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Effects of citrus essential oil compounds on management leaf spot diseases on sugar beet under field conditions

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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 under field condition was evaluated In laboratory experiments, results indicated completely reduction in linear growth of *A. tenuis* and C. *beticola* which were obtained with Citral, Methyl anthranate and Nerol at concentration of 5.0 ml/l. In field trials , results indicated that the most effective treatments was 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. Results showed that Citral, Methyl anthranate and Nerol at 5.0 ml/l. and fungicides (Ridomyl at 2 g/l) reduced the Cercospora leaf spot severity more than 67.5 and 78.1% respectively. 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. Citral at concentration of 5.0 ml/l. increased the total soluble solids (TSS) of sugar beet yield by 6.7% .While, slightly increase TSS 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 as the second sugar crop for sugar production in Egypt followed by sugarcane. Recently, sugar beet crop has become 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

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the most economically important diseases of sugar beets (Bugbee, 1995; Dexter, *et al*., 1998; Enikuomehin, 2005 and Harveson, 2007).Controlling theses diseases depend mainly on chemical fungicidal. Avoiding environmental pollution, fungicide alternative application 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 *Getrichum candidum*, *Penicillium digitatum* and *Penicillium 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 treatment 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. In field experiment, the pronounced treatment was potassium bicarbonate plus nerol at 0.5 % which reduced the disease incidence more than 86.8 %. The main objectives of the present research were studies the effect of Citral, Methyl anthranate and Nerol against Cercospora and Alternaria leaf spots diseases of sugar beet under field conditions .

Materials and methods

Source of pathogenic fungi and sugar beet seeds

Pathogenic isolate 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 5.0ml/l were added individually to conical flasks containing sterilized potato dextrose agar (PDA) medium to obtain the proposed concentrations, then mixed gently and dispensed in sterilized Petri plates (10 cm–diameter). Plates were individually transferred culture 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 treatments. The tested plates were incubated at 25±2 °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 at 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 during 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. The experiment was conducted in a completely randomized block design with three replicates (plots) for each particular treatment.

Citral, Methyl anthranate and Nerol at three concentrations of 0.00, 2.50 and 5.0 ml/l in addition to fungicides (Ridomyl at 2 g/l as comparison treatment) were applied under field conditions. All treatments were applied as foliar application on sugar beet every 30 days.

Disease assessment

Cercospora leaf spot scale modified from Jones and Windels (1991) as follow: 0 = no leaf lesions. 1 = 25% or less, 2 = 26 to 50, 3 = 51 to 75 and 4 = 76 to 100% infected leaf area.

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 lesion,. 1 = 25% or less, 2 = 26 to 50, 3 = 51 to 75 and 4 = 76 to 100% infected leaf area. Diseases were 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* under laboratory condition

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 indicate 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)	*A. tenuis*		*C. beticola*	
		Linear growth (mm)	Reduction (%)	Linear growth (mm)	Reduction (%)
Citral	1.25	40.0 c[1]	55.6	52.0 b	38.0
	2.50	20.0 d	77.8	27.6 c	69.3
	5.00	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	0.0	90.0 a	0.0	90.0 a	0.0

[1]Mean followed by a common letter are not significantly different by DMRT at P = 0.05

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

Results indicated that al treatments significantly reduced the disease severity of sugar beet plants (Table 2). The most effective treatments were 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. Moderate effect was obtained with Citral, Methyl anthranate and Nerol at 2.5 ml/l. and fungicides (Ridomyl at 2 g/l) which reduced the disease severity more than 56.5 and 55.0% during 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.	Alternaria leaf spot			
		First season 2009		Second season 2010	
		Disease severity	Reduction %	Disease severity	Reduction %
Citral	2.5 ml/L	0.8 b[1]	65.2	0.7 b	65.0
	5.0 ml/L	0.4 c	82.6	0.2 c	90.0
Methel anthranate	2.5ml/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
Nerol	2.5ml/L	1.0 b	56.5	1.0 b	50.0
	5.0 ml/L	0.5 c	78.3	0.4 c	80.0
Fungicide	2 g/L	1.0 b	56.5	0.9 b	55.0
Control	0.0	2.3 a	0.0	2.0 a	0.0

[1]Mean followed by a common letter are not significantly different by DMRT at P = 0.05

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

Results showed that al treatments significantly reduced the disease severity of sugar beet plants (Table 3). The most effective treatments were Citral, Methyl anthranate and Nerol at 5.0 ml/l. and fungicides (Ridomyl at 2 g/l) which reduced the Cercospora leaf spot severity more than 67.5 and 78.1% respectively. 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 growing seasons.

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	2.5ml/L	1.5 c[1]	62.5	1.2 b	62.5
	5.0 ml/L	1.1 df	72.5	0.7 c	78.1
Methel anthranate	2.5ml/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	2.5ml/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	2 g/L	1.1 df	72.5	0.7 c	78.1
Control	0.0	4.0 a	0.0	3.2 a	0.0

[1]Mean followed by a common letter are not significantly different by DMRT at P = 0.05

Effect of citrus essential oil compounds on sugar beet yield

It is indicated that all treatments significantly increase sugar beet yield. (Table 4). The highest increased was obtained with Citral ans Methyl anthranate at 5.0 ml/l. which increased the sugar beet yield more than 11.5 and 10.5% respectively. Treated sugar beet 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 tow growing seasons. Meanwhile, Other treatments were less effective.

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[1]	9.1	22.0 b	10.0
	5.0 ml/L	24.5b	11.4	23.1a	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
	2.5 ml/L	23.5c	6.8	21.5 c	7.5
Nerol	5.0 ml/L	24.0 b	9.1	22.0 b	10.0
Fungicide	2 g/L	23.5c	6.8	21.5 c	7.5
Control	0	22.0 d	0.0	20.0 d	0.0

[1]Mean followed by a common letter are not significantly different by DMRT at P = 0.05

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

Result showed that the highest increase in TSS was obtained with Citral at concentration 5.0 ml/l which increased the total soluble solids (TSS) of sugar beet yield by 6.7%. Slight increased 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. Effect of citrus essential oil compounds on total soluble solids (TSS) of sugar beet yield under field conditions.

Treatments	Concentrations	T.S.S. %	Increase %
Citral	2.5 ml/L	15.5 b[1]	3.3
	5.0 ml/L	16.0 a	6.7
Methel anthranate	2.5 ml/L	15.0 c	0.0
	5.0 ml/L	15.6 b	4.0
Nerol	2.5 ml/L	15.0 c	0.0
	5.0 ml/L	15.5 b	3.3
Fungicide	2 g/L	15.0 c	0.0
Control	0	15.0 C	0.0

[1]Mean followed by a common letter are not significantly different by DMRT at P = 0.05

Discussion

In present study , results indicated complete reduction in linear growth of *A. tenuis* and C. *beticola* after treated 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 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 can complete inhibition of *Alternaria solani* caused organisms of early blight disease of potato. Moreover, Citral at 5.0 ml/l. could complete growth reduction of *P. 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 showed that Citral, Methyl anthranate and Nerol at 5.0 ml/l could dramatically reduction in Cercospora and Alternaria leaf spot diseases of sugar beet and significantly increased the sugar beet yield and total soluble solids (TSS). Essential oils of citrus or their constituents are shown to be 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 gave more toxic against fungi than commercial fungicides (Singh *et al*., 1993, French *et al*., (1978) stated that citral is the most effective constituent of citrus essential oils. Furthermore, Rodov *et al*. (1995) reported that young mature – green lemon fruit manifests a significant lower level of postharvest decay compared with older yellow fruit. The 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. During long term storage of lemon fruit, citral can be decreased in parallel with the decline of antifungal activity in the peel which reflected in the increase of decay incidence. 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) noted that using Nerol alone or in combination with potassium or sodium bicarbonates can be reduced the early blight incidence of potato under greenhouse and field conditions

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