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Efficacy of certain fungicide alternatives for controlling sugar beet powdery mildew

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Abstract

In order to study the importance of selection of suitable sugar beet cultivars for growing in Upper Egypt, ten sugar beet cultivars were tested for their susceptibility to *Erysiphe betae*, the causal fungus of powdery mildew Among them, Sirona (453 AUPMPC; the Area Under Powdery Mildew Progress Curve) was significantly the most resistant cultivar to powdery mildew disease while, FD.0807 (1484 AUPMPC) was the most susceptible one. Field experiment was conducted to evaluate five plant extracts, three chemical antioxidants and four microelement compounds for their efficacy in controlling *E. betae*. Results confirmed that all tested treatments caused significant reduction in AUPMPC values when sugar beet plants were sprayed with these tested compounds and the increasing of the concentration increased resistance of sugar beet plants against powdery mildew disease. Among the tested plant extracts, the highest protection (69.9% & 66.9%) on both cultivars Sirona and FD.0807 respectively was achieved by 30% of basil extract followed by 30% of garlic extract (53% & 60.4%) while, the least protection (33.9% & 52.8%) was obtained by 10% of black cumin seed extract. Concerning the tested inducers, the highest protection (56.7% & 70.6%) was achieved by 300 ppm of salicylic acid followed by 200 ppm of salicylic acid (51% & 63.7%), while, 100 ppm of ascorbic acid gave the least protection (2.2% & 34.1%). On the other hand, 40 ppm of cupric sulfate achieved the best percentage of protection (72% &78.7%) whereas, the least protection (8%) was obtained by 10 ppm of zinc sulfate on Sirona cultivar and the least protection (43.6%) was obtained by 10 ppm of magnesium sulfate on FD.0807 cultivar. The best comparative treatment was the tested fungicide Bellis® 38% WG (89.5% &90% protection). Usage of plant extracts, antioxidants and micro elements are well recommended as fungicide alternatives for controlling the disease in parallel with their safe influence on human health due to reduction of the accumulated chemical hazards in the plant tissues.

Keywords: sugar beet, powdery mildew, *Erysiphe betae*, plant extracts, micronutrients.

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Introduction

Powdery mildew of sugar beet caused by *Erysiphe betae* (Vanha) Weltzien is a common disease in many countries (Hills *et al.,* 1980). In Egypt, environmental conditions are favorable for the fungus to spread rapidly especially in the late sowings after September (El-Fahhar, 2008).Disease spread occurs mostly by conidial infections, which dispersed by wind (Kontradowitz & Verreet, 2010).The infection of sugar beet by powdery mildew is economically significant for growers worldwide and can cause sugar yield losses up to 30% (Francis, 2002). Utilizing disease resistance is an important strategy for controlling plant pathogens because it may be relatively easy to use, cost-effective and environmentally more acceptable than reliance on pesticides (Hogenboom, 1993). Moderate resistance to the disease is obtainable in commercial varieties appearing in "slow-mildewing" phenotypes (Kontradowitz & Verreet, 2010). Many chemical constituents extracted from different plant genera were tested in the past years and found to be successful in controlling powdery mildew fungi on several crops (Moharam & Ali, 2012; El-Fahhar et al., 2009).The activation of a wide range of disease resistance mechanisms and the production of a wide range of defense compounds; it is race non-specific and is often effective against a broad spectrum of pathogenic agents (Ismail et al., 2012; Galal & Abdou, 1996; Kuc, 1995; Elad, 1992).The objective of this work was to evaluate some sugar beet cultivars for their susceptibility to powdery mildew disease and to evaluate some chemical and plant extracts for controlling the disease under field conditions.

Materials and methods

Reaction of ten sugar beet cultivars to powdery mildew: Field experiment was carried out at the experimental field of the Faculty of Agriculture, Al-Azhar University, Assiut governorate, Egypt, during 2013/2014 and 2014/2015 growing seasons. Seeds of ten sugar beet cultivars (Belatos, Capel, Oscarpoly, FD.0807, Pleno, Glorius, Sirona, Hilospoly, Samba and Athospoly) which were kindly gifted from Agricultural Research Station, Sakha, Kafr El-Sheikh were sown in plots consisted of ten rows (9-meters long, interspace between plant and another 20 cm and 3 seeds/hole were sown) in a completely randomized design with three replicates of each cultivar. Sugar beet plants were fertilized and irrigated as recommended by Egyptian Ministry of Agriculture. Plants were thinned to one plant/hole and left for natural infection. Disease severity was estimated three times, when plants were closing the rows (120 days after sowing) then estimated twice again (140 and 160 days after sowing**)** to calculate the Area Under Powdery Mildew Progress Curve (AUPMPC) values.

Powdery mildew assessment: Evaluation of disease severity was accomplished by examining both sides of leaves and rating disease intensity as the extent of leaf area covered by the fungus mycelium on a scale of 0 to 4 according to the scale by Whitney *et al.,* (1983). Scale ranged from 0-4, categories whereas 0= no mildew colonies observed, 1=1-25%, 2=26-50%, 3=51-75% and 4=76-100% of matured leaf area covered by mildew.

Area Under Powdery Mildew Progress Curve (AUPMPC) was calculated for the assessment period using the following equation adopted by Chiha *et al.* (1997).

$$AUPMPC = D\ (1/2\ (Y_1 + Y_k) + (Y_2 + Y_3 + \ldots\ldots Y_{k-1})$$

Where: D = Time interval; Y_1= First disease score; Y_k = Last disease score; Y_2 and Y_3 = Intermediate disease score.

Evaluation of some plant extracts, antioxidants and micronutrients for controlling sugar beet powdery mildew: Field experiment was carried out at the experimental field of the Faculty of Agriculture, Al-Azhar Univ., Assiut Governorate, Egypt, during 2014/2015 and 2015/2016 growing seasons. Sirona and FD.0807 sugar beet cultivars were selected for the experiment which exhibited resistant and susceptible reaction, respectively to powdery mildew disease based on their interactions in the previous experiment in the reaction of sugar beet cultivar. Field plots consisted of two rows (9-meters long and interspace between plant and another 20 cm) and arranged in a split plot design with three replicates per treatment. One plot was specified for each tested compound and one plot was left as a check for all treatments. Agricultural practices were applied as recommended by Ministry of Agriculture. Plants were thinned to one plant / hole and left for natural infection. Large area around the plots was left without treatment to avoid any contamination by any treated chemicals from neighboring fields (Gado, 2013).

Preparation of the tested compounds: Aqueous plant extracts were prepared from all parts (leaves, seeds and /or cloves) of five plants *i.e.,* basil, camphor, garlic, black cumin and mint as following:

Tested plants	The used part
Basil (*Ocimumbasilicum*)	Leaves
Camphor (*Eucalyptus globulus*)	Leaves
Garlic (*Allium sativum*)	Cloves
Black cumin (*Nigella sativa*)	Seeds
Mint (*Mentha piperita*)	Leaves

The plant parts were washed several times with sterilized distilled water, cut into small pieces, then 100 g of each sample were macerated in 100 ml sterilized distilled water by using mortar, after that, resulting extract was squeezed twice through four layers of cheese cloth as described by Hassan (2006) with slight modification. Filtrates of aqueous plant crude extracts were kept in dark bottles in refrigerator until used. Each aqueous plant extract was used as foliar spraying at the concentrations of 10, 20 and 30 % attributed to the crude extract. Sugar beet plants were sprayed after 105, 125, 145, 165 and 185 days from sowing date.

The efficacy of three chemical inducers *i.e.* ascorbic acid, salicylic acid and citric acid were tested to study their effect against powdery mildew disease of sugar beet plants (Sirona cv. and F.D.0807 cv.). Each chemical inducer was used as foliar spraying at the concentrations of 100, 200 and 300 ppm (Ismail *et al.*, 2012). Sugar beet plants were sprayed after 105, 125, 145, 165 and 185 days from sowing date.

To assess the efficiency of microelements, as foliar sprays on the control of powdery mildew disease on sugar beet plants (Sirona cv. and F.D.0807 cv.), solutions of cupric sulfate, ferrous sulfate, magnesium sulfate, zinc sulfate and mixture of all of them at the concentrations of 10, 20 and 40 ppm (Radwan, 2017) were used as a foliar spray after 105, 125, 145, 165 and 185 days from sowing date.

The used fungicide was commercial formulation of 25.2% w/w boscalid and 12.8% w/w pyraclostrobin (Bellis® 38% WG). Fungicide was applied in the dosage (0.5 gm / liter) as cited in its user manual sheet which is recommended by the manufacturer (BASF™) as described by Moustafa et al. (1990).

Time of application: Treatment applications were started 105 days after sowing (the first sign of the disease has appeared). Plants were sprayed five times during each season with 20 days intervals. Tap water was used for spraying untreated plants (control). Disease severity was determined (five times) in order to evaluate treatments after ten days from each spraying of tested compounds. Solutions of each tested compounds were applied using a hand sprayer, at a volume of 2 Liters of tap water per plot (until run off). Thirty plants were used for each treatment. Plants without spraying served as control. AUPMPC values were calculated as described before.

Statistical analysis: Analysis of variance of the data was carried out on the mean values of the tested treatments according to the procedures described by Gomez and Gomez (1984). The least significant difference (L.S.D) at 5% probability was used for testing the significance of the differences among the mean values of the tested treatments for each character.

Results

Reaction of sugar beet cultivars for powdery mildew infection: Most of tested sugar beet cultivars significantly responded with varied degrees to powdery mildew infection (Table 1). Plants of line FD.0807 were most susceptible followed by Athospoly cv., Hilospoly cv., Oscarpoly cv., Belatos cv. and Pleno cv. respectively. Capel cv., Glorius cv. and Samba cv. showed intermediate reaction to powdery mildew while, Sirona cv. showed the lowest AUPMPC value. In that trend of reactions to the disease, all cultivars took place in the same orders during both growing seasons tested.

Table 1: Mean of area under powdery mildew progress curve (AUPMPC) values for ten sugar beet cultivars during 2013/2014 and 2014/2015 growing seasons under natural infection.

Cultivars	AUPMPC		
	2013/2014	2014/2015	Mean
Athospoly	1315	1312	1313
Belatos	917	939	928
Capel	798	757	777
FD.0807	1467	1502	1484
Glorius	703	718	710
Hilospoly	1090	1125	1107
Oscarpoly	981	942	961
Pleno	895	861	878
Samba	673	676	674
Sirona	443	462	453
LSD at 0.05 %	36.7	55.7	

Efficacy of plant extracts to natural sugar beet powdery mildew: Data in (Table 2) showed that the five plant extracts at the three tested concentrations 10, 20 and 30% reduced the AUPMPC significantly during both 2014/2015 and 2015/2016 growing seasons as compared with the control but each to a different extent. The concentration of 30% was more effective in reducing AUPMPC than the concentrations of 20% and 10% respectively in each particular plant extract. Repeated sprays of basil extract at the concentration of 30% significantly achieved the lowest AUPMPC on both sugar beet cultivars Sirona and FD.0807, followed by garlic extract. Moderate effects were achieved by camphor and mint extracts while, black cumin seed extract came in the last order. The best treatment (Basil 30%) was higher in AUPMPC than the tested fungicide Bellis® 38% WG.

Table 2: Mean of AUPMPC values to sugar beet (Sirona & FD.0807 cultivars) as affected by foliar spray with plant extracts under field conditions during 2014/2015 and 2015/2016 growing seasons.

Treatment	Conc. (%)	AUPMPC							
		Sirona cv.				FD.0807 L.			
		2014/2015	2015/2016	Mean	Protection (%)	2014/2015	2015/2016	Mean	Protection (%)
Basil	10	508	496	502 ±1	28	842	851	847 ±1	48.7
	20	401	406	403 ±1	42.2	703	724	713 ±1	56.8
	30	218	203	210 ±1	69.9	553	538	546 ±1	66.9
Black cumin	10	700	657	678 ±1	2.8	1212	1165	1188 ±1	28.1
	20	541	515	528 ±1	24.3	927	929	928 ±1	43.8
	30	474	448	461 ±1	33.9	774	784	779 ±1	52.8
Camphor	10	636	613	625 ±1	10.4	1131	1143	1137 ±1	31.2
	20	485	505	495 ±1	29	803	842	822 ±1	50.2
	30	395	400	397 ±1	43.1	655	683	669 ±1	59.5
Garlic	10	598	592	595 ±1	14.7	934	932	933 ±1	43.5
	20	475	452	463 ±1	33.6	772	776	774 ±1	53.1
	30	336	321	328 ±1	53	641	665	653 ±1	60.4
Mint	10	671	649	660 ±1	5.4	1188	1199	1193 ±1	27.8
	20	494	477	485 ±1	30.5	821	838	830 ±1	49.7
	30	429	402	416 ±1	40.4	726	757	741 ±1	55.1
Bellis® 38% WG		78	68	73 ±1	89.5	191	138	165 ±1	90
Control		721	675	698 ±1	0.0	1652	1653	1653 ±1	0.0
LSD at 0.05 %									
Treatment(T)		28.9	24.7			27	32.3		
Concentration(C)		10.9	7.8			16.1	12.4		
(T x C)		25.1	20.5			42.5	32.8		

Efficacy of antioxidants to natural sugar beet powdery mildew: All tested resistance inducers caused significant reduction in AUPMPC values when sugar beet plants were sprayed with these tested inducers (Table 3). Increasing inducers concentration increased resistance of sugar beet plants against powdery mildew disease. The lowest AUPMPC on both cultivars Sirona and FD.0807 respectively was achieved by 300 ppm of salicylic acid followed by 200 ppm of salicylic acid and 300 ppm of citric acid respectively. The highest AUPMPC was obtained by 100 ppm of ascorbic acid. The best treatment (300 ppm salicylic acid) was higher in AUPMPC than the tested fungicide Bellis® 38% WG. The same trend was observed on the both growing seasons.

Table 3: Mean of AUPMPC values to sugar beet (Sirona& FD.0807 cultivars) as affected by foliar spray with antioxidants under field conditions during 2014/2015 and 2015/2016 growing seasons.

Treatment	Conc. (ppm)	AUPMPC							
		Sirona cv.				FD.0807 L.			
		2014/2015	2015/2016	Mean	Protection (%)	2014/2015	2015/2016	Mean	Protection (%)
Ascorbic acid	100	700	664	682 ±1	2.2	1100	1077	1089 ±1	34.1
	200	648	638	643 ±1	7.8	1003	1013	1008 ±1	39
	300	572	558	565 ±1	19	885	880	882 ±1	46.6
Citric acid	100	604	562	583 ±1	16.4	940	946	943 ±1	42.9
	200	521	503	512 ±1	26.6	782	798	790 ±1	52.2
	300	470	471	470 ±1	32.6	707	718	713 ±1	56.8
Salicylic acid	100	537	516	527 ±1	24.4	813	820	816 ±1	50.6
	200	343	341	342 ±1	51	604	594	599 ±1	63.7
	300	309	295	302 ±1	56.7	485	486	485 ±1	70.6
Bellis® 38% WG		78	68	73 ±1	89.5	191	138	165 ±1	90
Control		721	675	698 ±1	0.0	1652	1653	1653 ±1	0.0
LSD at 0.05 %									
Treatment(T)		40	25.5			32.5	31.1		
Concentration(C)		6.2	15.4			16.6	18.3		
(T x C)		14	34.4			37.2	41		

Efficacy of micronutrients to natural sugar beet powdery mildew: All tested microelements caused significant reduction in AUPMPC values when sugar beet plants were sprayed with any of these tested compounds (Table 4). Increasing microelements concentration increased resistance of sugar beet plants against powdery mildew disease. The lowest AUPMPC value on Sirona cultivar was achieved by 40 ppm of cupric sulfate followed by 20 ppm of cupric sulfate and 40 ppm of combination respectively. The highest AUPMPC value was obtained by 10 ppm of zinc sulfate. The best treatment (40 ppm, cupric sulfate) was higher in AUPMPC value than the tested fungicide Bellis® 38% WG. Data also showed that, the lowest AUPMPC value on FD.0807 cultivar was achieved by 40 ppm of cupric sulfate followed by 40 ppm of combination and 40 ppm of ferrous sulfate respectively. The highest AUPMPC value was obtained by 10 ppm of magnesium sulfate. The best treatment (40 ppm, cupric sulfate) was higher in AUPMPC value than the tested fungicide Bellis® 38% WG. The same trend was observed on the both growing seasons.

Discussion

The present study was carried out to evaluate ten sugar beet cultivars for their reaction to powdery mildew disease and to investigate the effect of five plant extracts *i.e.* basil, camphor, garlic, black cumin and mint at 10, 20, and 30 % concentrations on AUPMPC caused by *Erysiphe betae*. Also, the effect of three antioxidants (*i.e.* ascorbic acid, salicylic acid and citric acid) at 100, 200 and 300 ppm and four microelements (*i.e.* cupric sulfate, ferrous sulfate, magnesium sulfate, zinc sulfate and mixture of all of them) at the concentrations of 10, 20 and 30 ppm were studied Bellis® 38% WG fungicide was applied at the recommended concentration to be used as a comparative treatment.

Table 4: Mean of AUPMPC values to sugar beet (Sirona & FD.0807 cultivars) as affected by foliar spray with micronutrients under field conditions during 2014/2015 and 2015/2016 growing seasons.

Treatment	Conc. ppm	AUPMPC							
		Sirona cv.				FD.0807 L.			
		2014/2015	2015/2016	Mean	Protection %	2014/2015	2015/2016	Mean	Protection %
Cupric sulfate	10	437	385	411 ±1	41.1	741	755	748 ±1	54.7
	20	217	209	213 ±1	69.4	417	436	426 ±1	74.2
	40	206	184	195 ±1	72	348	355	352 ±1	78.7
Ferrous sulfate	10	487	499	493 ±1	29.3	831	847	839 ±1	49.2
	20	294	309	302 ±1	56.7	523	514	518 ±1	68.6
	40	255	268	261 ±1	62.6	422	415	418 ±1	74.7
Magnesium sulfate	10	556	518	537 ±1	23	934	928	931 ±1	43.6
	20	390	356	373 ±1	46.5	673	676	674 ±1	59.2
	40	346	314	330 ±1	52.7	593	572	582 ±1	64.7
Zinc sulfate	10	662	623	642 ±1	8	926	883	904 ±1	45.3
	20	431	414	423 ±1	39.3	727	696	711 ±1	56.9
	40	357	336	347 ±1	50.2	605	602	603 ±1	63.5
Combination CS+FS+MS+ZS	10	454	436	445 ±1	36.2	782	815	799 ±1	51.6
	20	263	250	257 ±1	63.1	487	467	477 ±1	71.1
	40	246	225	236 ±1	66.1	414	377	396 ±1	76
Bellis® 38% WG		78	68	73 ±1	89.5	191	138	165 ±1	90
Control		721	675	698 ±1	0.0	1652	1653	1653 ±1	0.0
LSD at 0.05 %									
Treatment (T)		13.9	22.7			22.2	19.3		
Concentration (C)		12	11.3			15.8	17.3		
(T x C)		31.8	29.7			41.9	45.8		

The results indicate that Sirona (453 AUPMPC) was significantly the most tolerant cultivar to powdery mildew disease while, FD.0807 (1484 AUPMPC) was significantly the most susceptible cultivar, these results were in accordance with those reported by several researchers that concluded that plant cultivars are variants in their reactions to powdery mildew infection (Matsuda & Takamatsu, 2003; Abd-ElKareem et al., 2001). Fungicides have been used for a long time as the main strategy for controlling powdery mildew disease on sugar beet and subsequently increase yield production (Hassan & Berger, 1980; Docea & Fratila, 1980; Kontaxis, 1978 ; Abol-Wafa et al., 1976), Bellis® 38% WG fungicide when applied at the recommended concentration gave the best protection from powdery mildew on both sugar beet cultivars Sirona and FD.0807 respectively (89.5% & 90%), the highest effect of Bellis fungicide could be attributed to its mode of action due to its active ingredients which are related to the chemical fungicidal groups (Succinate dehydrogenase inhibitors and Quinone outside Inhibitors) those affect the respiration process in the fungal cell, those groups were found to be very effective against powdery mildew fungi in previous studies of (Karaoglanidis & Karadimos, 2006; Hollomon & Wheeler, 2002; Bartlett et al., 2002). On the other hand the fungicides resistant races of some pathogens have been reported by (Fernández-Aparicio et al., 2009; Weiland & Koch, 2004). As well as the side effects of fungicides on human health (Durmusoglu *et al.*, 1997; Eckert & Ogawa, 1988) and the environment (Garcia, 1993; Horst *et al.*, 1992). Despite that, fungicides are still the most dependable method in controlling such diseases although all their undesirable effects. Hence, there is a growing trend many years ago to involve other successful methods in disease management program depending on compounds which are useful, nontoxic and safe either on human health or on the environment. Among the tested plant extracts, the highest protection (69.9% & 66.9%) on both cultivars Sirona and FD.0807 respectively was achieved by 30% of basil extract followed by 30% of garlic extract (53% & 60.4%) while, the least protection (33.9% & 52.8%) was obtained by 10% of black cumin seed extract. The effect of plant derived constituents could be attributed to changes in plant metabolism which are dependent on the plant (Schmitt, 2002), such constituents lead to increase phenylalanine ammonia lyase activity in some plant leaves (Singh & Prithiviraj, 1997), ajoene (a compound derived from garlic) was used to control powdery mildew (Singh *et al.*,1995), the host plants exhibit delayed senescence and increases in chlorophyll contents, ethylene production and various enzyme activities were found in some treated plants (Herger & Klingauf, 1990).The importance of plant derived agents is not only for the inhibitory effect on the pathogen, but in way due to their ability to induce host resistance through increasing the activity of many enzymes which playing a defense role against invading pathogens (Nawar & Kuti, 2003; Caruso et al., 2001). The compounds responsible for the preventative and curative effects could be fraction from these agents in relation to host resistance. Concerning the tested inducers, the highest protection (56.7% & 70.6%) was achieved by 300 ppm of salicylic acid followed by 200 ppm of salicylic acid (51% & 63.7%), while, 100 ppm of ascorbic acid gave the least protection (2.2% & 34.1%). Data are in agreement with those reported by several researchers when they used such compounds against several plant diseases caused by various pathogens (Sklodowska et al., 2010; Vimala et al., 2009; El-Samawaty & Galal, 2009; Ismail et al., 2006; Shaat & Galal, 2004; Sparla et al., 2004; Galal & Abdou, 1996). The reason for that effectiveness could be explained that many factors may act on plants to induce high levels of systemic resistance to subsequent pathogen attack. Some chemicals like benzothiadiazole, β-aminobutyric acid, chitosan, salicylic acid or even some plant extracts have been reported to induce resistance in a number of plant–pathogen interactions that can be very effective (Barilli et al., 2009,2010; Bélanger & Labbé, 2002; Dann & Deverall, 2000; Frey & Carver, 1998). They may provide commercially useful broad-spectrum plant protection that is stable, long-lasting and environmentally benign. Exogenous application of salicylic acid solutions to pea leaves induced systemic resistance to E. pisi and the percentages of fungal germlings that successfully infected untreated leaves were reduced by 20–30% (Frey & Carver, 1998). On the other hand, 40 ppm of cupric sulfate achieved the best percentage of protection (72% & 78.7%) whereas, the least protection (8%) was obtained by 10 ppm of zinc sulfate on

Sirona cultivar and the least protection (43.6%) was obtained by 10 ppm of magnesium sulfate on FD.0807 cultivar. Finally, it is recommended to conduct further studies on the possibilities of applying useful compounds such as plant derived compounds, resistance inducers, macro and micro nutrients in the open field and to produce them commercially in order to reduce the risk of fungicides on human health and environment.

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