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Evaluation of different chemicals to control *Erysiphe betae* the causal pathogen of sugar beet powdery mildew

Mohamed M. El-Sheikh Aly[1], Ali H. ElShaer[2], Anwar A. Galal[3], Harbi M. Abd-Alla[3], Mohamed A. Eliwa[1*]

[1]Agricultural Botany Department, Faculty of Agriculture, Al-Azhar University, 71524 Assiut, Egypt
[2]Plant Pathology Research Institute, Agricultural Research Center, Giza, Egypt
[3]Plant Pathology Department, Faculty of Agriculture, Minia University, Minia, Egypt

Abstract

Survey on sugar beet plantations at Minia and Assiut governorates, Egypt revealed that powdery mildew disease was the most epidemic threat on sugar beet plantations. It was noticed that the highest Area Under Powdery Mildew Progress Curve (AUPMPC) value was detected in Abnob locality, Assiut governorate while the lowest one was found in Maghagha locality, Minia governorate. Data revealed that five months' post collection conidia of *Erysiphe betae* failed to infect sugar beet leaves cultivar FD.0807. Results of conidial germination showed that the percent germination in darkness was lower than in light. Also a high percentage of germinating conidia formed appressorium on dry glass slides. The examination of powdery mildew infected sugar beet leaves using scanning electron microscopy showed that the fungus penetrates the epidermis of the leaves by the haustoria which are folded in many patches forming a complex web almost completely covers the leaf. Field experiment was conducted to evaluate three chemical compounds containing plant macronutrients, along with five fungicides against powdery mildew disease. Results showed that sodium bicarbonate achieved the best disease control among the macronutrient-containing compounds followed by calcium chloride and potassium silicate, respectively. Sodium bicarbonate achieved the highest total soluble solids (TSS) percentage and root weight at all rates of application followed by calcium chloride, while potassium silicate achieved the least TSS % and root weight. Concerning fungicides, Bellis 38%WG gave noticeable result in disease reduction followed by Collis 30% SC and Tilt 25% EC, respectively. The results showed that the highest TSS % and root weight were detected in the roots of sugar beet plants treated with Bellis 38% fungicide followed by Collis 30%. Meanwhile, the lowest significant of TSS % and root weight was detected after treatment with Permatrol 99%.

Keywords: sugar beet, powdery mildew, *Erysiphe betae*, macronutrients, fungicides.

[*]**Corresponding author:** Mohamed A. Eliwa,
E-mail: mohamedeliwa.5419@azhar.edu.eg

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1. Introduction

Sugar beet (*Beta vulgaris* L.), is an herbaceous dicotyledonous plant belongs to family Amaranthaceae (formerly Chenopodiaceae). It is considered as one of the two major sugar crops around the world and an important crop of temperate climates which provides nearly 40% of the world's annual sugar production and is a source for bioethanol and animal feed (Bastas and Kaya, 2019). Under field conditions, several pathogenic fungi attack sugar beet plants causing serious diseases *i.e.*, Cercospora leaf spot (*Cercospora beticola* Sacc.) and rust (*Uromyces betae* Press). Sugar beet powdery mildew which is caused by *Erysiphe betae* (Vanha) Weltzien is among the most important foliar diseases of sugar beet worldwide (Gobarah & Mekki, 2005). The disease is economically significant for growers worldwide and can cause sugar yield losses up to 30 % (Francis, 2002). Gado (2013) reported that powdery mildew is considered as a major foliar disease of sugar beet in areas with dry and relatively warm weather conditions throughout the world, devastating foliar disease affecting plant growth and consequently sugar production. Egyptian environmental conditions help the fungus to spread rapidly specially in the late sowings after September. Losses in sucrose could reach 82.9 % for some cultivars due to powdery mildew infection. Total soluble solids percent and root weight were dramatically affected by disease severity under infected conditions (El-Fahhar, 2008). Grimmer et al. (2007) reported that if the disease is not controlled it can cause a 20 to 35 percent loss in sugar yield. Crop loss is due to a reduced root yield and often to a lower concentration of sugar in roots. Both effects apparently are due to a reduced efficiency of diseased leaves and to their premature death, when roots are rapidly enlarging. As part of the environment, nutrients influence plant, pathogen and microbial growth to remain an important factor in disease control. The interaction of nutrition in these components is dynamic and all essential nutrients are reported to influence the incidence or severity of some diseases, mineral nutrients are the components of plants and regulate metabolic activity associated with resistance of a plant and virulence of a pathogen. Adequate nutrition is generally required to maintain a high level of disease resistance. Nutrient sufficiency also may shorten a susceptible growth stage for some plant-pathogen interactions (Huber & Haneklaus, 2007). Macronutrients are well recommended as fungicide alternatives for enhancing plant health, subsequently inducing plant resistance and controlling the disease in parallel with their safe influence on human health (Huber & Haneklaus, 2007). Control of sugar beet powdery mildew is mainly achieved by applications of broad spectrum systemic fungicides (Byford, 1996). Although, the wide spread use of the chemical fungicides has become a subject of research concern due to their harmful effect on non-target organisms as well as their possible carcinogenicity (Ziedan & Farrag, 2011). However, further studies should concern safe, applicable, reliable and efficient replacement of chemical fungicides by other safer chemical or natural compounds harmless to plants or human health. The objectives of this study were to (1) investigate the spread of sugar beet powdery mildew disease in some governorates in Upper Egypt and (2) to assess the role of some different chemical compounds on reducing powdery mildew disease incidence on sugar beet.

2. Materials and methods

2.1 Survey of sugar beet powdery mildew

Survey of sugar beet powdery mildew was conducted in different districts of two Governorates (3 districts) namely Abnob, Dayrot and Manfalot (Assiut Governorate) and Maghagha, Samallot and AbuQurkas (El-Minia Governorate), Egypt. At least 3 fields of each district were concerned. Each field under survey was determined with a field map, 5 sampling sites were designated per field tested and one of each of the four corners plus one in the center of the field. Sampling sites were located at least 5 meter from the edge of the field (Ray & McLaughlin, 1942). Severity of powdery mildew was monitored 4 times at 20 days' intervals. Area under disease progress curve was conducted.

2.2 Powdery mildew disease 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. Disease severity was determined 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.

2.3 Estimating conidia survival

Greenhouse experiment was conducted in 3 m × 3 m area well-isolated protected one to study how long the time of powdery mildew conidia still able to attack sugar beet plants and initiate the disease. Only powdery mildewed sugar beet leaves were collected from the most susceptible cv. FD.0807 grown under open field and dried carefully on sterilized benches with 70 % ethyl alcohol to avoid rottenness, then packed in plastic bag and stored at room temperature until used. Two sugar beet cultivars Sirona and F.D.0807 were sown (4 seeds /pot) in 30 cm diameter plastic pots, filled with sterilized sandy clay soil, nine pots for each cultivar in three replicates, each replicate consisted of three pots and nine pots of each cultivar which were isolated apart by thin white plastic sheets served as control. Artificial inoculation was done 60 days from planting by shaking dried diseased sugar beet leaves (after five months of storage) over the growing plants at a height of about 30cm. Disease severity was estimated after seven days of inoculation.

2.4 *In vitro* conidia germination tests

Conidia germination test was carried out using light microscope, slides were washed in 50 percent alcohol and wiped with a cloth to remove inert particles in order to prevent condensation of free moisture on the glass surface at very high relative humidity levels. The conidia were detached and collected by

vigorously shaking infected leaves over the glass slides placed at the bottom of a plastic container 20×20×10 cm^3. In order to reduce variation in germination and to obtain reproducible results, only 24 h old conidia were utilized. For this purpose, the plants were shaken every day to prevent accumulation of old and shriveled spores. Each slide was placed on a U-shaped glass rod in a moist chamber made up of sterile Petri dish lined with filter paper saturated with sterile distilled water. Petri dishes were incubated at 25±2°C (Awad et al., 1990) for 24 h before examination. One set of chambers was kept in the light and another in darkness. Three slides were used as replicates for each particular treatment. The percentage of germination was based on the following formula:

$$\text{Germination \%} = \frac{\text{Number of germinated spores}}{\text{Total number of examined spores}} \times 100$$

2.5 Scanning electron microscopic examination

Sample preparation: In order to study the three dimensional structure through scanning electron microscope (SEM) of the haustoria and the infection mode on the susceptible cultivar FD.0807, fresh infected leaves (120 days old, 48 h after symptom appearance) were collected, put in paper bag and carried to Electron Microscope Unit at Assiut University, Egypt. The leaves were cut into appropriate samples and were subjected to fixation in 5 % cold buffered gluteraldehyde for 2 days. The samples were then washed by cacodylate buffer for three times thirteen minutes for each and post fixed in 1% osmium tetroxide for 2 h. Samples were then washed in cacodylate buffer for three times thirteen minutes each and then dehydrated by using ascending series of ethanol 30, 50, 70, 90 for 2 h, 100 % for two days, and then to amyl acetate for two days. Critical point drying was applied to the samples by using liquid carbon dioxide. Each sample was sticked on metallic blocks by using silver paint. By using gold sputter coating apparatus, samples were evenly gold coated in a thickness of 15 nm (Bozzola et al., 1991).

2.6 Control of powdery mildew disease

Experiments were carried out at the experimental field of the Faculty of Agriculture, Al-Azhar University, Assiut, Egypt during 2014/2015 and 2015/2016 growing seasons. Resistant and susceptible sugar beet cultivars Sirona and FD.0807, respectively were selected for the experiments of powdery mildew disease control. Field plots consisted of two rows (9 m 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 one tested compound and one plot was left for control. Field was fertilized and irrigated as usual. 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).

2.6.1 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. Disease severity

was determined (5 times) in order to evaluate treatments after ten days from each time of 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 were served as control. AUPMPC values were calculated as described before.

2.6.2 Estimation of total soluble solids (TSS) percentage and root weight of the treated sugar beet plants

At harvest, three replicate samples, each sample of thirty roots for five sprays treatments were randomly collected for determination of root weight and sugar analysis. Juice analysis were done by using a digital refractometer to determine TSS % of root juice and a precise hand scale was used to measure root weight.

2.6.3 Effect of applying macronutrients on the disease severity

Three chemical compounds containing macronutrients *i.e.* calcium chloride, potassium silicate and sodium bicarbonate were tested to study their effect against powdery mildew disease of sugar beet plants Sirona cv. and F.D.0807 line. Each compound was used as foliar spraying at the concentrations of 0.1, 0.2 and 0.3 g /l. Sugar beet plants were sprayed after 105, 125, 145, 165 and 185 days from sowing date. Bellis® 38 % WG Fungicide was used as a comparative treatment which applied in the dosage (0.5 g/l) as cited in its user manual sheet as recommended by the manufacturer (BASF™).

2.6.4 Effect of fungicides

In the study, the used fungicides were Bellis 38 % (25.2 % w/w boscalid and 12.8 % w/w pyraclostrobin), Collis 30 % (20 % w/v boscalid and 10 % w/v kresoxim–methyl), Camzin 50 % (50 % w/w carbendazim), Tilt 25 % (25 % w/v Propiconazole) and Permatrol 99 % (99 % v/v Jojoba oil). Fungicides were applied at the recommended dosage as summarized in Table (1).

Table 1: Trade name, group name, chemical group, common name, recommended doses and production Company of tested fungicides.

Trade name	Group name	Chemical group	Common name	Recommended dose	Production company
Bellis® 38% WG	Succinate dehydrogenase inhibitors	Pyridine-carboxamides	Boscalid	50 g/l	BASF™
	Quinone outside Inhibitors	Methoxy-carbamates	Pyraclostrobin		
Collis® 30% SC	Succinate dehydrogenase inhibitors	Pyridine-carboxamides	Boscalid	50 ml/l	BASF™
	Quinone outside Inhibitors	Oximino-acetates	Kresoxim-methyl		
Camzin® 50% WP	Methyl Benzimidazole Carbamates	Benzimidazoles	Carbendazim	75 g/l	CAM™
Tilt® 25% EC	DeMethylation Inhibitors	Triazoles	Propiconazole	15 ml/l	Syngenta ™
Permatrol™ 99% Oil	---------	-------	Jojoba oil	1000 ml/l	Soiltech™

2.6.5 Disease reduction

Disease reduction percent was calculated according to Ismail et al. (2012) as follows:

$$\text{Disease reduction} = \frac{\text{AUPMPC of control} - \text{AUPMPC of treatment}}{\text{AUPMPC of control}} \times 100$$

2.7 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 (LSD) at 5% probability was used for testing the significance of the differences among the mean values of the tested treatments for each character.

3. Results

3.1 Survey of sugar beet powdery mildew

Data in Table (2) represent the survey of powdery mildew disease which took place in Assiut and Minia governorates during 2012/2013 growing season. Data showed that the highest AUPMPC value was detected in Abnob locality followed by Manfalot then Dayrot while, the lowest AUPMPC value was found in Maghagha locality followed by AbuQurkas and Samallot localities respectively.

3.2 Survival of conidia

Greenhouse experiment was conducted on 2014/2015 growing season to determine the overwintering capability of vegetative mycelia and conidia of *Erysiphe betae* and their role in the dissemination of the fungus. Obtained results from consecutive observations confirmed that the conidia collected from the previous season (2013/2014) could not initiate any type of infection or disease symptoms which means that the conidia could not survive as long as it were stored in this experiment and the conidia that remains on the crop debris at the end of the season are not one of the means which used by the fungus for its overwintering.

Table 2: Area under powdery mildew progress curve (AUPMPC) values on sugar beet plants (Glorius, Sirona and Samba cultivars) grown in different districts of Assiut and El-Minia governorates, Egypt.

Governorate	District	Cultivar	AUPMPC
Assiut	Abnob	Glorius	660 ±1
	Dayrot	Sirona	322 ±1
	Manfalot	Samba	442 ±1
Minia	Maghagha	Sirona	118 ±1
	Samallot	Glorius	190 ±1
	AbuQurkas	Samba	228 ±1
Mean	----------		327 ±1
LSD at 0.05			53.9

3.3 *In vitro* conidia germination tests

The purpose of this experiment was to make a preliminary study on the germination percentages of *E. betae* conidia on glass slides at 100 % relative

humidity at room temperature (25±2°C), in light and in darkness. As shown in Table (3) the percent germination in darkness was lower than in light. A high percentage of germinating conidia formed appressorium on dry glass slides. One appressorium was formed by the germ tube of each conidium. The appressorium formation was not affected by light or darkness. The conidia of *Erysiphe betae* germinated at a fast rate within 8 to 10 h of incubation. In the same time 100 % relative humidity (RH) was sufficient enough to prevent conidia from shriveling.

Table 3: *In vitro* conidia germination percent at light and darkness conditions.

Incubation conditions	Germination (%)
Light	74
Darkness	58
LSD at 0.05	18.27

3.4 Scanning electron microscope examination

First observation on the scanning electron microscopy (SEM) images of the infection method of sugar beet with powdery mildew pathogenic fungus *E. betae* is that it penetrated the epidermis of the leaves by the haustoria as shown in Figure (1). On upper leaf surface, a great amount of conidia was visible and haustoria as well. Outer surface of the haustoria is rough and wavy. The haustoria are folded in many patches forming a complex web which almost completely covers the leaf. Haustoria penetrated the leaf as a drilling machine resulting at the side parts pieces of mesophyll which are folded at the bottom of the haustoria. It was clearly observed that haustoria penetrated the stomata of the leaf easily and successfully. The convoluted haustoria penetrated the leaf epidermis in many points infecting the entire leaf surface. The haustoria were convoluted and folded in multiple ways. They entered perpendicularly the leaf from the top. There were visible several conidiphores formed, preparing new source of secondary infection. The entering zone of the haustoria, the hyphal part is thickened, being like a connection tube between the fungus and the leaf.

3.5 Effect of applying macronutrients on disease severity of sugar beet powdery mildew

Three compounds containing macronutrients were tested for their ability to control powdery mildew disease on sugar beet. Data in Table (4) showed that all the tested macronutrients significantly reduced AUPMPC values when sugar beet plants were sprayed with them. It was noticed that increasing macronutrients concentration sub-sequently increased resistance of sugar beet plants against powdery mildew disease. The lowest (AUPMPC) on both cultivars Sirona and FD.0807 respectively was achieved by 0.3 g/l of sodium bicarbonate followed by 0.3 g/l of calcium chloride and 0.2 g/l of sodium bicarbonate respectively. The highest (AUPMPC) was obtained by 0.1 g/l of potassium silicate. The best treatment (0.3 g/l sodium bicarbonate) was higher in (AUPMPC) value than the tested fungicide Bellis® 38 % WG.

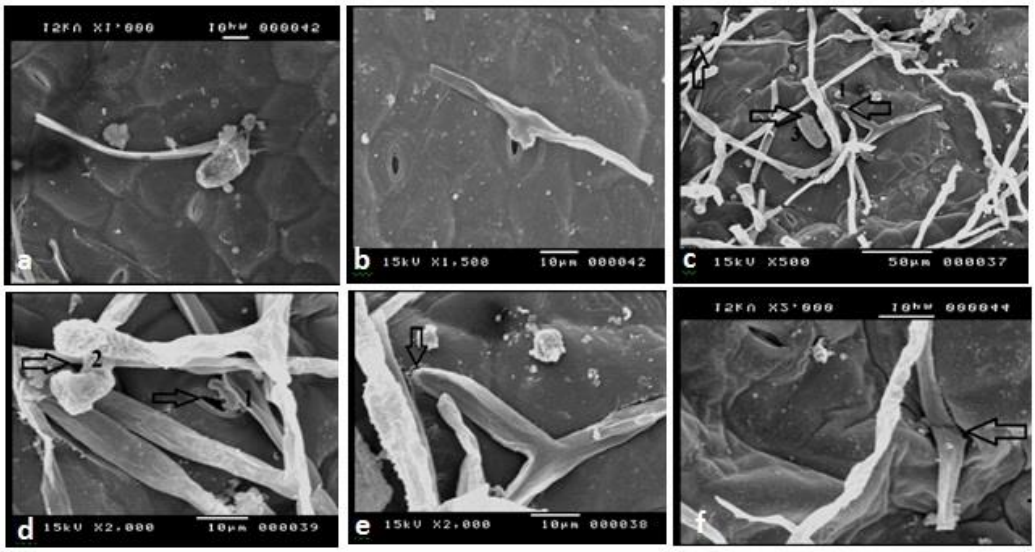

Figure 1: Scanning microscope of haustoria and conidia where **a:** mature conidiospore freshly separated, initiating germination tube turned towards a stoma on the adaxial leaf surface, note the root-like appendages at the bottom trying to attach itself to the leaf, **b:** detached mycelial fragment with single haustorium in the middle and about to enter a stoma, **c:** a network of hyphae on the abaxial surface of leaf 48 h after inoculation, successful penetration into the mesophyll (1), single haustorium seeking for stoma (2) and fresh separated conidiospore ready for germination (3), **d:** magnifying successful penetration into the mesophyll, **e:** one single haustorium about to enter an opened stoma on the abaxial leaf surface (1) and two short conidiophores, each has single aged shriveled conidiospore at the top (2) and **f:** Enlarged view of haustorium actually penetrated the mesophyll; using physical pressure is clear from the bending backward resulted in visible folding in the upper side of the haustorium.

Table 4: AUPMPC values of sugar beet cultivars Sirona and FD.0807 as affected by macro nutrients foliar spray under field conditions during 2014/2015 and 2015/2016 growing seasons.

Treatment	Conc. (g/l)	AUPMPC							
		cv. Sirona				cv. FD.0807			
		2014/2015	2015/2016	Mean	Disease reduction (%)	2014/2015	2015/2016	Mean	Disease reduction (%)
Calcium chloride	0.1	574	552	563 ±1	19.3	842	851	846 ±1	48.8
	0.2	472	464	468 ±1	32.9	687	702	694 ±1	58
	0.3	360	354	357 ±1	48.8	618	606	612 ±1	62.9
	Mean	468	456	462 ±1	33.71	715	719	717 ±1	56.58
Potassium silicate	0.1	651	630	641 ±1	8.1	1070	1068	1069 ±1	35.3
	0.2	582	597	589 ±1	15.6	922	910	916 ±1	44.5
	0.3	514	495	504 ±1	27.7	801	772	786 ±1	52.4
	Mean	582	574	578 ±1	17.16	931	916	923 ±1	44.11
Sodium bicarbonate	0.1	541	530	536 ±1	23.2	855	819	837 ±1	49.3
	0.2	430	410	420 ±1	39.8	665	686	675 ±1	59.1
	0.3	339	298	318 ±1	54.4	608	582	595 ±1	64
	Mean	436	412	424 ±1	39.15	709	695	702 ±1	57.5
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)	29.3	20.2	-----	-----	24.3	16.9	-----	-----
	Concentration (C)	7.1	24.5	-----	-----	16.4	15.5	-----	-----
	(T × C)	15.8	54.7	-----	-----	36.8	34.7	-----	-----

3.6 Estimation of total soluble solids (TSS) contents of sugar beet plants treated with macronutrients

Data in Figure (2) showed that the TSS percentage in the roots of infected sugar beet plants (Sirona and FD.0807 cultivars) and treated with the compounds containing microelements was increased significantly by all used compounds as compared to control. Sodium bicarbonate achieved the highest

TSS percentage at all rates of application followed by calcium chloride, while potassium silicate achieved the least TSS percentage.

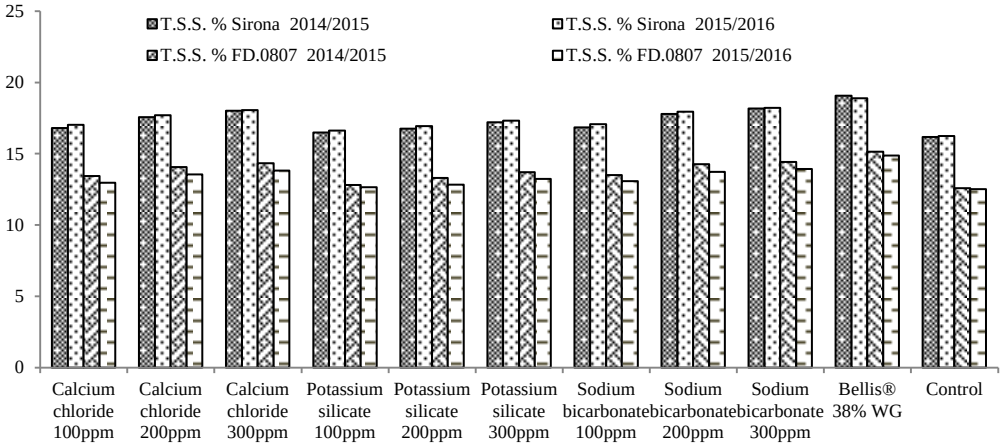

Figure 2: TSS percent in roots of sugar beet cultivars (Sirona and FD.0807) as affected by macronutrients foliar spray under field conditions during 2014/2015 and 2015/2016 growing seasons.

3.7 Evaluation of root weight of sugar beet plants treated with macro-nutrients

Data in Figure (3) showed that the root weight of infected sugar beet plants (Sirona and FD.0807 cultivars) and treated with the compounds containing microelements was increased significantly by all used compounds as compared with the control. Sodium bicarbonate achieved the highest root weight at all rates of application followed by calcium chloride, while potassium silicate achieved the least root weight. In general, the root yield representing in their weight and TSS content inversely related to the severity of the disease. Significant differences among the tested compounds and each other were noticed. It was also noticed that there was significant differences between the treatments with the three different concentrations.

3.8 Evaluation of the effect of fungicides on controlling sugar beet powdery mildew

Commercial fungicides were tested for controlling sugar beet powdery mildew on Sirona and FD.0807 cultivars under field conditions in both 2014/2015 and 2015/2016 growing seasons. Data represented in Table (5) revealed that in case of Sirona cultivar Bellis 38 % was significantly the most effective fungicide in controlling the disease followed by Collis 30 % then Tilt 25 %, Camzin + Tilt and Camzin 50 % respectively while, Permatrol 99 % came in the last rank. Concerning FD.0807 cultivar in both 2014/2015 and 2015/2016 growing seasons, Bellis 38 % WG was significantly the most effective fungicide followed by Collis 30 % SC then Tilt 25 % EC, Camzin + Tilt and Camzin 50 % WP respectively while, Permatrol 99 % came in the last order. In general, all fungicides reduced the disease significantly as compared with the control, but each to a different extent.

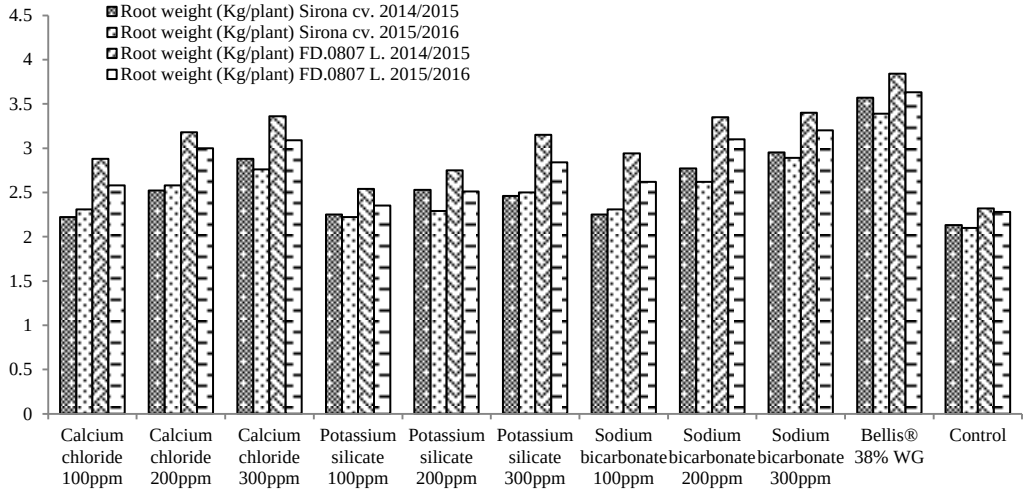

Figure 3: Root weight (Kg /plant) of sugar beet cultivars (Sirona and FD.0807) as affected by macronutrients foliar spray under field conditions during 2014/2015 and 2015/2016 growing seasons.

Table 5: AUPMPC values for sugar beet cultivars Sirona and FD.0807 affected by fungicides foliar spray under field conditions during 2014/2015 and 2015/2016 growing seasons.

Fungicides (Trade name)	Concentration	AUPMPC values for cultivars							
		Sirona				FD.0807			
		2014/2015	2015/2016	Mean	Disease reduction (%)	2014/2015	2015/2016	Mean	Disease reduction (%)
Bellis 38% WG	50 g	78	68	73±1	89.5	191	138	165±1	90
Collis 30% SC	50 ml	116	125	121±1	82.6	245	210	228±1	86.2
Camzin 50% WP	75 g	256	269	263±1	62.3	548	561	555±1	66.4
Tilt 25% EC	15 ml	123	139	136±1	80.5	310	275	293±1	82.2
Camzin 50% + Tilt 25%	75 g + 15 ml	186	189	188±1	73	372	391	381±1	76.9
Permatrol (Jojoba oil) 99%	1000 ml	663	592	628±1	10	944	885	915±1	44.6
Control	--------	721	675	698±1	0.0	1652	1653	1653±1	0.0
LSD at 0.05		17.5	22.6	--------	--------	52.6	36.5	--------	--------

3.9 Total soluble solids (TSS) contents of sugar beet plants treated with fungicides

Data in Figure (4) showed that the highest TSS percentage was detected in the roots of sugar beet plants (cv. Sirona) naturally infected with powdery mildew and treated with Bellis 38 % WG fungicide followed by Tilt 25 % EC then Collis 30 % SC, Camzin + Tilt and Camzin 50 % WP respectively while, the lowest significant TSS percentage was detected after treatment by permatrol 99%. Concerning sugar beet cultivar (FD.0807), the highest TSS percentage was detected in the roots of sugar beet plants treated with Bellis 38% WG followed by Collis 30 % SC then Tilt 25 % EC, Camzin + Tilt and Camzin 50 % WP respectively. On the other hand, Permatrol 99 % treatment significantly recorded the lowest TSS percentage.

3.10 Estimation of root weight of sugar beet plants treated with fungicides

Data in Figure (5) showed that the highest root weight of sugar beet plants (cv. Sirona) infected with powdery mildew was achieved by treating plants with Bellis 38 % WG followed by Collis 30 % SC then Tilt 25 % EC, Camzin + Tilt and Camzin 50 % WP respectively

while, Permatrol 99 % significantly recorded the lowest root weight of sugar beet plants naturally infected with powdery mildew.

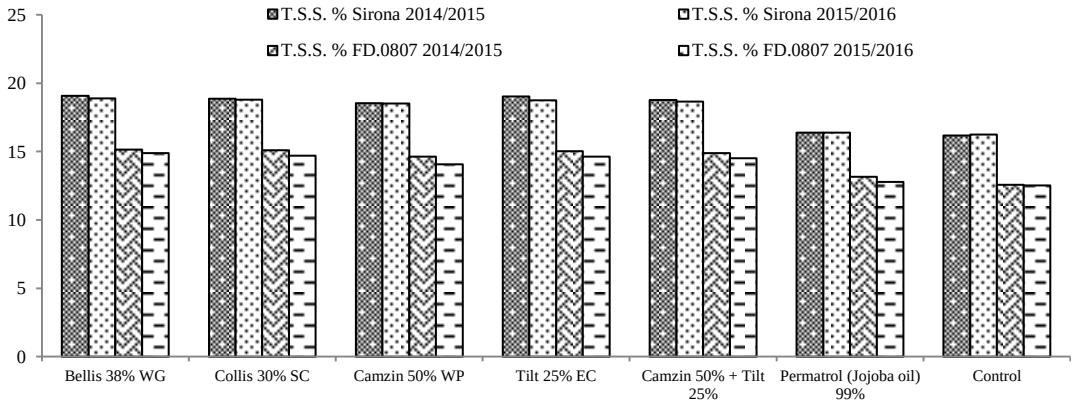

Figure 4: TSS % in roots of sugar beet cultivars Sirona and FD.0807 as affected by fungicides foliar spray under field conditions during 2014/2015 and 2015/2016 growing seasons.

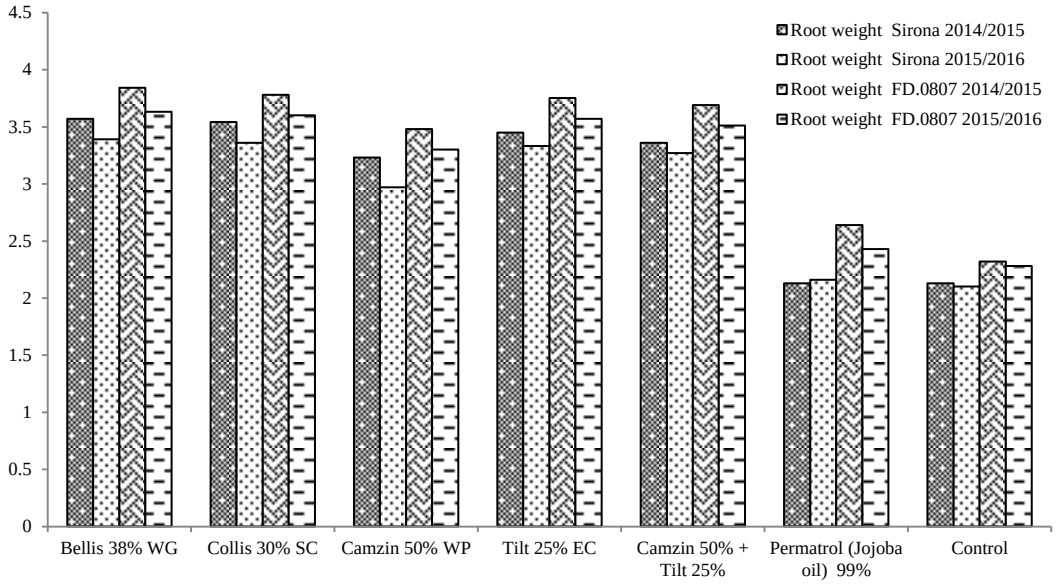

Figure 5: Root weight (Kg/plant) of sugar beet cultivars (Sirona and FD.0807) as affected by fungicides foliar spray under field conditions during 2014/2015 and 2015/2016 growing seasons.

Whereas, the highest root weight of sugar beet plants (cv. FD.0807) infected with powdery mildew was achieved by treating plants with Bellis 38 % WG followed by Collis 30 % SC then Tilt 25 % EC, Camzin + Tilt and Camzin 50 % WP respectively while, Permatrol 99 % significantly recorded the lowest root weight of sugar beet plants naturally infected with powdery mildew. In general, all treatments significantly increased root yield as compared to control, except Permatrol 99 % in the case of Sirona cultivar.

4. Discussion

The present study was carried out to investigate the spread of *Erysiphe betae* the causal fungus of sugar beet powdery mildew disease grown in Upper Egypt and to find a best proper method to control the disease. Survey at sugar beet plantations around Minia and Assiut governorates was resulted to confirm that powdery mildew disease is the most destructive among the foliar diseases which attack sugar beet plantations. The percentage of the disease was higher in Assiut governorate plantations than in Minia, which diversity could be attributed to the arid hot climate prevailing in Assiut region, providing high temperature and low relative humidity those are preferred by powdery mildew on the whole. These results were in accordance with those reported by several researchers (Matsuda & Takamatsu, 2003; Abd-ElKareem et al., 2001). Laboratory experiment was carried out to study on the germination percentages of *Erysiphe betae* conidia on glass slides. The percent germination in darkness was lower than in light and all germinating conidia formed appressorium on dry glass slides. The obtained high germination percentage and the rapid germination of the conidia indicate that the sugar beet powdery mildew does not require that high moisture of germination applied in the experiment. Minassian (1967) reported that only 50 percent relative humidity is sufficient to prevent conidia from shriveling in the atmosphere. Upon reaching the host leaf the microclimate probably provides efficient moisture which prevents shriveling of conidia during germination. It is therefore clear that the atmospheric humidity in Assiut region is favorable for the spread of the fungus and suitable for its development on sugar beets. To learn more about the epidemiology of sugar beet powdery mildew in Upper Egypt, greenhouse experiment was conducted to determine the overwintering capability of vegetative mycelia and conidia of *Erysiphe betae* and their role in the dissemination of the fungus. Obtained results confirmed that the conidia collected from the previous season could not initiate any type of infection or disease symptoms which means that the conidia could not survive as long as it were stored in this experiment and the conidia that remain on the crop debris at the end of the season are not one of the means which used by the fungus for its overwintering. So, the initiation of infection could be caused by long distance dissemination of the conidia during the suitable growing date of sugar beets. Long distance dissemination of *Erysiphe* spp. has also been recorded in Europe (Hermansen & Stix, 1974). The conidia of *E. betae* are not viable after short periods at low temperature (Dzhanuzakov, 1965). If the sexual stage of the fungus is rare, overwintering by this means may be of little importance (Kontaxis et al., 1974). Observation on the scanning electron microscopy (SEM) images of the infection method of sugar beet powdery mildew (*E. betae*) show that the fungus penetrates the epidermis of the leaves by the haustoria. It was clearly observed that haustoria penetrate the stomata of the leaf easily and successfully. The convoluted haustoria penetrated the leaf epidermis in many points infecting the entire leaf surface. The haustoria were convoluted and

folded in multiple ways. They entered perpendicularly the leaf from the top. There were visible several conidiphores formed, preparing new source of secondary infection. The entering zone of the haustoria, the hyphal part was thickened, being like a connection tube between the fungus and the leaf. These results are in accordance with those of Hickey and Yoder (1990), Biggs et al. (2009), Pintye et al. (2011) and Jakabilyefalvi (2016) who studied powdery mildew infection steps. The effects of three macronutrients (*i.e.* calcium chloride, potassium silicate and sodium bicarbonate) at the concentrations of 0.1, 0.2 and 0.3 g/l were studied. The results indicated that 0.3 g/l of sodium bicarbonate achieved the best percentage of disease reduction whereas; the least disease reduction was obtained by 0.1 g/l of potassium silicate on both Sirona and FD.0807cultivars. It is well known that mineral nutrients are essential for the growth and development of plants and microorganisms, and are important factors in plant-disease interactions. Any nutritional deficiency hinders plant metabolism and results in a weakened plant, which lowers disease resistance. Plant nutrients may affect disease susceptibility through plant metabolic changes, thereby creating a more favorable environment for disease development. When a pathogen attacks a plant, it alters the plant's physiology, particularly with regard to mineral nutrient uptake, assimilation, translocation, and utilization. There are two primary resistance mechanisms that mineral nutrition can affect; First by formation of mechanical barriers, primarily through the development of thicker cell walls, Second by synthesis of natural defense compounds, such as phytoalexins, antioxidants, and flavanoids that provide protection against pathogens (Spann & Schumann, 2010). Calcium compounds play an essential role in the formation of healthy, stable cell walls. Adequate Ca also inhibits the formation of enzymes produced by fungi and bacteria, which dissolve the middle lamella, allowing penetration and infection. Ca deficiencies trigger the accumulation of sugars and amino acids in the apoplast, which lowers disease resistance (Kelman et al., 1989). Silicon is combined with other components to give cell walls greater strength as physical barriers aginst penetration by *Pyricularia grisea* (rice blast) and *Erysiphe* spp. (mildews), and is involved in physiological responses to infection by increasing the availability of K and mobility of Mn (Savant et al., 1997; Datnoff et al., 1991). Mineral nutrition also affects the formation of mechanical barriers in plant tissue. As leaves age the accumulation of silicon (Si) in the cell walls helps in forming a protective physical barrier to fungal penetration. Potassium (K) is essential for the synthesis of proteins, starch, and cellulose in plants. Cellulose is a primary component of cell walls, and K deficiency causes cell walls to become leaky, resulting in high sugar (starch precursor) and amino acid (protein building blocks) concentrations in the leaf apoplast. Unlike for other nutrients, the generalization can be made for K that an adequate supply usually results in an increased resistance to attack by all parasites and pests. Potassium deficiencies created by over application of dolomite or magnesium lowers this resistance (Spann & Schumann, 2010).

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, 1979). Five commercial fungicides were tested for their effectiveness against powdery mildew disease on sugar beets. The results indicate that, all tested fungicides significantly reduced the disease severity as compared to the control treatment. The high noticeable significant disease reduction was achieved by Bellis® and Collis® fungicides followed by Tilt®, Camzin® and Permatrol™ respectively. The highest similar effect of Bellis and Collis fungicides could be attributed to their similar mode of action due to their active ingredients which are related to the same groups of fungicides (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 Bartlett et al. (2002), Hollomon and Wheeler (2002) and Karaoglanidis and Karadimos (2006). Propiconazole, the active ingredient of Tilt fungicide is related to (Demethylation Inhibitors) fungicide group that affect sterol biosynthesis in membranes of the fungal cells, which explain its role in decreasing disease severity, this result is in accordance with those of Kontaxis (1978), Kolbe (1981), Paulus *et al*. (1986), El-Desouky (1988), El-Shami *et al*. (1995), Warkentin *et al*. (1996) and Gado (2013). Acceptable disease reduction was achieved by Camzin fungicide which contains carbendazim that affect cytoskeleton and motor proteins of the fungal cell this is in agreement with Iqbal et al. (1994), Ahmed (1995), He et al. (1998) and Ziedan and Farrag (2011). The combination of Camzin and Tilt fungicides is more effective than Camzin only due to the potential of propiconazole the active ingredient of Tilt fungicide which is clearly better than Camzin as individual treatments. The inhibitory effect of jojoba oil, the active ingredient of Permatrol is also recorded in previous findings (Moharam & Obiadalla Ali, 2012; Alahakoon et al., 2010; Nuñez-Palenius et al., 2009; Singh, 2008; Konstantinidou-Doltsinis et al., 2006; Rettinassababady et al., 2000). The importance of plant derived agents such as jojoba oil 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. On the other hand, the fungicides resistant races of some pathogens have been reported by Fernández Aparicio et al. (2009) and O'Brien (1994). As well as the side and undesirable effects of fungicides on human health and the environment (Durmusoglu et al., 1997; Garcia, 1993). Despite that, fungicides are still the most dependable method in controlling such diseases. In this research, all studied treatments, macronutrients or fungicides, recorded the highest values concerning sucrose and root weight. This may be referred to the effect of the treatments on the general health of the beets. Meanwhile, the different treatments may

have effect on the storage parenchyma tissues where the sucrose is stored, but this point need further study in the future.

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