة سكور، امييننت، مونترو واوبس والتى تتبع مجموعة الترايازول. بينما كانت أميستار(ازوكسى ستروبين) وفوليوجولد (كلوروثاتينول) أقل كفاءة في تثبيط النمو الفطرى. وكذلك أظهر سكور، امييننت أعلى كفاءة في مقاومة المرض بالحقل بالمقارنة بباقي المبيدات المستخدمة. هذا وقد أدت معاملة النباتات بسكور وامييننت إلى أعلى زيادة معنوية فى المحصول وصفات الجودة (وزن الجذور، المواد الصلبة الذائبة %، السكروز % والنقاوة %) بالمقارنة بالكنترول (غير المعاملة) سواء وقت الحصاد أو بعد ١٥ يوم من الحصاد. وبدراسة متبقيات هذين المبيدين في الجذور الناتجة من النباتات المعاملة تبين أنهما أقل من الحد المسموح به (MRL) حسب منظمة الصحة العالمية والفاو والاتحاد الأوروبى في هذا الخصوص.