

Induction of Resistance Against Cercospora Leaf Spot Disease of Sugar Beet Plants Under Field Conditions

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Cercospora leaf spot caused by *Cercospora beticola* is one of the most economically important disease of sugar beet plants. Effect of di-basic potassium phosphate (K_2HPO_4), ascorbic acid and potassium bicarbonates ($KHCO_3$) against *Cercospora* leaf spot disease of sugar beet plants under field conditions was evaluated.

In laboratory trial, results indicate that ascorbic acid at concentration of 2 and 3 g/l had no inhibitory effect on the tested fungus while, complete reduction in the linear growth was obtained with $KHCO_3$ at 20 g/l. In field trials, results indicated that the most effective treatments were K_2HPO_4 at concentration 100 mM and $KHCO_3$ at 20 g/l which reduced *Cercospora* leaf spot severity more than 70.0 and 76.7%, respectively. Treated sugar beet plants with 50.0mM K_2HPO_4 , 3g/l ascorbic acid, 10 g/l $KHCO_3$ and Fungicides (Ridomil-plus at 2g/l) resulted in reducing *Cercospora* leaf spot severity more than 50.0%. All treatments significantly increased sugar beet yield. The highest increase was obtained with 100 mM K_2HPO_4 and 20 g/l $KHCO_3$ which increased sugar beet yield more than 15.2 and 17.4% and the total soluble solids (TSS) of sugar beet roots by 18.7 and 20.0%, respectively. Chitinase activity was stimulated by all treatments. Highest increase was obtained with K_2HPO_4 at concentration of 100 mM, $KHCO_3$ at 10 and 20 g/l and ascorbic acid at 3 g/l which increased the chitinase activity more than 113.0%.

It could be suggested that some chemical inducers may be used for controlling *Cercospora* leaf spot disease of sugar beet plants under field conditions.

Keywords: Ascorbic acid, *Cercospora beticola*, di-basic potassium phosphate, potassium bicarbonate and sugar beet.

Sugar beet (*Beta vulgaris* L.) is considered the second sugar crop for sugar production in Egypt after sugar cane. Recently, sugar beet crop has been an important position in Egyptian crop rotation as a winter crop not only in fertile soils, but also in poor, saline, alkaline and calcareous soils (Gobarah and Mekki, 2005). *Cercospora* leaf spot caused by the fungus *Cercospora beticola* is the most economically important diseases of sugar beets (Bugbee, 1995; Dexter *et al.*, 1998; Enikuomihin, 2005 and Harveson, 2007).

Dibasic potassium phosphate (K_2HPO_4) is commonly used for induction of resistance against foliar diseases in various plants (Mucharromah and Kuc, 1991; Reuveni *et al.*, 1996; Abd-El-Kareem *et al.*, 2001, 2002 and 2004). In this concern,

spraying potato plants with K_2HPO_4 and chitosan induced resistance against late blight disease and increased tuber yield under field conditions (Abd-El-Kareem *et al.*, 2002).

Moreover, the content of ascorbic acid in plant tissues was associated with resistance to some diseases (Zacheo *et al.*, 1977). Ascorbic acid was reported for inducing resistance in many plants against fungal diseases (Daisy *et al.*, 2000; Shahda, 2000; Khan *et al.*, 2001 and El-Gamal *et al.*, 2007).

Furthermore, bicarbonates are widely used in the food industry (Lindsay, 1985) were found to suppress several fungal diseases of cucumber plants (Ziv and Zitter, 1992). Using of potassium bicarbonates for controlling several plant diseases was reported by Horst *et al.* (1992), Ziv and Zitter (1992) and Abd-El-Kareem (2007).

The main objective of the present research was studying the effect of potassium phosphate, ascorbic acid and potassium bicarbonate against *Cercospora* leaf spot of sugar beet plants under field conditions.

Materials and Methods

Source of the pathogenic fungus and sugar beet seeds:

Pathogenic isolate of *Cercospora beticola* the causal agent of *Cercospora* leaf spot was kindly obtained from the Plant Pathol. Dept., National Res. Centre, Giza, Egypt. Meanwhile, sugar beet seeds (cv. Rosana) were obtained from the Dept. of Sugar Crop Res., Agric. Res. Centre, Giza, Egypt.

Effect of different chemical inducers on linear growth of C. beticola under laboratory conditions:

Chemical inducers, *i.e.* K_2HPO_4 , ascorbic acid and potassium bicarbonate were tested to study their inhibitory effect on the linear growth of *C. beticola* under laboratory conditions. Two concentrations of K_2HPO_4 (50 and 100 mM, ascorbic acid (2 and 3 g/l) and potassium bicarbonate (10 and 20 g/l) were added individually to conical flasks containing sterilized PDA medium to obtain the desired concentrations, then mixed gently and dispensed in sterilized Petri plates (9 cm diameter). Plates were individually inoculated at the centre with equal disks (6mm diameter) of 10 day old culture of *C. beticola*. Five plates were used as replicates for each particular treatment. Inoculated plates were incubated at $25\pm 2^\circ C$. Linear growth of fungus was measured, when the control plates reached full growth and the average growth diameter was calculated.

Effect of different chemical inducers on Cercospora leaf spot disease of sugar beet plants under field conditions:

Experiments were carried out in the experimental farm of the National Research Centre at El-Noubariya, Behera governorate. The chemical inducers were applied as foliar spraying under field conditions to study the possibility of their effect under large scale for safe control against *Cercospora* leaf spot disease during two growing seasons, first during October 2007 to April 2008 and second from October 2009 to April 2010. Sugar beet yield and total soluble solids (TSS) were also determined at the two growing seasons.

Field experiments were conducted under conditions of natural infection. Sugar beet seeds cv. Rosana were sown in plots (4x8 m), each comprised of 8 rows and 32 holes/row. A completely randomized block design with three replicates (plots) for each particular treatment was used. Agricultural practices were carried out as recommended.

Treatments:

Two concentrations of each chemical inducer, *i.e.* K_2HPO_4 at 50.0 and 100.0 mM, ascorbic acid at 2.0 and 3.0 g/l and potassium bicarbonate at 10.0 g/l and 20.0 g/l in addition to fungicides Ridomil-plus at 2 g/l as recommended dose (metalaxyl-M and S-isomer, produced by trademark of United States Borax and Chemical Corporation)

Application:

All treatments were applied as foliar spray on sugar beet plants after 40 and 70 and 100 days from sowing while, the fungicide was sprayed every 15 days from 40 days to 100 days of sowing.

Disease assessment:

Cercospora leaf spot scale adopted by Jones and Windels (1991) was modified as follows:

- 0 = No leaf lesions.
- 1 = 5% or less of leaf area.
- 2 = More than 25 to 50 %
- 3 = More than 50 to 75 %
- 4 = More than 75 to 100 % infected leaf area.

Disease was recorded three times until the 130th day of sowing.

Determination of sugar beet yield:

Sugar beet yield (tons/feddan) for each treatment was determined.

Determination of total soluble solids (TSS) of sugar beet yield:

At harvest time (190 days from sowing) a random sample of ten roots were taken from each replicate to determine total soluble solids (T.S.S %) by using a hand refractometer, according the method described by Carruthers and Oldfield (1961).

Effect of different chemical inducers on chitinase activity of sugar beet plants under field conditions:

Two concentrations of each chemical inducer, *i.e.* K_2HPO_4 at 50 and 100 mM, ascorbic acid at 2 and 3 g/l and potassium bicarbonate at 10 and 20 g/l were applied to study their effect on chitinase activity in sugar beet plants.

Extraction of enzyme:

Chitinase activity was determined after 110 days from sowing. The enzyme was extracted from sugar beet leaves and the supernatant was prepared according to the method described by Tuzun *et al.* (1989).

Chitinase assay:

Chitinase activity was determined by the colorimetric method adopted by Boller and Mauch (1988). Colloidal chitin was used as substrate and dinitrosalicylic acid as

reagent to measure reducing sugars. Chitinase activity was expressed as mM N-acetylglucose amine equivalent released / gram fresh weight tissue / 60 minutes.

Statistical analysis:

Tukey test for multiple comparisons among means was utilized (Neler *et al.*, 1985).

Results

Effect of chemical inducers on the linear growth of C. beticola:

Two concentrations of each treatment, *i.e.* K_2HPO_4 at 50 & 100 mM, ascorbic acid at 2 & 3 g/l and potassium bicarbonate at 10 & 20 g/l were tested to study their inhibitory effect against *C. beticola* under laboratory conditions. Results in Table (1) indicate that both tested concentrations of ascorbic acid had no inhibitory effect on the tested fungus. However, complete reduction in the linear growth of *C. beticola* was obtained with $KHCO_3$ at 20 g/l. The highest reduction was achieved with $KHCO_3$ at 10 g/l and K_2HPO_4 at 100 mM which reduced the linear growth more than 72.2%. Meanwhile, other treatments were less effective.

Table 1. Effect of chemical inducers on the linear growth of *C. beticola* under laboratory conditions

Chemical inducer	Concentration	Linear growth (mm) *	Reduction
K_2HPO_4	50 mM	70.2 b	22.0
	100 mM	25.0 c	72.2
Ascorbic acid	2 g/l	90.0 a	0.0
	3 g/l	90.0 a	0.0
$KHCO_3$	10 g/l	20.0 c	77.8
	20 g/l	00.0 d	100.0
Control	0.0	90.0 a	---

* Figures with the same letter are not significantly different (P= 0.05).

Effect of different chemical inducers on Cercospora leaf spot disease of sugar beet plants under field conditions:

Two concentrations of the chemical inducers, *i.e.* K_2HPO_4 at 50.0 and 100.0 mM, ascorbic acid at 2 and 3 g/l and potassium bicarbonate at 10 & 20 g/l in addition to the fungicide Ridomil-plus at 2 g/l were applied under field conditions to study their effect on severity of *Cercospora* leaf spot disease of sugar beet plants, in addition to sugar beet root yield and total soluble solids (T.S.S %). Results in Table (2) indicate that all treatments have significantly reduced the disease severity of sugar beet plants. The highest reduction was obtained with K_2HPO_4 at the concentration of 100 mM and $KHCO_3$ at 20 g/l which reduced *Cercospora* leaf spot severity by 70.0 and 76.7 %, respectively during the two growing seasons. Treated sugar beet plants with K_2HPO_4 at 50 mM, ascorbic acid at 3 g/l, $KHCO_3$ at 10 g/l and the fungicides Ridomil-plus at 2 g/l resulted in reduction in *Cercospora* leaf spot severity by 50.0%, during the two growing seasons. Meanwhile, other treatments were less effective.

Table 2. Effect of chemical inducers on Cercospora leaf spot of sugar beet plants under field conditions

Treatment	Conc.	Cercospora leaf spot severity *			
		First season 2008/09		Second season 2009/10	
		Disease severity	Reduction (%)	Disease severity	Reduction (%)
K ₂ HPO ₄	50 mM	1.2 d **	60.0	1.3 bc	59.4
	100 mM	0.9 f	70.0	0.8 d	75.0
Ascorbic acid	2.0 g / l	1.8 b	40.0	1.7 b	46.9
	3.0 g / l	1.2 cd	60.0	1.1 c	65.7
KHCO ₃	10.0 g / l	1.5 c	50.0	1.3 bc	59.4
	20.0 g / l	0.7 f	76.7	0.6 d	81.3
Ridomil – plus	2 g / l	1.2 cd	60.0	1.1 c	65.7
Control	0.00	3.0 a	---	3.2 a	---

* Disease severity scale from 0 to 4 according to Jones and Windels (1991) based on infected leaf area.

** Figures with the same letter are not significantly different (P = 0.05).

Effect of chemical inducers on sugar beet yield under field conditions:

Results in Table (3) indicate that all treatments significantly increased sugar beet yield. The highest increase was obtained with K₂HPO₄ at the concentration of 100 mM and KHCO₃ at 20 g/l which increased the sugar beet yield more than 15.2 and 17.4%, respectively, during the two growing seasons. Treated sugar beet plants with K₂HPO₄ at 50 mM, ascorbic acid at 3 g/l, KHCO₃ at 10 g/l and the fungicides Ridomil-plus at 2 g/l resulted in increasing the sugar beet yield more than 10.0% during the two growing seasons. While, other treatments were less effective.

Table 3. Effect of chemical inducers on sugar beet yield under field conditions

Treatment	Conc.	Sugar beet yield (ton/feddan)*			
		First season 2008/09		Second season 2009 /10	
		Yield	Increase (%)	Yield	Increase (%)
K ₂ HPO ₄	50 mM	25.5 b	10.9	23.5 b	11.9
	100 mM	26.5 a	15.2	25.5 a	21.4
Ascorbic acid	2 g / l	25.0 bc	8.7	23.0 bc	9.5
	3 g / l	25.5 b	10.9	24.0 b	14.3
KHCO ₃	10 g / l	25.0 bc	8.7	23.1 bc	10.0
	20 g / l	27.0 a	17.4	26.0 a	23.8
Ridomil-plus	2 g / l	25.5 b	10.9	23.1 bc	10.0
Control	0.0	23.0 f	---	21.0 d	---

* Figures with the same letter are not significantly different (P = 0.05).

Effect of chemical inducers on total soluble solids (TSS) in sugar beet roots under field conditions:

Results in Table (4) indicate that the highest increase in TSS was obtained with K_2HPO_4 at the concentration of 100 mM and $KHCO_3$ at 20 g/l which increased the total soluble solids (TSS) of sugar beet roots by 18.7 and 20.0%, respectively. While spraying with K_2HPO_4 at 50 mM and $KHCO_3$ at 10 g/l increased the total soluble solids (TSS) of sugar beet roots by 13.3 and 14.7%, respectively. Meanwhile, other treatments were less effective.

Table 4. Effect of chemical inducers on the total soluble solids (TSS) of sugar beet yield under field conditions

Treatment	Concentration	T.S.S. (%)	Increase (%)
K_2HPO_4	50 mM	17.0 b *	13.3
	100 mM	17.8 a	18.7
Ascorbic acid	2 g/l	15.0 f	0.0
	3 g/l	16.0 d	6.7
$KHCO_3$	10 g/l	17.2 b	14.7
	20 g/l	18.0 a	20.0
Ridomil - plus	2 g/l	15.0 f	0.0
Control	0.0	15.0 f	---

* Figures with the same letter are not significantly different ($P = 0.05$).

Effect of chemical inducers on chitinase activity of sugar beet plants under field conditions:

Two concentrations of each chemical inducer, *i.e.* K_2HPO_4 at 50 and 100 mM, ascorbic acid at 2 and 3 g/l and potassium bicarbonate at 10 and 20.0 g/l were applied to study their effect on chitinase activity in sugar beet plants. Results in Table (5) indicate that all treatments have significantly increased the chitinase activity in sugar beet leaves. The highest increase was obtained with K_2HPO_4 at concentration 100 mM, $KHCO_3$ at 10 & 20 g/l and ascorbic acid at 3.0 g/l which increased the chitinase activity by 113.0, 113.0, 126.7 and 113.0%, respectively. Spraying with K_2HPO_4 at 50 mM, increased the chitinase activity by 100.0%. Ascorbic acid at 2 g/l and the fungicide Ridomil plus at 2 g/l caused slight increase in chitinase activity by 66.7%.

Table 5. Effect of chemical inducers on chitinase activity of sugar beet plants

Treatment	Concentration	Chitinase activity	Increase (%)
K_2HPO_4	50 mM	3.0 b **	100.0
	100 mM	3.2 a	113.0
Ascorbic acid	2 g/l	2.5 c	66.7
	3 g/l	3.2 a	113.0
$KHCO_3$	10 g/l	3.2 a	113.0
	20 g/l	3.4 a	126.7
Ridomil - plus	2 g/l	2.5 c	66.7
Control	0.0	1.5 e	---

* Chitinase activity was expressed as mM N-acetyl glucose amine equivalent released/gram fresh weight/60 min.

** Figures with the same letter are not significantly different ($P = 0.05$)

Discussion

Cercospora leaf spot caused by *Cercospora beticola* is one of the most economically important diseases of sugar beets (Bugbee, 1995; Dexter *et al.*, 1998; Enikuomehin, 2005 and Harveson, 2007). Induced resistance against several diseases was reported by many researchers (Reuveni *et al.*, 1995; Khan *et al.*, 2001; Abd-El-Kareem, 2007 and Haggag and Abd-El-Kareem, 2009). In laboratory trials, results indicated that both tested concentrations of ascorbic acid had no inhibitory effect on the tested fungus. While, complete reduction in linear growth of *C. beticola* was obtained with KHCO_3 at 20 g/l.

Dibasic potassium phosphate (K_2HPO_4) was commonly used for induction of resistance against several diseases (Mucharromah and Kuc, 1991; Reuveni *et al.*, 1996 and Abd-El-Kareem *et al.*, 2001 & 2002 and 2004). In present study, 100 mM of K_2HPO_4 caused a pronounced reduction in *Cercospora* leaf spot disease of sugar beet plants and significantly increased the sugar beet yield and total soluble solids (TSS). Treated potato plants with K_2HPO_4 and Chitosan induced resistance against late blight disease and increased tuber yield under field conditions (Abd-El-Kareem *et al.*, 2002). The complete underlying mechanisms of phosphate salts for controlling plant diseases have not clearly determined (Mucharromah and Kuc, 1991). There is a possibility that phosphate salts might increase the synthesis of host metabolites such as phytoalexins, in view of evidence that phytoalexin production can be induced by phosphate salts (Reuveni *et al.*, 1995). In the present study, the highest increase in chitinase activity was obtained with K_2HPO_4 at concentration 100 mM, KHCO_3 at 10 & 20 g/l and ascorbic acid at 3 g/l which increase the chitinase activity more than 113.0%. The increased accumulation of chitinase and β -1,3-glucanase in treated plant leaves with K_2HPO_4 strongly indicate the possible role of both enzymes in defence mechanism for controlling plant diseases (Irving and Kuc, 1990 and Abd-El-Kareem *et al.*, 2001).

Content of ascorbic acid in plant tissues has been associated with resistance to some diseases (Zacheo *et al.*, 1977). In present study, ascorbic acid concentration 3 g/l caused dramatically reduction in *Cercospora* leaf spot disease of sugar beet plants and significantly increased the sugar beet yield and total soluble solids (TSS). In this concern, El-Gamal *et al.* (2007) reported that potato plants sprayed with ascorbic acid caused significant reduction in late and early blight under greenhouse and field conditions. In our results, ascorbic acid had no inhibitory effect on the tested fungus but stimulation of chitinase activity by 113%. This enzyme involved in resistance against plant pathogens (Datta *et al.*, 2001). Using ascorbic acid for controlling several plant fungal diseases was reported by (Shahda 2000; Khan *et al.*, 2001 and El-Gamal *et al.*, 2007).

Potassium bicarbonate was found to suppress several fungal diseases of cucumber plants (Ziv and Zitter, 1992). In the present study, potassium bicarbonate caused more reduction in *Cercospora* leaf spot disease of sugar beet plants and significantly increased the sugar beet yield and total soluble solids (TSS). This result may be due to: the antifungal activity of bicarbonate and increased the activity of chitinase enzyme against fungal pathogens. In this respect, Punja and Grogan (1982)

reported that the improved effectiveness of control with bicarbonates was attributed to fungicidal characteristics of bicarbonates ions.

Hypotheses have been proposed for the inhibitory mechanisms of bicarbonates. Hydrogen ion concentration of bicarbonates salts has been shown to have a profound inhibitory effect on sclerotia and conidia germination of *S. rolfsii* and *S. fuliginea*, respectively, (Punja and Grogan, 1982 and Homma *et al.*, 1981). The bicarbonate causes the collapse of hyphal walls and shrinkage of conidia (Punja and Grogan, 1982; Ziv and Zitter, 1992 and Horst *et al.*, 1992). On the other hand the role of potassium ions (potassium bicarbonate) in increasing crop resistance to diseases caused by bacteria and fungi was widely reviewed by Perrenoud (1990). In general, potassium application improves plant health and vigour, making infection less likely or enabling a quick recover (Perrenoud, 1993). Potassium probably exerts its greatest effects on disease through specific metabolic functions that alter compatibility relationships of the host-parasite environment and increases the production of disease inhibitory compounds, such as phenols, phytoalexins and auxins around the infection sites of resistant plants, (Kiralý, 1976).

It could be suggested that some chemical inducers may be used for controlling *Cercospora* leaf spot disease of sugar beet under field conditions.

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المقاومة المستحثة ضد تبقع الأوراق السركوسبوري في نباتات بنجر السكر تحت ظروف الحقل

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** قسم النبات الزراعي - كلية الزراعة - جامعة بنها - مصر.

تمت دراسة تأثير عدد من المستحضرات الكيماوية وهي فوسفات البوتاسيوم ثنائية القاعدية (K_2HPO_4) وحمض الأسكوربيك وبيكربوات البوتاسوم ($KHCO_3$) في مكافحة مرض تبقع الأوراق السركوسبوري في نباتات بنجر السكر تحت ظروف الحقل وأوضحت النتائج ما يلي :-

في تجارب المعمل أدت معاملة بيكربونات البوتاسيوم بتركيز ٢٠ جم / لتر إلى التثبيط الكامل للنمو الطولي للفطر سرкосبورا بيتيكولا بينما لم يؤد حمض الأسكوربيك بتركيز ٢ و ٣ جم / لتر إلى تثبيط للنمو الطولي للفطر المختبر. أوضحت النتائج تحت ظروف الحقل أن المعاملتان فوسفات البوتاسوم ثنائية القاعدية بتركيز ١٠٠ ملليمول و بيكربونات البوتاسيوم بتركيز ٢٠ جم / لتر أدتا إلى تثبيط مرض تبقع الأوراق السركوسبوري بواقع ٧٠ و ٧٦,٧% على الترتيب على الأقل خلال موسمي النمو.

معاملة نباتات البنجر بفوسفات البوتاسوم ثنائية القاعدية بتركيز ٥٠,٠ ملليمول وبيكربونات البوتاسيوم بتركيز ١٠ جم / لتر وحمض الأسكوربيك بتركيز ٣٠,٠ جم / لتر والمبيد ريدوميل بلس بتركيز ٢ جم / لتر أدت إلى تثبيط مرض تبقع الأوراق السركوسبوري بواقع ٥٠% على الأقل.

أدت جميع المعاملات إلى زيادة معنوية في كمية المحصول وأعلى نسبة في كمية المحصول تم الحصول عليها بواسطة المعاملتان فوسفات البوتاسوم ثنائية القاعدية بتركيز ١٠٠ ملليمول و بيكربونات البوتاسيوم بتركيز ٢٠ جم / لتر حيث أدتا إلى زيادة معنوية بمقدار ١٥,٢ و ١٧,٤% وكذلك المواد الصلبة الذائبة الكلية في الجذور (TSS) بمقدار ١٨,٧ و ٢٠,٠% على الترتيب. أدت جميع المعاملات إلى زيادة معنوية في نشاط انزيم الكيتينيز وأدت المعاملات فوسفات البوتاسوم ثنائية القاعدية بتركيز ١٠٠ ملليمول و بيكربونات البوتاسيوم بتركيز ١٠ و ٢٠ جم/لتر وحمض الأسكوربيك بتركيز ٣٠,٠ جم / لتر إلى زيادة معنوية بمقدار ١١٣ و ١١٣ و ١٢٦,٧ و ١١٣% على الترتيب. وتشير النتائج إلى إمكانية استخدام بعض المستحضرات الكيماوية في مكافحة مرض تبقع الأوراق السركوسبوري في نباتات بنجر السكر تحت ظروف الحقل.