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ORIGINAL PAPER

Enhancing the Ability of the Sugar Beet Plants to Control Powdery Mildew by Using some Chemical Fungicides

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ABSTRACT

Sugar beet (*Beta vulgaris*L.) is an important cash crop for the production of sugar beet grown in temperate regions. Often, fungal leaf diseases infect the sugar beet crop during the season, causing significant yield reductions. The efficacy of some chemical fungicides to control the infection by the disease and their impact on yield and sugar content was studied. The obtained data showed that plants and yield traits were significantly exceeded by applying fungicides used. Plants treated with Eminent recorded the highest efficiency regarding disease management as well as root weight, TSS, sugar percentage and purity followed by Nativo then Microthiol. In addition, plants sprayed with Eminent recorded the highest content of total phenols and peroxidase activity followed by Nativo and Microthiol. Only Nativo was safe for human after harvest that detected maximum residue level (MRL) ratio 0.02 at harvest. On the other hand, Eminent was the most dangerous to human, where recorded the highest residues at harvest and after 14 days of harvest. Foliure fungicide didn't show any decline in their residue at 14 days after harvest.

Keywords: Sugar beet, Powdery mildew, Chemical fungicides, Maximum Residue Level (MRL), Total phenols, Peroxidase.

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INTRODUCTION

Sugar beet (*Beta vulgaris* L.) is a dicotyledonous herbaceous plant in the Amaranthaceae family. Sugar beet together with sugarcane are the two largest sources of sugar production worldwide. Sugar beet is a temperate climate crop that produces approximately 40% of the world's annual sugar production and can be used as a source of bioethanol and animal feed.(Bastas and Kaya, 2019).

Egypt's main crop for producing sugar has become sugar beet. Every year, it expands to accommodate the demands of the increasing population. The cultivated area with sugar beet during 2021/2022 growing season reached about 682771 feddans, which produced about 1708400 tons sugar (Anonymous,2022). Sugar beet plants under field conditions are attacked by many foliage diseases such as Cercospora leaf spot caused by *Cercospora beticola* Sacc., powdery mildew caused by *Erysiphe betae* (Vanha) Weltzeinand , rust caused by *Uromyces betae* Press causing serious diseases and severely reduced root yield and sugar quality subsequently. However, powdery mildew is the most constrain one Under Egyptian conditions **(Ata *et al.*, 2023).**This disease causes economic damage on which inflicts farmers worldwide with sugar crop losses under severe disease infection reaching 30% (Francis, 2002).Sugar beet roots are severely affected by the disease, which resulting in up to a 22% reduction in root yield and a 13% reduction in sucrose content (El-Fahhar and Abou El-Magd, 2008). Meantime, powdery mildew is one of the significant diseases that affect sugar beet foliage growth and significantly reduce root weight

(Kontradowitz and Verreet, 2010 and Ata *et al*, 2023).Powdery mildew is a severe fungus of sugar beet in places with generally warm and dry climatic conditions globally, and it causes damage that affects plant growth as well as sugar production (Gado, 2013 and Ata *et al*, 2023).

Environmental conditions in Egypt are suitable for the spread of powdery mildew, particularly in late planting after October. Powdery mildew infection causes sucrose losses of up to 82.9%. George and Karadimos (2006) evaluated four fungicides belonging to Strobilurin chemical group: azoxystrobin, kresoxim-methyl, pyraclo-strobin and trifloxystrobin in three different application doses (100, 150 and 200 mg a.i. ha–1) for the control of powdery mildew, caused by *Erysiphe betae* in sugar beet. Among the four strobilurin fungicides tested, trifloxystrobin and kresoxim-methyl were the most effective with control efficiency values higher than 94% compared to the control treatment even when applied at lower application dose of 100 mga.i. ha^{-1}. Azoxystrobin and pyraclostrobin showed a poor to modest activity against the disease even when applied at the highest application dose of 200 g a.i. ha^{-1}.

If the disease is not controlled, it can cause a loss of 20 to 35 percent in the yield (Grimmer*et al.*, 2007). On the other hand, under suitable conditions, total soluble solids and root weight are greatly impacted by disease severity(El-Fahhar, 2008).

The aim of the current study was to determine efficacy of some chemical fungicides oncontrolling powdery mildew infection of sugar beet plants with estimationtheir impact on yield, sugar content and purity, as well as theirmaximum residue level (MRL) in the harvested yield.Also, determination the effect of spraying these fungicides on the total phenols content and activity of peroxidase in the leaves of the treated plants.

MATERIALS AND METHODS

Field trials were carried out in the Experimental Station of Gemmeza Agricultural Research Station, to estimate the role of some fungicides in controlling powdery mildew in sugar beet during 2019/2020 and 2020/2021 seasons.

1.The experiments layout:

The trials were designed with three replicates in a randomized complete block design. Sugar beet cv. Hercules was cultivated at the recommended density (35000 plant fed^{-1}) in rows of three meters length and 60 cm apart with four rows per each experimental plot. Four seeds were planted in each hill on one side of the row in September 15^{th}. Four weeks after sowing the emerged seedlings were thinned into one plant per hill.

Before the first and second irrigations, sugar beet got 100 kg N fed^{-1} (80 N unit fed^{-1}) in two similar dosages. Nitrogen was applied from source of urea (46.5%), while calcium super phosphate (15.5% P_2O_5) was applied at soil preparation. Sugar beet was additionally treated with 50 kg potassium sulphate (48% K_2O) as a single dosage just before the second irrigation.

2.Chemical fungicides:

The effectiveness of six fungicides *i.e*., Alliet, Eminent, Folicure ,Microthiol, Prolectus, , and Nativoon on controlling powdery mildew caused by *Erysiphe betae* on sugar beet cv. Hercules under natural infection circumstances grown under open field was assessed(Table, 1). Also, the effect of these treatments on yield and sugar content as well as sugar purity was taken into consideration. Moreover, estimation the effect of these fungicides on total phenols content and activity of peroxidase in the leaves of the treated plants.

Table (1): Trade name, Common Name, active ingredient %, dosage and chemical name of the tested fungicides

Trade name	Common name	Formulation %	dosage 100 L^{1-} water
Nativo	Tebuconazole 50% + Trifloxystrobin 25%	75 % WG	25 g
Microthiol	Sulphur	80 % WG	250 g
Prolectus	fenpyrazamine	50 % WG	50 g
Alliet	fosetyl-aluminium	80 % WP	250 g
Folicure	tebuconazole	25 % EC	50 ml
Azostar	azoxystrobin	25 % SC	50 ml
Eminent	tetraconazole	12.5 % EW	100ml

3.Assessment of powdery mildew severity:

All tested fungicides were sprayed three sprays at the recommended dose started after the first appearance of the disease with 15 days intervals between each spray (Kontradowiz and Verreet, 2010).

The following measurements were recorded as follows:

For each fungicide at three different times during the development of vegetative growth, the visual estimation of the affected leaf surfaces of at least 40 leaves (10 plants per test, replications entirely randomized) were used to estimate the severity of the disease.

3.1 Disease assessment:

To evaluate the severity of powdery mildew, a six-category disease index devised by Hills *et al.* (1980) was used. The mycelium-covered percentage of leaves area was indicated by the scale categories (R0=0%, R1=10%, R2=35%, R3=65%, R4=90%, and R5=100%). Disease severity was calculated according the following formula:

3.2 Disease severity %=

$$\frac{\sum(\text{Each category x number of leaves in each category})}{\text{The total leaf number x the highest category}} \times 100$$

The Efficacy of each treatment in reducing powdery mildew severity was calculated as a percentage using the formula of Derbalah *et al*. (2011).

Efficacy%= [(DSC-DST)/DSC] ×100

Where:

DSC: Disease severity in control.

DST: Disease severity in treatment.

At the end of the trials (180 days after planting), plants were harvested, roots were weighed and quality traits *i.e.*,percentages of T.S.S., sucrose content and purity were assessed in fresh roots.

4.Evaluation of yield and quality:

4.1.Average of one root weight (kg):

The average of one root weight was calculated as the average of ten randomly selected roots (kg) from each plot.

4.2.Total soluble solids (T.S.S %):

Random samples, each of 10 roots have been taken randomly from each plot to evaluate the total soluble solids using hand refractometer at harvesting.

4.3.Sucrose %:

The percentage of sucrose was determined in fresh samples of sugar beet roots using a saccharometer based on the method given by Anonymous (1995).

4.4.Sugar purity:

Sugar purity was determined using an automatic sugar polarimeter according to methods described by Le-Decote (1971) and McGinnus (1982).

5. Determination of total phenols and Peroxidase activity in the leaves:

5.1Total phenols:

Total phenols were measured using a Jenway England UV/Vis spectrophotometer at 750 nm, as reported by Singleton *et al*.(1999).

5.2.Peroxidase activity:

The peroxidase activity was measured colorimetrically using the method given by Amako *et al*. (1994). Peroxidase enzyme activity was measured as a change in absorbance at 430 nm per minute per gram of fresh leaves.

6. Determination of fungicides residue in sugar beet roots:

A certified laboratory used liquid chromatograph y/ mass spectrometry (LC-S/MS), acetonitrile extraction, and the QuEChERS technique to determine the amount of fungicide residues in sugar beet roots were **used** (Alder *et al*., 2006 and Anonymous, 2008). For this approach, the limit of quantification (LOQ) is 0.01 mg kg^{-1}. Acetonitrile was used to extract the fungicides from the homogenized sample, and the Agilent Technologies 6460 Triple Quad LC/MS system was used to analyze the filtered extract in order to determine their presence**(Simon-Delso *et al*., 2015).**

7.Statistical analysis:

All data were subjected to analysis of variance (ANOVA) for the whole randomized block design, followed by a comparison of means using LSD at the 0.05 level of probability, as described by Gomez and Gomez (1984).

RESULTS AND DISCUSSION

Powdery mildew caused by *Erysiphe betae* is the most serious and destructive foliar disease in many sugar beet growing regions of the world including Egypt, causing sugar yield losses of up to 30% (**Ruppel, 1995and Ata *et al*, 2023).**

1- Efficacy of some fungicides against powdery mildew:

Presented data (Table, 2) show that all used fungicides significantly decreased the infection by powdery mildew caused by *E. betae* compared with the control in both seasons (2019/2020 and 2020/2021). Eminent fungicide was the most effective fungicide against powdery mildew, where it resulted in the lowest disease severity, being 0.83 and 0.93% with the largest reduction efficacy of 99.13 and 98.97% followed by Nativo with disease severity of 4.19 and 3.98% and reduction efficacy of 95.64 and 95.59% then Microthiol with disease severity of 9.21 and 9.89% and reduction efficacy reached 90.41 and 89.03% in both seasons, respectively. On the other side, Prolectus fungicide seemed to be an ineffective fungicide against powdery mildew. Eminent fungicide exaptation in reducing sugar beet powdery mildew and improving growth metrics. The maximum impact of Eminent fungicide might be linked to its active ingredient mode of action, which is tetraconazol, which gives sterol dimethylation inhibiting group (DMI) (AbdElhady and Bondok, 2017). Chemical fungicides have been utilized as the primary technique for controlling powdery mildew infection and, as a result, increasing yield production (Hassan and Berger, 1980 and Wolf and Verreet, 2008). Chemical treatment is strongly advised since powdery mildew is an aggressive and damaging disease that is unlikely to be controlled without the application of fungicides. Fungicides are well known for their role in disease control (McGrath2004). Finally, Avižienytė *et al*. (2016) found that the fungicide Opus is the most effective against powdery mildew when treated twice. Various active ingredients of the fungicides had insignificant effects on sugar beet yield and sucrose content in the roots. A single application of the fungicide Artea 0.5 L ha^{-1} (a.i. propiconazole+ cyproconazole) and a double application of Opus 1.0 L ha^{-1} (a.i. epoxiconazole) and Folicur 1.0 L ha^{-1} (a.i., tebuconazole) significantly decreased the content of impurities (potassium, sodium, alpha amino nitrogen) in the sugar beet roots compared with the untreated control treatment.

2. Effect of some fungicides on sugar beet yield and quality.

Results in Table (3) show that all tested fungicides significantly exceeded expectations in increasing all sugar beet yield traits in both seasons (2019/2020 and 2020/2021). Sugar beet plants treated with the fungicide Eminent recorded the highest root weight, being 1.09 and 1.14 kg $root^{-1}$, TSS being 20.89 and 21.88%, sugar percentage, being 18.11 and 17.68% and sugar purity, being 83.22 and 81.24% in both seasons, respectively, followed by Nativo then Microthiol in all previous traits in both seasons. In the present study, all tested fungicides showed positive effects on root yield, sugar yield and qualities. Fungicides have been used for a long time as the main strategy for controlling powdery mildew on sugar beet and subsequently increased yield production (Hassan and Berger 1980 and Wolf and Verreet, 2008). Our results are in the same line with those of George and Karadimos (2006) who, evaluated four fungicides belonging to the relatively new class of strobilurin fungicides, azoxystrobin, kresoxim-methyl, pyraclostrobin and trifloxystrobin for the control of powdery mildew, caused by *E. betae* in sugar beet. Among the four strobilurin fungicides tested, trifloxystrobin and kresoxim-methyl were the most effective with control efficiency values higher than values of control treatment even when applied at lower application dose of 100 mg a.i. ha^{-1}. Disease severity, in terms of AUDPC values was significantly correlated to decreased root yield, while no significant correlation existed among disease severity values and sugar content of the roots or sucrose yield. Also, Andr (2009) showed that control of diseases appears to be best achieved using preparations that combine benzimidazole and triazole antifungal preparations. Topsin-M 70WP (a benzimidazole) and Impact (a triazole) combined preparations act systemically, have a relatively a significant and positive influences on yields and sugar contents. Topsin-M 70 is associated with improved yield, sugar content and keeping quality in beets. In the same way, Gado, (2013) evaluated three triazole derivative fungicides (Score, Eminent and Opus) against sugar beet powdery mildew and found that Eminent and Score were the best fungicides, they increased root weight and sugar content followed by Opus. sugar purity, *i.e.*, potassium, sodium and alpha amino acids were greatly decreased due to spraying the tested compounds compared to control. While, Gouda and El-Naggar (2014) found that spraying Eminent increased root yield, sucrose percentage and gross sucrose more than 90, 56 and 214 %, respectively compared with the untreated plots. However, Montoro caused more than 70, 35 and 136% increases in yield, respectively. Sprays with Galben provided less increases in yield components up to 37, 30 and 80%, respectively. Also, Khan (2014) reported that application triphenyltin hydroxide or pyraclostrobin, generally provided better leaf spot disease control compared to control, in addition to root yield ranged from 12.1 to 16.4 tones ha^{-1} higher, and sucrose concentration 2.4 to 3.8% higher,than the check treatment. According to Khan and Khan (2010), the maximum leaf spot disease control was by Nativo and Triazole treatments. The efficacy of Chlorothalonil was, also, better than Mancozeb and Propineb.

Fungicides have been used for a long time as the main strategy for controlling powdery mildew disease. In the present study, all tested fungicides showed positive effect on disease control, root yield, TSS%, sucrose% and purity%.

Table (2): Efficacy of some fungicides against powdery mildew of sugar beet caused by *E. betae*

Fungicides	Disease severity % during		Efficacy % during	
	2019/2020	2020/2021	2019/2020	2020/2021
Nativo	4.19 ef	3.98 ef	95.64 ab	95.59 ab
Microthiol	9.21 de	9.89 de	90.41 bc	89.03 bc
Prolectus	82.71 b	73.27 b	13.90 e	18.73 e
Alliet	77.91 b	69.44 b	18.89 e	22.98 e
Folicure	14.79 d	13.31 d	84.60 c	85.24 c
Eminent	0.83 f	0.93 f	99.13 a	98.97 a
Control	96.06 a	90.16 a	-	-
LSD at 0.05	7.93	7.17	7.25	6.79

Table (3): Effect of some fungicides on sugar beet yield and quality

Fungicides	Root weight (Kg)		TSS %		Sucrose %		Purity %	
	2019/20	2020/21	2019/20	2020/21	2019/20	2020/21	2019/20	2020/21
Nativo	1.07 a	1.03 b	20.56 a	19.77 b	17.15 b	16.49 b	83.58 a	80.37 a
Microthiol	0.99 b	0.95 c	19.09 b	18.23 c	15.92 c	15.21 c	80.61 c	77.00 b
Prolectus	0.64 f	0.71 f	12.28 f	13.63 f	11.71 f	12.99 d	59.28 f	65.77 c
Alliet	0.70 e	0.73 ef	13.44 e	14.01 ef	12.45 e	12.98 e	63.03 e	65.73 c
Folicure	0.91 c	0.85 d	17.38 c	16.31 d	16.10 c	15.12 c	81.51 bc	76.53 b
Eminent	1.09 a	1.14 a	20.89 a	21.88 a	18.11 a	17.68 a	83.22 ab	81.24 a
Control	0.56 g	0.51 g	10.75 g	9.79 g	10.95 g	11.66 f	50.32 g	53.58 d
LSD at 0.05	0.04	0.03	0.86	0.79	0.46	0.36	2.05	1.40

3. Effect of some fungicides on sugar beet content of total phenols and peroxidase activity:

The obtained results (Table, 4) indicate that contents of total phenols and activity of peroxidase in sugar beet plants significantly varied under the effect of different used fungicides. All tested fungicides significantly increased sugar beet plant contents of total phenol and peroxidase in comparison to the control. Sugar beet plants sprayed with Eminent fungicide had the highest content of total phenol (0.79 mg g^{-1}fw) and peroxidase (0.75 mg g^{-1}fw) followed by Nativo and Microthiol fungicides. In contrast, sugar beet plants under the control treatment had the lowest contents of total phenol (0.44 mg g^{-1}fw) and peroxidase (0.41 mg g^{-1}fw). All used fungicides can reduce fungi growth by two methods. The first method was the direct effect on fungi growth or reduces spore production and this effect is associated with the fungicide mode of action. While, the second method was on host plant enzymatic defense reactions. Some fungicides increase the host plant reaction by increasing the production of defense enzymes such as peroxidase or polyphenol oxidase. In this study all used fungicides increased total phenols content and peroxidase in sugar beet plants in comparison to the control. Ebrahim and Helmy (2016) found that enzymes, phenols, sugars, proteins and chlorophyll contents in leaves exhibited low contents of sugars, phenols, protein, pigments and antioxidant enzymes under Eminent fungicide. While, AbdElhady and Bondok, (2017) found that Eminent fungicide was the superior in reducing sugar beet powdery mildew and improving growth metrics. This fungicide efficacy is associated with the high defense enzymes production such as peroxidase and polyphenol oxidase. Eliwa *et al.* (2018) mentioned that Bellis fungicide's mode of action could be attributed to its active ingredients, which are related to chemical fungicides groups (succinate dehydrogenase inhibitors and quinone outside inhibitors) that affect the respiration process in the fungal cell, and these groups have been proven to be very effective to control powdery mildew infection. Sugar beet plants treated with Bellis produced higher peroxidase and phenol oxidase than the control. The resistance has been associated with a high activity of defensive enzymes such as peroxidase (Farrag and El-Mansoub, 2020).

4. Residues of some fungicides in sugar beet roots:

Data (Table, 5) show that only Nativo fungicide is safe on human after harvest, where it declines to the maximum residual level (MRL) ratio 0.02 at harvest. Eminent was the most dangerous fungicide for human, where this fungicide recorded the highest residues (1.34 and 0.30 mg kg^{-1}) at harvest and after 14 days of harvest. Folicure fungicide did not show any decline in their residue after 14 days of harvest. After 14 days of harvest both Folicure and Eminent residues were more than the M LR effect of different used fungicides. All tested fungicides significantly increased sugar beet plant contents of total phenols and peroxidase activity in comparison to the control. Sugar beet plants sprayed with Eminent fungicide had the highest content of total phenol (0.79 mg g^{-1}fw) and peroxidase (0.75 mg g^{-1}fw) followed by Nativo and Microthiol fungicides. In contrast, sugar beet plants under the control treatment had the lowest contents of total phenols (0.44 mg g^{-1}fw) and peroxidase (0.41 mg g^{-1}fw).

Table (4): Effect of some fungicides on sugar beet contents of total phenol and peroxidase activity.

Fungicides	Total phenols (mg g^{-1}f.w)	Enzyme activity
Nativo	0.72 ab	0.70 ab
Microthiol	0.71 ab	0.69 b
Prolectus	0.61 cd	0.50 d
Alliet	0.53 d	0.45 de
Folicure	0.67 bc	0.66 bc
Eminent	0.79 a	0.75 a
Control	0.44 e	0.41 e
LSD at 0.05	0.09	0.06

There is no enough information's for fungicide residues in plant tissues as most fungicides had short

Table (5): Residues of some fungicides in sugar beet roots.

Fungicides	Residues at harvest (mg kg^{-1})	Residues 14 days after harvest (mg kg^{-1})	MRL EU of pesticides
Nativo	0.02 c	0.01 c	0.02 c
Folicure	0.21 b	0.21 b	0.02 c
Eminent	1.34 a	0.30 a	0.05 b
LSD at 0.05	0.07	0.02	0.01

half live periods .Andr (2009) showed that, benzimidazole and triazole have a relatively long residual effect and a significant and positive influences on yields and sugar contents.

CONCLUSION

This study demonstrates the importance of using fungicides in managing powdery mildew in beets, as they are the most effective in suppressing diseases, taking into consideration the use of pesticides that are most effective in suppressing the disease, as these pesticides led to an increase in phenols and the activity of peroxidase enzyme, which in turn increases the root yield and the subsequent increase sugar extracted from the roots.However, the wide spread use of the chemical fungicides has become a subject of research concern due to their environment pollution, harmful effect on non-target organisms as well as their possible carcinogenicity.

CONFLICTS OF INTEREST

The author(s) declare no conflict of interest.

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