
Effects of citrus essential oil compounds on management leaf spot disease on sugar beet plant under field condition

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Effect of Citral, Methyl anthranate and Nerol as fractions of citrus essential oils for controlling *Cercospora* and *Alternaria* leaf spot diseases of sugar beet plants under field conditions was evaluated. In laboratory experiments, results indicated complete reduction in linear growth of *A. tenuis* and *C. beticola* was obtained with Citral, Methyl anthranate and Nerol at concentration of 5.0 ml / l. In field trials, results indicated that the most effective treatments are Citral, Methyl anthranate and Nerol at 5.0 ml / l. reduced the *Alternaria* leaf spot severity more than 78.3 and 80.0 % respectively during two growing seasons. As for *Cercospora* leaf spot, results indicated that Citral, Methyl anthranate and Nerol at 5.0 ml/l. and Fungicides (Redomyl – plus at 2 g/l) reduced the *Cercospora* leaf spot severity more than 67.5 and 78.1% respectively during two growing seasons. The highest increase in sugar beet yield was obtained with Citral and Methyl anthranate at 5.0 ml / l. which increase the sugar beet yield more than 11.5 and 10.5% respectively during two growing seasons. Citral at concentration 5.0 ml / l. increased the total soluble solids (TSS) of sugar beet yield by 6.7%. While slight increase was obtained with Citral at 2.5 ml/l, Methyl anthranate and Nerol at 5.0 ml / l. for each treatment. It could be suggested that constituents of essential oils may be used as eco friendly natural compounds for controlling *Cercospora* and *Alternaria* leaf spot diseases of sugar beet plants under field conditions.

Key words: Sugar beet, *Cercospora beticola*, *Alternaria tenuis*, Citrus essential oils, Citral, Methyl anthranate, Nerol.L

Introduction

Sugar beet (*Beta vulgaris* L.) is considered the second sugar crop for sugar production in Egypt followed by sugarcane. 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-

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Mervat and Mekki, 2005). *Cercospora* and *Alternaria* leaf spot caused by the fungi *Cercospora beticola* and *Alternaria tenuis* respectively are the most economically important diseases of sugar beets (Bugbee, 1995.; Dexter, *et al.*, 1998; Enikuomihin, 2005 and Harveson, 2007). Controlling these diseases depends mainly on fungicidal treatments. Avoiding environmental pollution fungicides alternatives are needed (El-Gamal- Nadia, *et al.*, 2007 and Haggag-Wafaa and Abd-El-Kareem, 2009). Citral, Methyl anthranate and Nerol are some fractions of citrus essential oil caused complete inhibition of the linear growth of *Giotrichum candidum*, *Penicillium digitatum* and *P. italicum* as causal agents of fruit citrus diseases (El-Mohamedy *et al.*, 2002). Abd-El-Kareem, (2007) reported that in greenhouse experiments, the most effective treatments were potassium or sodium bicarbonates and nerol at 0.50% which reduced the early blight incidence more than 70.6% as compared with untreated plants. They added that, in field experiments, the pronounced treatment was potassium bicarbonate plus nerol at 0.5% which reduced the disease incidence more than 86.8%. The main objectives were to study the effect of Citral, Methyl anthranate and Nerol against *Cercospora* and *Alternaria* leaf spots diseases of sugar beet plants under field conditions.

Materials and methods

Source of pathogenic fungi and sugar beet seeds

Pathogenic isolates of *Cercospora beticola* and *Alternaria tenuis* the causal agents of *Cercospora* and *Alternaria* leaf spot diseases were kindly obtained from Department of Plant Pathology National Research Centre, Giza, Egypt. Meanwhile, sugar beet seeds cv. Rosana were obtained from Department of Crop Research, Agricultural Research Centre, Giza, Egypt.

Testing of some essential oils on linear growth of A. tenuis and C. beticola under laboratory conditions

Three compounds of citrus essential oil i.e., Citral, Methyl anthranate and Nerol were tested to study their inhibitory effect on linear growth of *A. tenuis* and *C. beticola* under laboratory conditions. Four concentrations of each treatment i.e. 0.00, 1.25, 2.50 and 5.0 ml/l were added individually to conical flasks containing sterilized PDA medium to obtain the proposed concentrations, then mixed gently and dispensed in sterilized Petri plates (10 cm; diameter). Plates were individually inoculated at the center with equal disks (6-mm; diameter) of 10-days old culture of *A. tenuis* and *C. beticola*. Five plates were used as replicates for each particular treatment. Inoculated plates

were incubated at $25 \pm 2^{\circ}\text{C}$. The average linear growth of fungus was calculated after 10 days.

Effect of citrus essential oil compounds on Cercospora and Alternaria leaf spot diseases under field conditions

Experiments were carried out, in Experimental Farm of National Research Centre at El-Noubareia, Behera governorate during two successive seasons 2009 and 2010. Three compounds of citrus essential oils i.e. Citral, Methyl anthranate and Nerol were applied under field conditions to study the possibility of their effect under large scale for safe control against *Cercospora* and *Alternaria* leaf spot diseases during tow cultivation seasons. Sugar beet yield and total soluble solids (TSS) were also determined at two cultivation seasons. Field experiments were conducted under natural infection in plots (4x8 m) each comprised of 8 rows and 32 holes/row, was conducted in a completely randomized block design with three replicates (plots) for each particular treatment.

Citral, Methyl anthranate and Nerol at three concentrations *i.e.* 0.00, 2.50 and 5.0 ml/l in addition to Fungicides (Redomyl-plus at 2g/l as comparison treatment) were applied under field conditions. All treatments were applied as foliar application on sugar beet plants every 30 days.

Disease assessment

Cercospora leaf spot scale described by Jones and Windels, (1991) was modified as follow : 0 = No leaf lesions., 1 = 25% or less., 2 = 26 to 50., 3 = 51 to 75., 4 = 76 to 100%., infected leaf area. Disease was recorded until 130 days of sowing.

Alternaria leaf spot scale from 0 to 4 according to Vakalunakis, (1990) based on the leaf area infected was used, as follows: -
0 = No leaf lesions 1 = 25%, lesion 2 = 26 to 50, lesion 3 = 51 to 75, lesion 4 = 76 to 100% infected leaf area. Disease was recorded until 130 days of sowing.

Determination of sugar beet yield

Sugar beet yield (Ton / 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 plants were taken from each replicate to determine total soluble solids (T.S.S %) by using Hand Fractometer.

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

Results

Effect of citrus essential oil compounds on linear growth of A. tenuis and C. beticola in Vitro

Four concentrations of Citral, Methyl anthranate and Nerol *i.e.* 0.00 1.25, 2.50 and 5.0 ml/l were tested to study their inhibitory effect on linear growth of *A. tenuis* and *C. beticola*. Results indicated that complete reduction in linear growth of both fungi was obtained with Citral, Methyl anthranate and Nerol at 5.0 ml/l concentration (Table 1). The highest reduction was achieved with Citral, Methyl anthranate and Nerol at 2.5 ml/l., which reduced the linear growth more than 71.7 and 66.7% for *A. tenuis* and *C. beticola* respectively. Meanwhile, other treatments were less effective.

Table 1. Effect of *citrus* essential oil compounds on linear growth of *A. tenuis* and *C. beticola* under laboratory conditions

Treatments	Conc (ml/l)	<i>A. tenuis</i>		<i>C. beticola</i>	
		Linear growth (mm)	Reduction (%)	Linear growth (mm)	Reduction (%)
Citral	1.25	40.0 c	55.6	52.0 b	38.0
	2.5	20.0 d	77.8	27.6 c	69.3
	5.0	0.00e	100.0	0.00 d	100.0
Methel anthranate	1.25	52.0 b	38.0	57.0 b	36.7
	2.5	19.5 d	78.3	30.0 c	66.7
	5.0	0.00 e	100.0	0.00 d	100.0
Nerol	1.25	54.0 b	40.0	61.0 b	33.2
	2.5	25.5 d	71.7	28.5 c	68.3
	5.0	0.00e	100.0	0.00 d	100.0
Control		90.0 a	0.0	90.0 a	0.0

Means with the same letter are not significantly different (p= 0.05)

Effect of citrus essential oil compounds on Alternaria leaf spot under field conditions

Citral, Methyl anthranate and Nerol at three concentrations *i.e.* 0.00 , 2.50 and 5.0 ml/l in addition to Fungicides (Ridomyl – plus at 2g/l) were applied under field conditions to study their effect on Cercospora and Alternaria leaf spot diseases of sugar beet plants. In addition to sugar beet yield and total soluble solids (TSS %).

Results indicated that all treatments significantly reduced the disease severity of sugar beet plants (Table 2). The most effective treatments are Citral, Methyl anthranate and Nerol at 5.0 ml/l. which reduced the *Alternaria* leaf spot severity more than 78.3 and 80.0% respectively during two growing seasons. Moderate effect was obtained with Citral, Methyl anthranate and Nerol at 2.5 ml/l. and Fungicides (Ridomyl-plus at 2g/l) which reduced the disease severity more than 56.5 and 55.0% during two growing seasons.

Table 2. Effect of citrus essential oil compounds on *Alternaria* leaf spot of sugar beet plants under field conditions during 2009 and 2010 seasons.

Treatments	Conc.	<i>Alternaria</i> leaf spot			
		First season 2009		Second season 2010	
		Disease severity	Reduction (%)	Disease severity	Reduction (%)
Citral	2.5 ml/L	0.8 b	65.2	0.7 b	65.0
	5.0 ml/L	0.4 c	82.6	0.2 c	90.0
Methyl anthranate	2.5 ml/L	1.0 b	56.5	0.9 b	55.0
	5.0 ml/L	0.5 c	78.3	0.3 c	85.0
	2.5 ml/L	1.0 b	56.5	1.0 b	50.0
Nerol	5.0 ml/L	0.5 c	78.3	0.4 c	80.0
Fungicide	2g/L	1.0 b	56.5	0.9 b	55.0
Control		2.3 a	0.0	2.0 a	0.0

Means with the same letter are not significantly different ($p=0.05$)

Effect of citrus essential oil compounds on Cercospora leaf spot under field conditions

Results indicated that all treatments significantly reduced the disease severity of sugar beet plants (Table 3). The most effective treatments are Citral, Methyl anthranate and Nerol at 5.0 ml/l. and Fungicides (Ridomyl-plus at 2g/l) which reduced the *Cercospora* leaf spot severity more than 67.5 and 78.1% respectively during two growing seasons. Moderate effect was obtained with Citral, Methyl anthranate and Nerol at 2.5 ml/l. and which reduced the disease severity more than 55.0 and 56.3% during two growing seasons.

Effect of citrus essential oil compounds on sugar beet yield

Results indicated that all treatments significantly increase sugar beet yield (Table 4). The highest increase was obtained with Citral and Methyl anthranate at 5.0 ml/l. which increase the sugar beet yield more than 11.5 and 10.5%

respectively during two growing seasons. Treated sugar beet plants with Citral at 2.5 ml/l. and Nerol at 5.0 ml/l. increased the sugar beet yield more than 9.1 and 10.0 % during two growing seasons. Meanwhile, other treatments were less effective.

Table 3. Effect of citrus essential oil compounds on Cercospora leaf spot of sugar beet plants under field conditions during 2009 and 2010 seasons

Treatments	Conc.	Cercospora leaf spot			
		First season 2009		Second season 2010	
		Disease severity	Reduction (%)	Disease severity	Reduction (%)
Citral	2.5 ml/L	1.5 c	62.5	1.2 b	62.5
	5.0 ml/L	1.1 df	72.5	0.7 c	78.1
Methel anthranate	2.5 ml/L	1.7 bc	57.5	1.3 b	59.4
	5.0 ml/L	1.2 d	70.0	0.6 c	81.3
Nerol	2.5 ml/L	1.8 bc	55.0	1.4 b	56.3
	5.0 ml/L	1.3 d	67.0	0.6 c	81.3
Fungicide	2g/L	1.1 df	72.5	0.7 c	78.1
Control		4.0 a	0.0	3.2 a	0.0

Means with the same letter are not significantly different ($p=0.05$)

Table 4. Effect of citrus essential oil compounds on sugar beet yield under field conditions during 2009 and 2010 seasons.

Treatments	Conc.	Sugar beet yield (Ton/feddan)			
		First season 2009		Second season 2010	
		Sugar beet yield	Increase (%)	Sugar beet yield	Increase (%)
Citral	2.5 ml/L	24.0b	9.1	22.0 b	10.0
	5.0 ml/L	24.5b	11.4	23.1 a	15.5
Methel anthranate	2.5 ml/L	23.5c	6.8	21.5c	7.5
	5.0 ml/L	24.3 a	10.5	22.8 a	14.0
Nerol	2.5 ml/L	23.5c	6.8	21.5 c	7.5
	5.0 ml/l	24.0 b	9.1	22.0 b	10.0
Fungicide	2g/L	23.5c	6.8	21.5 c	7.5
Control		22.0 d	0.0	20.0 d	0.0

Means with the same letter are not significantly different ($p=0.05$)

Effect of citrus essential oil compounds on total soluble solids (TSS) of sugar beet yield

Results indicated that the highest increase was obtained with Citral at concentration 5.0 ml/l. which increase the total soluble solids (TSS) of sugar beet yield by 6.7%. Slight increase was obtained with Citral, at 2.5 ml/l.,

Methyl anthranate and Nerol at 5.0 ml/l. for each. Meanwhile other treatments had no effect (Table 5).

Table 5. Effect of citrus essential oil compounds on total soluble solids (TSS) of sugar beet yield under field conditions

Treatments	Concentrations	TSS %	Increase %
Citral	2.5 ml/ L	15.5 b	3.3
	5.0 ml/ L	16.0 a	6.7
Methyl anthranate	2.5 ml/ L	15.0 c	0.0
	5.0 ml/ l	15.6 b	4.0
	2.5 ml/ L	15.0 c	0.0
Nerol	5.0 ml/ l	15.5 b	3.3
Fungicide	2g/L	15.0 c	0.0
Control		15.0 C	0.0

Means with the same letter are not significantly different (p= 0.05)

Discussion

Sugar beet (*Beta vulgaris L.*) is considered as 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-Mervat and Mekki, 2005). Cercospora and Alternaria leaf spot caused by the fungi *Cercospora beticola* and *Alternaria tenuis* respectively are the most economically important diseases of sugar beets (Bugbee, 1995; Dexter, *et al.*, 1998; Enikuomihin, 2005 and Harveson, 2007). Citral, Methyl anthranate and Nerol as some fractions of citrus essential oil caused complete inhibition of the linear growth of *Giotrichum candidum*, *Penicillium digitatum* and *P. italicum* as causal agents of fruit citrus diseases (El-Mohamedy *et al.*, 2002). In present study, results indicated complete reduction in linear growth of *A. tenuis* and *C. beticola* was obtained with Citral, Methyl anthranate and Nerol at concentration 5.0 ml/l. The highest reduction was achieved with Citral, Methyl anthranate and Nerol at 2.5 ml/l. which reduced the linear growth more than 71.7 and 66.7% for *A. tenuis* and *C. beticola* respectively. In this respect Abd-El-Kareem *et al.*, (2007) Reported that Nerol as one fraction of citrus essential oil caused complete inhibition of the linear growth of *Alternaria solani* the causal organisms of early blight disease of potato plants. Moreover, Citral at 5.0 ml/l. caused complete reduction in linear growth of, *Penicillium digitatum* and *P. italicum* as causal agents of fruit citrus diseases Abd-El-Kareem and Abd- Alla, 2002). Under field conditions, in present study, results indicate that Citral, Methyl anthranate and Nerol at 5.0 ml/l caused dramatically reduction in Cercospora and Alternaria leaf spot diseases of sugar beet plants and

significantly increased the sugar beet yield and total soluble solids (TSS). Essential oils of citrus or their constituents are shown to have fungicidal activities against postharvest pathogens of citrus (French *et al.*, 1978; Caccioni *et al.*, 1998, and Rio del *et al.*, 1998). In this regards, some constituents of essential oils from citrus fruits more toxic against fungi than commercial fungicides (Singh *et al.*, 1993). In this regard, French, *et al.* (1978) stated that citral is the most effective constituent of citrus essential oils. Furthermore, Rodov *et al.*, (1985) reported that young mature–green lemon fruit manifests a significant lower level of postharvest decay compared with older yellow fruit. They added that, resistance of young fruit to decay development is related to citral level in lemon flavedo which contained 1.5-2 times higher levels of citral comparing with the yellow fruit. Also during long–term storage of lemon fruit, citral concentrations decreased in parallel with the decline of antifungal activity in the peel which reflected in the increase of decay incidence. Also, the inhibitory effect of citral on several fungi was also reported by Asthana *et al.*, (1988). In this respect Abd-El-Kareem and Abd-Alla, (2002) and Abd-El-Kareem (2007) they noted that using Nerol alone or in combination with potassium or sodium bicarbonates reduced the early blight incidence of potato plants under greenhouse and field conditions

Under field trials in two successive seasons, that were tested some citrus essential oil compounds such as Citral, Methyl anthranate and Nerol at 5.0 ml/l as alternatives of fungicides for controlling foliar diseases of sugar beet resulted in dramatically reduction in *Cercospora* and *Alternaria* leaf spot diseases of sugar beet plants and significantly increased the sugar beet yield and total soluble solids (TSS). It suggested that some essential oils may be used for controlling *Cercospora* and *Alternaria* leaf spot diseases of sugar beet plants under field conditions.

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