

EFFICACY OF SOME FUNGICIDES ON CONTROLLING CERCOSPORA LEAF SPOT AND THEIR IMPACT ON SUGAR BEET YIELD COMPONENTS

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ABSTRACT

Sugar beet leaf spot, caused by *Cercospora beticola*, is the most important and destructive foliar disease in North Nile Delta of Egypt. However, control of the disease in Egypt is mainly achieved by fungicide treatments. So, the control efficacy of two Sterol demethylation inhibitors (DMI) fungicides, tetraconazol (Eminent) and difenoconazole + propiconazole (Montoro), and one Multi-site activity (MSA) fungicide, benalaxyl + copper oxichloride (Galben), against *C. beticola* and their impact on sugar beet yield components were tested in this study. Fungicides were tested under natural field infection in four seasons (2008/2009, 2009/2010, 2011/2012 and 2012/2013) in commercial field at Sakha in Kafr El-Sheikh governorate. All the three fungicides suppressed *Cercospora* leaf spot significantly compared with untreated plots. However, there were significant differences in efficacy among them. The most effective fungicide was Eminent, which provided high levels of efficacy (from 95 to 96.5%) followed by Montoro which showed efficiency from 83 to 86%. Galben provided moderate control efficacy from 53 to 63%. Sprays with Eminent increased root yield, sucrose percentage and gross sucrose more than 90, 56 and 214 % respectively compared with the untreated plots. However, Montoro caused more than 70, 35 and 136% increases in yield respectively. Sprays with Galben provided less increases in yield components (up to 37, 30 and 80% respectively). Since fungicidal application considered as the main tool employed in sugar beet *Cercospora* leaf spot disease management in Egypt, the obtained results concluded that both DMI fungicides, Eminent and Montoro, were effective in controlling the disease. However, further studies are needed to determine the best application program to avoid appearance of DMI resistance strains of *C. beticola*.

Keywords: *Cercospora beticola*, sugar beet leaf spot, DMI fungicides, yield components.

INTRODUCTION

Sugar beet (*Beta vulgaris* L.) is planted in nearly 93,094 Fadden in Kafr El-Sheikh governorate, and considered as the leader in sugar beet production in Egypt, representing about 37.4% of the planted area and accounting for 39.8% of the tonnage produced annually (Sugar crop council, 2010). Sugar beet leaf spot caused by *Cercospora beticola* Sacc., is the most destructive foliar disease of sugar beet in warm and humid areas such as the Mediterranean basin (Mukhopadhyay & Rao, 1978; Rossi et al., 1995, Weiland & Koch, 2004 and Whiteny & Duffus, 1995). In Egypt, epidemical and biological control studies related to *Cercospora* leaf spot on sugar beet were reported by several investigators (EL-Fahhar 2003, El-Kholi 1995 and Yassin, 2008). In the absence of control measures, in areas with high disease severity, yield losses range from 25 to 50% (Byford et al., 1996, Shane and

Teng 1992). Crop losses attributable to *Cercospora* leaf spot (regardless of disease management and indirect costs) is manifested as a reduction in root weight and reduced sugar content (Smith and Martin, 1978; and Khan and Smith, 2005). *Cercospora* leaf spot is managed by planting disease tolerant varieties, reducing inoculum by crop rotation and tillage, and fungicide applications (Miller *et al.*, 1994). Combining high levels of *Cercospora* leaf spot resistance with high yield in sugar beet is difficult (Smith and Campbell, 1996 and Windels *et al.*, 1998). As a result, commercial varieties generally have only moderate levels of resistance and require fungicide application to obtain acceptable levels of control against *Cercospora* leaf spot (Miller *et al.*, 1994). Unfortunately, there is no breeding program now in Egypt due to the unfavorable conditions for seed production and Egypt depends on the exotics as a source of varieties. At the same time, these varieties changed after a short period. Thus we need to acquire knowledge about efficacy of some fungicides as a rapid and preventive measure in *Cercospora* leaf spot control. So the goal of this article was to compare the efficacy of three different fungicides for controlling *Cercospora* leaf spot of sugar beet and their impact on yield components.

MATERIALS AND METHODS

The relationship among efficacy of three fungicides for reducing disease severity of *C. beticola* and increasing yield components were determined by analysis of epidemics on sugar beets in four seasons (2008/2009, 2009/2010, 2011/2012 and 2012/2013) in commercial field at Sakha, Kafr El-Sheikh governorate. Trials were conducted in a field where sugar beets heavily infected by *C. beticola* in the previous seasons (referred as a hot spot for the disease). The experimental design was a randomized complete block with three replicates. Plots consisted of four 12-m rows spaced 60 cm apart. Plots were planted on 20- 25 August with the *Cercospora* leaf spot-susceptible cv. Pleno. Plants were hand-thinned to a spacing of 20cm. Symptoms of naturally infection by *C. beticola* were detected in plots at 90 days after planting.

Table 1: Fungicides used for control of *Cercospora* leaf spot of sugar beet.

Fungicide	Rate/100 liters of water	Active ingredient	Formulation	Company
Eminint	100cm	Tetraconazole	12.5% EW	Lots Agric. Develop. Co.
Montoro	50cm	Difenoconazole + propiconazole	30% EC	Star Chem. Co.
Galben	250g	Benalaxyl+copper oxichloride	46% WP	Lots Agric. Develop. Co.

Three fungicides varied in active ingredients, Eminent, Montoro and Galben (Table 1) were used in three timing treatments. The fungicides were applied every 15 days after disease severity appearance reached 1%.

Fungicide untreated control plots sprayed with water. Sprays were applied with a tractor-mounted boom sprayer.

Disease severity

Three days prior to harvest, *Cercospora* leaf spot severity was assessed on sugar beet plants in the center two rows of each plot using the standard area diagrams of Shane and Teng (1992) for disease severity.

Efficacy of fungicides

The efficacy of each fungicide was estimated by the following formula (El-Shemi, 2003 and Frolich, 1979):

$$\text{Efficacy\%} = \frac{\text{Average disease severity of untreated fungicide plot} - \text{Average disease severity of treated plot}}{\text{Average disease severity of untreated fungicide plot}} \times 100$$

Yield components

Yield components i.e., root weight, sucrose %, gross sucrose was estimated during the four tested seasons as an impact of fungicides efficacy. Avoiding sugar beet roots on the ends of each plot, ten roots selected randomly from the center two rows of each plot, hand defoliated, harvested and weighed for root yield. Increasing of root yield was computed according to the following formula (Ibrahim *et al.*, 2003):

$$\text{Yield increase \%} = \frac{\text{Average yield of fungicide treated plot} - \text{Average yield of fungicide untreated plot}}{\text{Average yield of fungicide untreated plot}} \times 100$$

To estimate sucrose %, three replications were taken with 3 roots selected randomly from roots used in yield assessment. Root slices (2mm in thickness) of the three roots were shredded with a kitchen grade and thoroughly mixed. After that, 26g of sample was taken for cold extraction procedure for sucrose determination (A.O.A.C., 1990). The sample was blended with 177ml of dilute basic lead acetate solution (3%) in an electric blender for 2 minutes. The mixture was then filtered through filter paper (Whatman No.1). The clear filtrate was measured by the aid of saccharometer. Gross sucrose was calculated as recorded by Abdel-Motagally and Attia, 2009 according to the following formula:

$$\text{Gross sucrose} = \text{root yield} \times \text{sucrose\%}$$

Increasing sucrose% and gross sucrose was estimated as an impact of fungicide efficacy using the same formula of increasing root weight described above.

Statistical analysis

To determine treatment effects, analysis of variance ANOVA was performed on the data. Analysis was performed on root yield (Kilogram per 10 roots); percent sugar content; and gross sucrose. Statistical analysis was done using COSTAT software version 9. Percentage data were transformed into arcsine before carrying out ANOVA.

RESULTS

During all seasons of the study, the disease epidemic was initiated naturally. Fungicide treatments in all experiment-plots during the four tested years resulted in significant decreases in disease severity of *Cercospora* leaf spot compared with the untreated plots (Tables 2, 3, 4 & 5). Treatments with Eminint exhibited high levels of leaf spot control since the disease severity was in between 2-4% and provided control efficacy higher than 95% during all seasons of the study. Montoro, also provided high control efficacy reached more than 83% with disease severity ranged from 9-11%. The remaining fungicide, Galben, was less effective than Eminint and Montoro hence disease severity was in between 21-30% reflecting control efficacy ranged from 53-63%. High levels of disease severity (56-78%) were observed on untreated fungicide plots (check) during all seasons of the study. Higher disease severity was occurred during 2012/2013 season compared with that observed during the previous seasons since it ranged from 4 to 78.3%.

Measurement of yield components showed that root weight, sucrose % and gross sucrose in all the fungicide-treated plots were higher ($p < 0.05$) compared with the check plots during all seasons of the study. Sprays with Eminint increased root yield, sucrose % and gross sucrose more than 93, 57 and 200 % respectively relative to the check plots. However, Montoro caused more than 70, 30 and 80% increases respectively. Meanwhile, sprays with Galben provided little increases in yield components (up to 37, 30 and 80% respectively). Negative correlation was found among disease severity and root weight, sucrose % as well as gross sucrose ($r = -0.978$, $p \leq 0.05$; -0.952 , $p \leq 0.05$ and -0.950 , $p \leq 0.05$ respectively).

Generally, yield component of Eminint treated plots was usually higher than either Montoro or Galben treated plots. At the same time plots sprayed with Montoro gave usually higher yield increase than Galben treated plots.

Table 2: Disease severity of *Cercospora* leaf spot on sugar beet, control efficacy (%) and yield components in plots treated with various fungicides in 2008/2009 season.

Treatment	Disease severity %	Efficacy %	Root weight		Sucrose		Gross sucrose**	
			Kg/10 root	Increase %	%	Increase%	Kg/10 root	Increase %
Eminint	2.5a*	96	23a	106	17.9a	70.3	4.1a	250.7
Montoro	10b	83.3	20.7b	85.6	15.7b	49.4	3.2b	176.2
Galben	28c	53.3	15.3c	38	13.8c	31.9	2.1c	81.7
Untreated plots	60d	0	11.2d	0	10.5d	0	1.2d	0

*Mean of each column followed by the same letter are not significantly different at $p = 0.05$ according to Duncan's multiple range test. ** Data of gross sucrose were not transformed.

Table 3: Disease severity of Cercospora leaf spot on sugar beet, control efficacy (%) and yield components in plots treated with various fungicides in 2009/2010 season.

Treatment	Disease		Root weight		Sucrose		Gross sucrose**	
	severity %	Efficacy %	Kg/10 root	Increase %	%	Increase %	Kg/10 root	Increase %
Eminint	2a*	96.5	21a	94.4	17a	62	3.6a	215.1
Montoro	9.3b	83.7	19b	75.9	14.7b	39.7	2.8b	145.8
Galben	21c	63.4	16.7c	54.5	14.3b	36.7	2.4c	111.1
Untreated plots	57.3d	0	10.8d	0	10.5c	0	1.1d	0

*Mean of each column followed by the same letter are not significantly different at $p=0.05$ according to Duncan,s multiple range test. ** Data of gross sucrose were not transformed.

Table 4: Disease severity of Cercospora leaf spot on sugar beet, control efficacy (%) and yield components in plots treated with various fungicides in 2011/2012 season.

Treatment	Disease		Root weight		Sucrose		Gross sucrose**	
	severity %	Efficacy %	Kg/10 root	Increase %	%	Increase %	Kg/10 root	Increase %
Eminint	2.3a*	95.8	22.3a	92.8	18.5a	57.2	4.1a	204.7
Montoro	9b	84.6	20b	72.5	16b	36.5	3.2b	137.7
Galben	22c	60.9	17c	46.6	15.7b	33.1	2.7c	95.6
Untreated plots	56d	0	11.7d	0	11.8c	0	1.4d	0

*Mean of each column followed by the same letter are not significantly different at $p=0.05$ according to Duncan,s multiple range test. ** Data of gross sucrose were not transformed.

Table 5: Disease severity of Cercospora leaf spot on sugar beet, control efficacy (%) and yield components in plots treated with various fungicides in 2012/2013 season.

Treatment	Disease		Root weight		Sucrose		Gross sucrose**	
	severity %	Efficacy %	Kg/10 root	Increase %	%	Increase%	Kg/10 root	Increase %
Eminint	4a*	94.9	21.3a	179.2	16.3a	104.2	3.5a	469.8
Montoro	11b	86	19a	144	14.3b	79.2	2.7b	336.9
Galben	30.3c	61.3	14.3b	88.7	12.7c	58.3	1.8c	200.4
Untreated plots	78d	0	7.6c	0	8d	0	1d	0

*Mean of each column followed by the same letter are not significantly different at $p=0.05$ according to Duncan,s multiple range test. ** Data of gross sucrose were not transformed.

DISCUSSION

In the area of Sakha, Kafr El-Sheikh governorate, where the experimental fields were established, Cercospora leaf spot is the most important foliar disease of sugar beet. Disease epidemics usually initiate during December and terminate in the middle of February, when cooler temperatures arrest disease development. Disease severity in the experimental fields generally was high during all 4 seasons of the study. All three fungicides Eminint, Montoro and Galben suppressed Cercospora leaf spot significantly compared with untreated plots. However, there were significant differences in efficacy among them. The most effective fungicide

was Eminint, which provided high levels of control efficacy followed by Montoro. Meanwhile, Galben provided moderate control efficacy. Differences in the level of control efficacy against fungal pathogens obtained by DMI fungicides have been observed previously (Gado, 2007 and Percich *et al.*, 1987) and can be explained by variation in the level of intrinsic activity of each fungicide against a specific pathogen. The locally systemic fungicides Eminent (tetraconazole) and Montoro (difenoconazole+ propiconazole) belonged to sterol demethylation inhibiting group (DMI) and triazole class. The DMI fungicides inhibit one specific enzyme, C14-demethylase, which plays a role in sterol production (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 eventually death. Also, most DMI fungicides have a residual period of approximately 14 days. Such results are in accordance with the hypothesis that the DMI fungicides are best applied prior to infection or in the early stage of the disease development (Lyr, 1987).

Meanwhile, the multi-site activity fungicides such as Galben (benalaxyl+ copper oxichloride) are considered protective or preventive fungicides. They inhibit fungi on the plant surface so the fungus will not be able to infect the plant. Contact fungicides affect multiple biochemical sites in fungi; they kill fungi by overwhelming them with poisonous materials. However, they should be applied preventively since they do not affect fungi once they have infected the plant (Meriggi, *et. al.* 2000). Also they do not penetrate into the plant as well as they remain active only as long as the fungicide remains on the plant surface in sufficient concentration to inhibit fungal growth, usually 7-14 days (Lyr, 1995). On the other hand, protectant fungicides are sensitive to environmental conditions like rainfall and solar radiation, in contrast to systemic fungicides, which are absorbed into the leaf after application and are not affected by rain wash-off and solar radiation (Lyr, 1987 and Lyr, 1995). May be for these reasons, Galben was not more effective against the disease. This result is similarly with that obtained by Meriggi *et al.*, (2003) who found that copper compounds have poor efficacy for *Cercospora* leaf spot control.

Our investigation revealed that, a decrease in disease severity attributed to fungicide efficacy caused a significant increased in root yield, sucrose concentration and gross sucrose. The obtained results revealed that the protectant fungicide, Galben, failed to give sufficient disease control compared with Eminint and Montoro and did not result in high root weight, sucrose concentration and gross sucrose. Also, under the impact of all fungicide treatments the increasing in gross sucrose appears to be caused more by increased root weight than by increased sucrose percentage. Rossi, *et al.*, (2000) 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 the sugar beet crop can be significantly reduced due to the loss of both root weight and sucrose

content. So, any suppression of disease development will lead to save yield reduction or increasing yield components than that of untreated plots. Our results are consistently with that of Gado (2007), Percich *et al.*, (1987) and Khan and Smith (2005) they reported that treated plots of sugar beet by fungicides resulted in increase in yield components, root weight and sucrose% , due to suppressing the causal agent of *Cercospora* leaf spot disease. Results of the present study agree with the previous investigators (Smith and Ruppel, 1971; Smith and Ruppel, 1973; and Shane and Teng, 1992). They reported that disease severity closely paralleled by reduction in root yield and sucrose content.

Since fungicidal applications continue to be the main tool employed in sugar beet *Cercospora* leaf spot disease management in foreign countries (Skaracis *et al.*, 2010) as well as in Egypt, the obtained results conclude that both MDI fungicides, Eminint and Montoro, were superior to control the disease. However, further studies needed to determine the best application program to avoid appearance of MDI resistance strains of *C. beticola* as reported elsewhere (Karaoglanidis *et al.*, 2002).

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كفاءة بعض المبيدات الفطرية في مقاومة تبقع الأوراق السركوسبوري وتأثيرها على مكونات محصول بنجر السكر
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يعتبر مرض تبقع الأوراق السركوسبوري المتسبب عن الفطر سرкосبورا بيتيكولا من أهم أمراض أوراق بنجر السكر الهامة في شمال دلتا مصر. وحيث أن مقاومة المرض في مصر تعتمد أساسا على المعاملة بالمبيدات الفطرية ، لذلك أختبرت كفاءة مبيدان من مبيدات الـديميثيلالاشن الفطرية المثبطة للاستيرول (دي إم أي)- تيتراكونازول- (إيمنت) وكذلك المبيد المركب من دايفينوكونازول+ بروبيكونازول (مونتورو) بالإضافة إلى مبيد ذو نشاط متعدد التأثير (إم إس آيه) -بينالاكسيل+ أوكسيكلورو النحاس-(جالين) ضد الفطر سرкосبورا بيتيكولا وتأثير ذلك على مكونات محصول بنجر السكر. أختبرت المبيدات تحت ظروف الحقل وكذلك الظروف الطبيعية للإصابة لمدة أربعة مواسم (2009/2008، 2010/2009 ، 2012/2011 ، 2013/2012) بحقل تجاري بسخا -كفر الشيخ. ثبقت المبيدات الثلاثة الإصابة بالمرض بدرجة معنوية مقارنة بالقطع الغير معاملة، وقد وجدت فروق معنوية بينها في الكفاءة. كان أكثر المبيدات كفاءة هو الإمنت والذي أظهر مستوى عالي من كفاءة المقاومة (من 95 إلى 96'5%) تلاه المبيد مونتورو(من 83 إلى 86%)، بينما أظهر المبيد جالين كفاءة متوسطة في المقاومة (من 53 إلى 63%). أدى الرش بالإمنت إلى زيادة وزن الجذور ، نسبة السكر، السكر الخام بنسب أكثر من 90، 56، 212 % على التوالي نسبة إلى القطع الغير معاملة. أيضا سبب الرش بالمونتورو أكثر من 70، 35، 135 % زيادة على التوالي. بينما كانت الزيادة الناتجة من الرش بالمبيد جالين أقل على المكونات المحصولية (بلغت 37، 30، 80 % على التوالي). وبما أن استخدام المبيدات الفطرية مازال هو الوسيلة الأساسية في مقاومة مرض تبقع الأوراق السركوسبوري في مصر، فقد أظهرت النتائج المتحصل عليها أن المبيدان إمنت و مونتورو مباشران وهما الأفضل في مقاومة المرض، ونحتاج لمزيد من التجارب للوصول إلى برنامج التطبيق الأمثل لتجنب ظهور سلالات مقاومة من الفطر سرкосبورا بيتيكولا لمجموعة المبيدات دي إم أي.

قام بتحكيم البحث

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