




ORIGINAL PAPER

## Management of Powdery Mildew Caused by *Erysiphe betae* in Sugar Beet using Algal Products

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### ABSTRACT

Powdery mildew, caused by *Erysiphe betae*, is one of the most serious diseases affecting sugar beet plants globally. It causes a great loss in the root yield, sugar percentage, and quality of produced sugar. The current study aimed to evaluate the three biocontrol agents, intracellular or extracellular products of microalgae (*Spirulina platensis*, *Nostoc muscorum* and *Anabaena oryzae*), over two successive growing seasons 2020/2021 and 2021/2022 against *E. betae* under greenhouse and field conditions. Results showed that *S. platensis* and *N. muscorum* gave a good potential to control powdery mildew of sugar beet and improve their productivity as well as root yield quality under field conditions. Furthermore, induced plant defense by increasing the activity of resistance enzymes (peroxidase (PO) and polyphenoloxidase (PPO)) and phenolic compounds under greenhouse conditions. The results also showed that, the superiority effect of cell culture on biomass ethanolic extract of *N. muscorum* which highly achieve efficiency in management of disease. In overall, this study indicated the potential benefits of using microalgae to control powdery mildew in sugar beet.

**Keywords:** Sugar beet, *Beta vulgaris*, Powdery mildew, *Erysiphe betae*, *Nostoc muscorum*, *Anabaena oryzae*, *Spirulina platensis*.

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### INTRODUCTION

In Egypt, *Beta vulgaris* L., known as sugar beet, is now a significant source of sugar. According to Cooke and Scott (1993), 27% of the world's sucrose is produced by the significant sugar beet crop. Azzazyet *et al.*, (2007) reported that root production and sugar ratios of the roots are the main determinants of sugar yield per unit area. *Erysiphe betae* fungus the cause of powdery mildew is considered one of the most severe foliar disease in the world, with 30% losses of production in sugar beet, (Francis, 2002). And also, Cause root yield reductions up to 22% as well as decreases in root sugar quantity up to 13% with (Forster, 1979).

Natural compounds and microorganisms like fungi and bacteria that often colonize living plants without creating visual damage have drawn a lot of attention recently (Haggag and Radwan, 2014). These factors can also trigger a plant's defense response. Similar to this, cyanobacteria (Blue green algae) have a part in soil fertility, system soil reclamation, bio-controlling agricultural diseases and pests.

Biofertilizers, biopesticides, and bio-fungicides made from algae have been developed (Stadnik and de Freitas, 2014). Diverse organisms known as blue-green algae (cyanobacteria) are widely found in maritime environments and typically yield large amounts of biomass. As evidenced by the success of already commercialized algae-derived benefit products such as biofertilizer, biopesticides, and biofungicides, blue-green algae (cyanobacteria) exhibit enormous potential for the production of novel agricultural technologies (Stadnik and Mateus de Freitas, 2014).

Algae are a key biotechnological source of compounds that can stimulate plant growth and protect plants from disease (Khan *et al.*, 2009). A sustainable, incredibly nutrient-dense, and environmentally friendly microalga has recently been suggested: *Spirulina platensis*, a microscopic and filamentous cyanobacterium (Eleiwa *et al.*, 2018). According to Marangoni *et al.* (2017), *Spirulina* has potent anti-inflammatory properties, is a free radical scavenger, and can inhibit the growth of some

gram-positive, gram-negative, and yeast bacteria as well as *Candida albicans*. *Anabaena variabilis*, a cyanobacterium, has an antifungal activity that inhibits the growth of plant pathogenic fungus species. *Aspergillus niger* and *Rhizopus stolonifer* are two plant pathogenic fungal strains, could grow and develop more slowly in the presence of *A. variabilis* extracts. Based on zone of inhibition formation, it was concluded that the extracts of *Anabaena* had significant antifungal efficacy (Tiwari and Sharma, 2013).

It has been demonstrated that *Anabaena* spp. (Farnkmoelle *et al.*, 1992) and *Nostoc* sp. (Bloor and England, 1989) are effective in suppressing damping-off and the growth of the soil fungus *Cunninghamella blakesleana*. Particularly, seeds treated with cell extracts or culture filtrates from cyanobacteria are shielded from damping-off fungus such *Fusarium* sp., *Pythium* sp., and *Rhizoctonia solani* (Kulik, 1995). 29 of the 298 micro-algal strains evaluated in a previous study's antifungal activity was discovered, this study's focus was chosen because *Nostoc commune* FA-103 has a wide range of antifungal action against plant pathogenic fungi, particularly *F. oxysporum* (Kim, 2006).

The goal of this study was to assess blue-green algae (cyanobacteria) potential for reducing powdery mildew disease severity and improvement sugar beet yield and yield characteristics throughout two growing seasons 2020/2021 and 2021/2022.

## MATERIALS AND METHODS

### Source and cultivation of micro-algae:

*Spirulina platensis*, *Nostoc muscorum*, and *Anabaena oryzae*, filamentous heterocystous cyanobacteria, used in this study, were obtained from Soil, Water, and Environmental Research Institute Agricultural Research Centre (ARC). This *S. platensis* was previously injected into Zarrouk's liquid medium (Zarrouk, 1966) and the other algae into BG-11 (Rippka *et al.*, 1979).

Cultures were separately maintained in 1L flasks with 300 mL of sterilized culture media. The cyanobacteria were prepared in 3 ml of each flask, and then incubated at  $29 \pm 2$  °C with a 12 h light/ 12 h dark cycle. Three to four times a day, cultures were manually shaken (Pandey, 2010). After 20 days, the cell culture media were homogenate by vortex and filtrated by sterile muslin cloth then kept at 4°C with a 12 h light/ 12 h dark cycle for further studies.

### Preparation of ethanolic extract:

After 20 days, the biomass was separated

from the cultural media by Centrifugation (40 min, 800 gravity, 10 °C), The algal biomass was harvested and washed thoroughly in sterilized distilled water, after that dried in oven at 40°C even weight stability, then uniformly grinded using mechanical grinder to make fine powder. Ten grams of the powder were extracted with 70 % ethanol. The extract was concentrated at 40°C under reduced pressure (72 mbar) with a rotary evaporator. In order to be used later, extracts were collected in airtight containers and kept at 4°C (Al-Aarajy *et al.*, 2012).

### Gas chromatography (GC), analysis for algae extracts and cell-cultures:

The biomass extracts and cell cultures of three applied cyanobacteria were purified by microfilter 22µm and pass through adsorbent material to remove the pigments and impurities, then analyzed by gas chromatography (GC) (Agilent 7000 Triple Quad) with a split automatic injector and silica capillary column DB-5 (length: 60 m; ID: 0.32 mm). Helium is used as carrier gas at a flow rate of 1 ml/min. The column is held at 150 °C for 1 min and ramped to 240 °C at a rate of 30 °C/min, then held at 240 °C for 30 min. The contents were identified by comparing the retention time of each peak to that of reference standards (Ahmed, *et al.*, 2019).

### Plant material:

The sugar beet cultivar selected for the experiment was "Pleno", a cultivar sensitive to powdery mildew (Ata, 2005). 12 hours before sowing, seeds were soaked in different treatments. Control soaked only in two applied media separately.

### Greenhouse tests:

This experiment was performed in the greenhouse of Maize and Sugar Crop Dis. Res. Dept. Plant Pathol. Res. Inst., ARC, Giza, (temp. 68° to 86°F, Shade or low light intensities as well as high relative humidity (greater than 95%), starting in October to the end of April in 2020/21 growing season, 120 pots (25 cm diameter) containing a 2:1 mixture of clay and sand were received normal irrigation and fertile once per week with 1% N: P: K (75:150:50) solution. Plants were thinned into two plants/pot 30 days after sowing. Three replicates were used for each treatment, each replicate had five pots.

### - Artificial inoculations in greenhouse:

For production of *Erysiphe betae* inoculum was obtained from a heavily infected field. Diseased leaves were transferred into the laboratory and conidia were washed off sporulating powdery mildew pustules and used to inoculate the test plants. Plants were

artificially inoculated with conidial Suspensions after four months of sowing. Before inoculation, one droplet of 0.1% Tween 80 was added to suspension.

#### **- Time of application:**

Treatments were applied preventively. Spray applications-initiated week before infection. Then repeated twice at intervals of 15-20 days (after appearing the symptoms on control plants).

#### **Field tests:**

Trials were carried out at the Research Experiment Farm at Sakha Agricultural Research Station under natural infection during 2020/21 and 2021/22 growing seasons. The experiment was designed in a complete block randomized. Three replicates were used for each treatment. Each replicate consisted of five rows 5 meter long and 50 cm spaced between plants.

#### **Time of application:**

Treatments were applied preventively. Sprayed applications initiated before expected infection after four months of seeding (at the beginning of March). Then repeated twice at intervals of 15-20 days or after appearing the symptoms on control plants.

#### **Treatments applications:**

The cell culture media and biomass extracts from each *S. platensis*, *N. muscorum* and *A. oryzae* cyanobacteria were applied separately as foliar application to evaluate their capabilities to induce resistance against powdery mildew of sugar beet in greenhouse and field. Control was sprayed with medium only (not inoculated with cyanobacteria). Before spraying, one droplet of 0.1% Tween 80 was added. The used fungicide (Tetraconazole 100 mL/ 100 L. water) as separate treatment Applied using an AZO precision sprayer, at a volume of 0.4L per plot and pressure 4 atm.

#### **Analysis two weeks after completion of treatment:**

##### **Disease assessment:**

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

##### **Biochemical research:**

##### **Activity of enzymes:**

Plants grown in greenhouse were used. Peroxidase (PO) and poly phenol oxidase (PPO) enzyme activity was measured according to Lobarzewski, (1990), PO activity was evaluated using guaiacol/H<sub>2</sub>O<sub>2</sub> as a substrate by spectrophotometer. PPO activity was assessed

using a spectrophotometer with catechol as a substrate, according to Fujta *et al.* (1995).

##### **Total phenolic content assay:**

The total phenolic content of the extracts and cell culture media were determined in accordance with a protocol described by Turkmen, and Velioglu (2005). Free phenols were determined in sugar beet leaves 10 days after the plants' treatment is complete Folin Ciocalteu reagent described by Bray and Thrope (1954). Conjugated phenols calculated according to the formula:

##### **Conjugated phenols**

$$= \text{Total phenols} - \text{Free phenols.}$$

##### **Scanning Electron Microscope (SEM)**

##### **Examination:**

Scanning electron microscope was utilized to examine the effects of the applied treatments on the development of conidia and spores, as well as the growth of *E. betae* on sugar beet leaves (Manzali *et al.*, 1993). Using a Jeol Scanning Electron Microscope model (SEM, Quanta FEG250, National Research Centre, Cairo, Egypt), interaction sites (spots) were noted and disc blocks of 1 cm<sup>2</sup> were obtained for SEM.

##### **Post-harvest analyses of field experiment for two seasons:**

##### **Root yield and its attributes:**

At harvest (200 days after sowing), 100 randomly selected plants from the third and fourth central ridges of each plot were picked to estimate root yield (ton/fed.) and measure fresh root weight.

##### **ii) Chemical components of roots:**

Ten guarded roots from each plot were randomly selected at each of the examined harvest ages and subjected to examination.

- The automatic saccharimeter was used to measure the amount of sucrose (LeDocte, 1977).
- Total soluble solids (T.S.S.) of fresh samples were measured using a fully automatic digital refractometer, model ATR-S (04320), with temperature correction between 15 and 40 °C and a Brix range of 0 to 95% (McGinnis, 1982).
- Purity percentage (%) was determined using the following formula (Devillers, 1988)

##### **Purity%=**

$$(\text{Sucrose\%/ Total soluble solid \%}) \times 100.$$

- The sugar yield (tons/fed) was determined using the formula:

Root yield (tons/fed) x sucrose%/100 equals sugar yield.

##### **Data Analysis:**

DSAASAT Version 1.1 software was used to do analysis of variance on the data (Snedecor and Cochran, 1980). Fisher's protected least

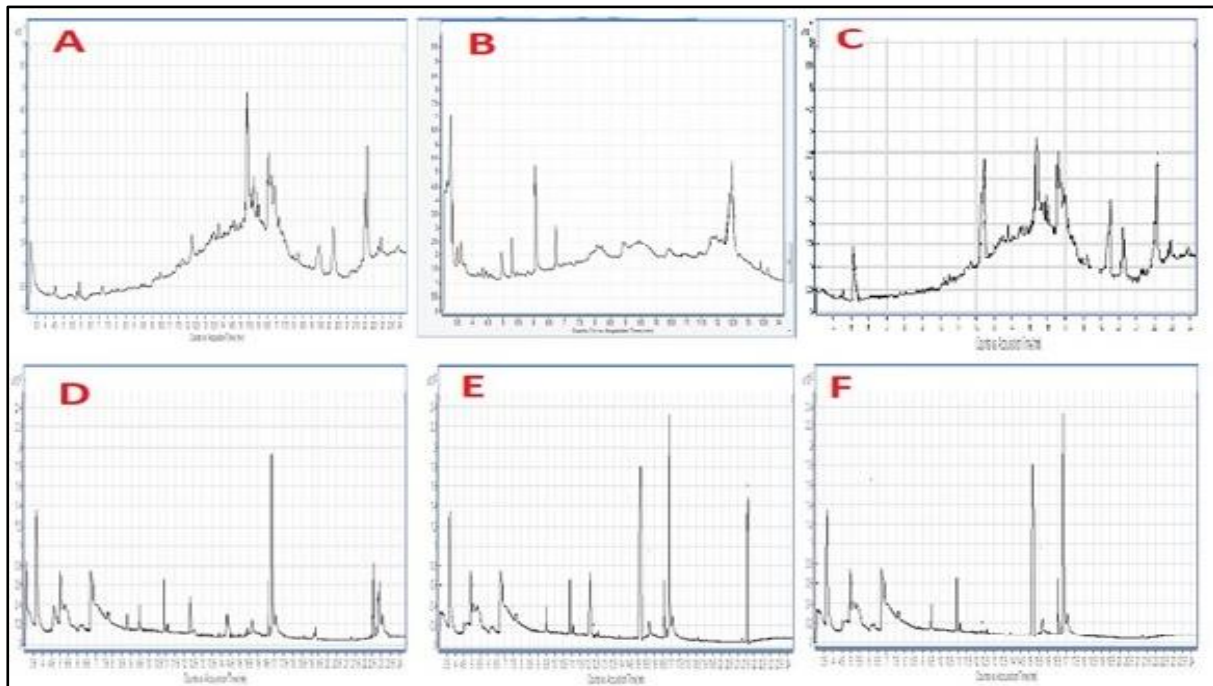
significant difference (LSD) at 5% significance was used to infer mean differences.

## RESULTS AND DISCUSSION

### Gas chromatography (GC) analysis:

During the progress of current investigation, both cell culture media and biomass extract were analyzed by GC analysis. The analysis of biomass extract of *Anabaena* showed twenty-

five components and twenty-two compounds for *Nostoc* extract while *Spirulina* extract produced twenty-three compounds (Fig., 1; A, B, and C respectively). In addition to that, nineteen, eighteen and twenty-one compounds were produced in cell culture of *Anabaena*, *Nostoc* and *Spirulina* (Fig., 1 D, E, F respectively). The analysis proved that these applied algae haven't the ability to produce any phycotoxins.



**Fig. (1): GC chromatography of ethanol extract of *A. oryzae* (A), *N. muscorum* (B) and *S. platensis* (C), and cell culture of *A. oryzae* (D), *N. muscorum* (E) and *S. platensis* (F).**

Data shown in Table, (1) reveal that the most abundant compounds of applied cyanobacteria have highly area sum % compared to other compounds that have been produced in our analysis. In the present study, Neophytadiene, Methyl stearate Stearic acid and Oxirane, decyl- were identified in cell culture and biomass ethanol extract of *Anabaena*, these compounds associated with the antimicrobial properties against a number of Gram-positive and Gram-negative bacteria were confirmed by Bhardwaj *et al.*, 2020 and Shi *et al.*, 2019 also reported strong effect against pathogenic fungi (Anjali *et al.*, 2019). In addition, analysis showed a presenting of Hexadecanoic acid, methyl ester which has antibacterial activity proved by Shaaban *et al.* (2021). Moreover, 1, 2-Benzenedicarboxylic acid which appears at retention time (RT) 22.1 min., has antifungal activity illustrated by Lotfi *et al.* (2021).

In the same context, the present study reported Undecane, 4,7-dimethyl, Heptadecane, Dimethyl Sulfoxide and Dibutyl phthalate in the biomass extract and cell culture of *N. muscorum*

that effects as antimicrobial compound against bacteria and fungi (Bukvicki *et al.*, 2013; Zara *et al.*, 2018 and Thejanuo *et al.*, 2020). Also, analysis of GC-mass in Table (1), reported the presence of 6-Methylheptan-2-one and Heptanal which have antibacterial activity (Xiaowei *et al.*, 2016 and Nafis *et al.*, 2019).

In the same vein, Cis-vaccenic, Crown-4-ether, Malonic acid, Octadecane, Phytol and phenol-d5 are the major components in the extract and cell culture of *S. platensis*. All of these compounds have antimicrobial behavior against pathogenic bacteria and fungi (Yildiz Mete, 2007; Prabhakar *et al.*, 2018 and Rowland *et al.*, 2018). A Remarkably, retention time (RT) 21.82 and 12.21 min referred to two main compounds Phytol and phenol-d5, respectively, in *Spirulina* ethanolic extract. phenol-d5 is growth promoting, antimicrobial substance (Pratyusha, 2022). Furthermore, Phytol which used a precursor for synthetic forms of vitamin-E and vitamin-K1 (Mandira and Bandyopadhyay, 2020).

From the foregoing, we conclude that all biomass extract and cell culture media of applied cyanobacteria in this study include antifungal compounds, Righini *et al.*, 2019 supported us about *A. oryzae* which inhibited *B. cinerea* colony growth in strawberry. *N.*

*muscorum* exhibited antagonistic activity against Gram-positive and Gram-negative bacteria and filamentous fungi (El-Sheekh *et al.*, 2006). Finally, Abdel-Moneim *et al.*, 2022 and Shedeed *et al.*, 2022, pointed to growth promoting and antimicrobial activity of *S. platensis*.

**Table (1): The major constituents in biomass extract and cultural filtrates of the studied cyanobacteria.**

Algae	Forms	Comp. name	RT (min)	Area sum%
<i>Anabaena oryzae</i>	biomass extract	Neophytadiene	15.181	19.65
		1,2-Benzenedicarboxylic acid	22.1	21.85
		Hexadecanoic acid, methyl ester	16.64	27.04
	cell culture	Hexadecanoic acid, methyl ester	16.48	19.64
		Methyl stearate Stearic acid	17.8	18.46
		Oxirane, decyl-	22.612	21.26
<i>Nostoc muscorum</i>	biomass extract	Undecane, 4,7-dimethyl-	6.31	18.54
		6-Methylheptan-2-one	8.746	16.45
		Heptadecane	12.419	19.74
	cell culture	Heptanal	6.82	17.06
		Dimethyl Sulfoxide	4.98	9.51
		Dibutyl phthalate	16.835	19.73
<i>Spirulina platensis</i>	biomass extract	phenol-d5	12.21	21.28
		Phytol	21.82	10.01
		Octadecane	15.58	16.6
	cell culture	Crown-4-ether	6.722	18.85
		Malonic acid	3.69	18.53
		Cis-vaccenic	16.86	14.54

#### The effect of cyanobacteria on the management of sugar beet powdery mildew under greenhouse and field conditions:

The effects of cell culture media and biomass extracts of filamentous heterocystous cyanobacteria (*S. platensis*, *N. muscorum* and *A. oryzae*) on the severity of sugar beet powdery mildew, caused by *E. betae* shown in (Table, 2). The efficacy of cell culture and biomass extracts differed significantly with the three types of cyanobacteria for the two successive seasons. The results illustrate superiority of the biomass extracts on the cell culture for disease management under greenhouse and field conditions during the two growing seasons with all cyanobacteria. The cell culture of *N. muscorum* showed remarkable superiority over all the other algal products used.

It has been established that algae are crucial to the protection and growth of crops. In addition, antifungal compounds of microalgal sources have replaced chemical pesticides. Table (1), put light spot-on high efficiency of *S.*

*platensis*, *N. muscorum* or *A. oryzae* which decrease the severity of powdery mildew due to superior of antimicrobial activity of these algae. Substances with antimicrobial effects may be insoluble in water, so that the biomass extract was superior in most cases. The results obtained can indicate that there are two types of cyanobacterial antifungal effects: the constitutive type, where antifungal substances are generally released by cyanobacteria, such as substances released by *S. platensis* and *A. oryzae* that act on the tested fungus (Table, 2), and the induced type, where antifungal substances are formed by cyanobacteria in the presence of the fungus, such as induced by *N. muscorum* against *E. betae*. The chemical nature of those constitutive and induced substances is not clear. Ibraheem and Abdel-Raouf, (2007) stated that in their study on the relation between cyanobacteria and bacteria. Nevertheless, the interaction of the exuded compounds with other metabolites in natural communities may enhance some of the observed effects (Suikkanen *et al.*, 2005).

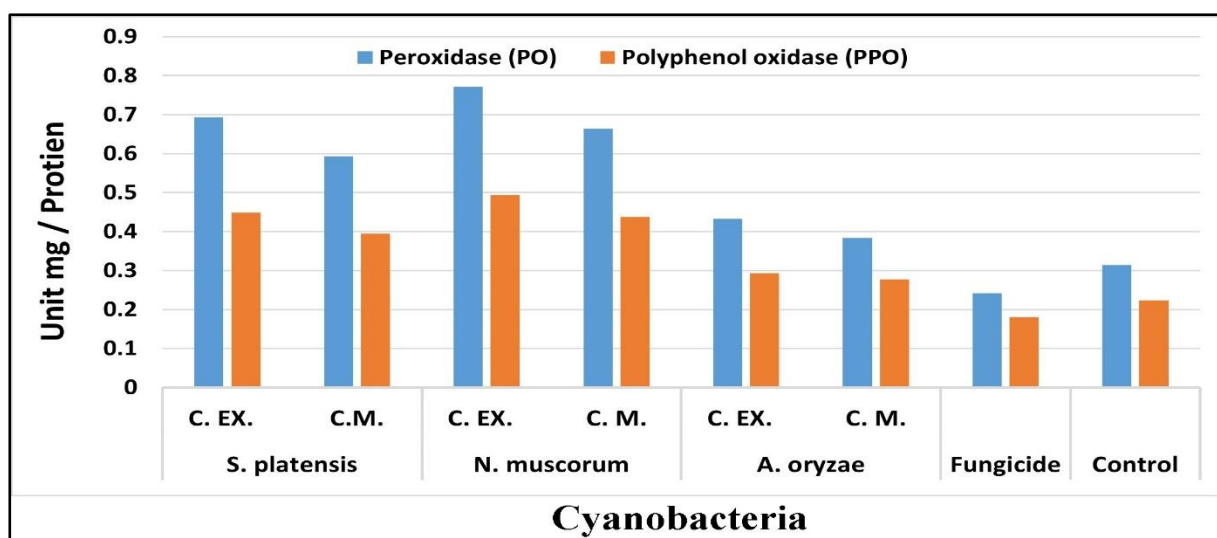
**Table (2): The effect of cyanobacteria on the management of Sugar beet powdery mildew under greenhouse and field conditions.**

Treatment	Forms	Greenhouse experiment		Field experiment			
				1 <sup>st</sup> season		2 <sup>nd</sup> season	
		DS.	Efficacy (%)	DS.	Efficacy (%)	DS.	Efficacy (%)
<i>S. platensis</i>	biomass extract	22.00	64.52	20.00	62.74	18.33	64.06
	cell culture	26.67	56.98	24.00	55.28	21.00	58.82
<i>N. muscorum</i>	biomass extract	23.00	62.90	19.33	63.98	17.33	66.02
	cell culture	19.33	68.82	15.00	72.05	12.00	76.47
<i>A. oryzae</i>	biomass extract	33.67	45.69	31.33	41.63	29.00	43.14
	cell culture	39.33	36.56	36.33	32.31	33.00	35.29
Fungicide	100cm <sup>3</sup> /100L.	15.00	75.81	10.33	80.75	8.33	83.67
Control		62.00	--	53.67	--	51.00	--
LSD at 0.05							
Treatments (A)		13.14		11.61		11.19	
Forms (F)		8.311		7.34		7.08	
A × F		18.58		16.42		15.83	

#### Effect of cyanobacteria on the activity of peroxidase (PO) and polyphenol oxidase (PPO) enzymes in leaves of sugar beet grown under greenhouse conditions:

Two weeks after completing treatments, their effects on two key enzymes in the enzymatic antioxidant machinery, peroxidase (PO) and polyphenoloxidase (PPO) (Fig. 2), were assessed in leaves of sugar beet in a greenhouse to better understand the biochemical mechanisms of the tested treatments. Generally, all tested bio-agents significantly increased the activity of defense related enzymes. Nonetheless, variable trend was observed at fungicide (Tetraconazole 100 cm<sup>3</sup>/100L. water) which inhibited the activity of both enzymes.

The maximum increase in PO and PPO activities was detected due to using *N. muscorum*, followed by treating with *S. platensis*. The lowest activity of these enzymes was detected in plants treated with *A. oryzae*. The biomass extract achieved a significant increased Peroxidase and polyphenol oxidase activities over culture media for all cyanobacteria. In response to microalgae treatment, our findings demonstrated a considerable positive association between peroxidase (PO) and polyphenol oxidase (PPO) activities (Fig. 2). These enzymes are important defense proteins that contribute to the disease resistance of plants. Our results are in harmony with those recorded by (Elsharkawy et al., 2022).



**Fig. (2): Peroxidase (PO) and polyphenol oxidase (PPO) enzyme activities in sugar beet leaves under greenhouse conditions are influenced by cyanobacteria (B. EX., Biomass extract; C. M., cell culture media).**

### Effect of cyanobacteria on phenols content in leaves of sugar beet grown under greenhouse conditions:

Data in (Fig., 3) show that leaves sprayed with the appropriate cyanobacteria resulted in an increase in the levels of free, conjugated, and total phenols. Additionally, there was a connection between the rise in sugar beet leaf phenolic content and resistance to *E. betae*. Additionally, research revealed that the younger leaves had higher levels of free and conjugated phenolic chemicals than the older leaves. With all of the tested blue-green algae, sugar beet treated with biomass extract were significantly better than sugar beet that had been sprayed with cell culture. The fungicide showed opposite behavior than previous treatments, as it reduced the total phenols to 14.085 compared to control

treatment. Biomass extract of *S. platensis* achieved the forefront on other microalgae followed by *N. muscorum*. Finally, *A. oryza* was the third one in influencing the phenol contents.

Phenolic compounds (PCs) represent the largest group of secondary metabolites in plants, ranging from simple aromatic rings to more complex molecules, also, including those extracted from microalgae, possess beneficial bioactivities such as antioxidant capacity, antimicrobial and immune modulatory activities in plant cells. To more thoroughly assess how microalgae affect plant stress tolerance. The results of the current study on microalgae are in agreement with Rachidi, (2021) about their potential to enhance plant defense responses and resistance to fungal diseases.

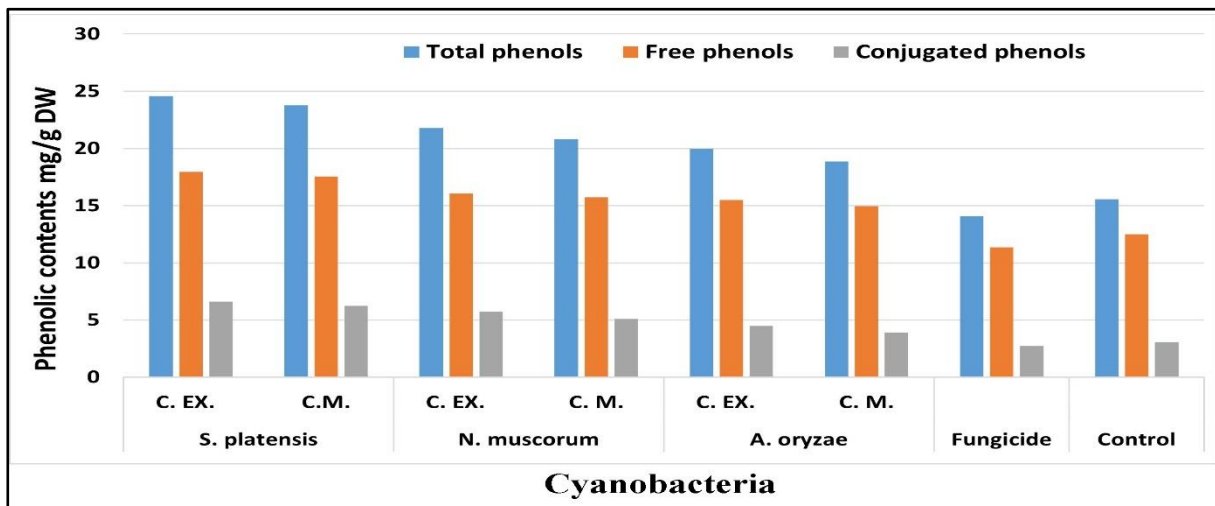


Fig. (3): Effect of cyanobacteria on phenols content in leaves of sugar beet grown under greenhouse conditions, (B. EX., Biomass extract; C. M., cell culture media).

### Scanning Electron Microscope (SEM) examination of the interaction among the most promising treatments and *E. betae* on leaves of sugar beet:

Several fungal morphological characteristics were also examined from the powdery mildew spots on treated plants compared to infected- not treated plants (control) using scanning electron microscope (SEM), in order to learn more about the potential mechanism(s) by which the tested treatments affected the fungal morphology. Growth, the density of conidiophores and conidia, and the disintegration of mycelium and conidia among the fungal morphological traits that were investigated. Micrograph SEM results show that *N. muscorum* was applied topically (Fig. 4B).

The density of the fungal mycelium was greatly reduced by *S. platensis*, Fig. (4 C), and *A. oryzae*, Fig. (4 D), particularly on leaves treated with *N. muscorum* and *S. platensis*.

Additionally, the phytopathogenic fungus' ability to produce conidiophores and conidia was deficient, and *E. betae* produced a much lower amount of conidia. Additionally, it resulted in plasmolysis and the breakdown of *E. betae's* mycelium and conidia. On treated leaves, it was interesting to observe that conidia, mycelium, and conidiophore showed signs of incompleteness and had twisted forms in their creation. It is worth mentioning disappearance of stomata in the leaf of sugar beet under the fungal hyphae in the control (Fig. 4A), and *vice-versa*, their appearance in the rest of the treatments (Fig., 4B, C, D). These results are inconvenient with Ziedan and Farrag, 2011 who studied the antagonistic action of *S. cerevisiae* on *E. betae* on sugar beet.

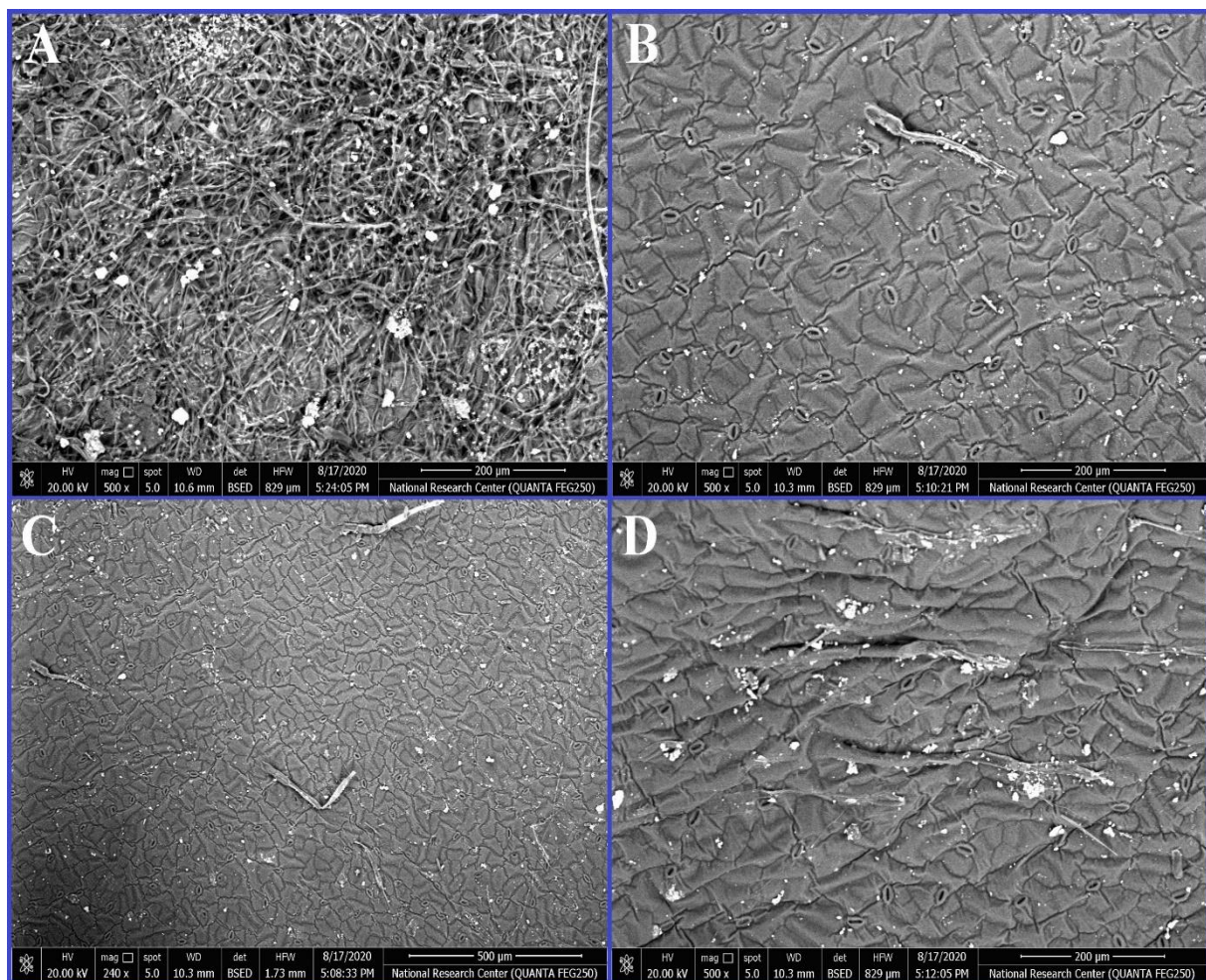
### Root yield and its attributes:

Data presented in Table (3) indicate that the effect of *N. muscorum*, *S. platensis*, *A. oryzae* or Tetraconazole on yield of sugar beet. There were

no great differences between the values of the two seasons of study concerning fresh weight of 100 roots and root yield. Table (3) clearly shows that all treatments led to considerable increase in fresh weight of 100 roots and root yield of the treated plants compared to non-treated ones (control). Also, ethanol extract remained superior over cell culture during 2020/21 and 2021/22 growing seasons with all applied cyanobacteria. Contrary to expectation, *S. platensis* ranked first at the rate% of increasing fresh weight of 100 roots and root yield during two seasons, followed by *N. muscorum*. The fungicide achieved the third rank, then *A. oryzae* occupied the last rank in affecting on increasing the percentage at rate % of fresh weight of 100 roots and root yield compared to control during the two seasons at Sakha Experiment Farm. These results are in harmony with those recorded by Jakienè *et al.*, (2015). The stimulatory effect of these microalgae may be attributed to elevating gibberellic acid (GA) and indole acetic acid (IAA) levels in treated plants (Aly *et al.*, 2008).

### Chemical composition of roots:

For the sugar beet sector, sugar purity is a crucial factor. Therefore, it is crucial to ascertain how agricultural practices affect the quality of the harvested sugar. Results in Table (4) show that all bio-agents or fungicides significantly increased sucrose% and total soluble sugar TSS% compared to control. The yield of sugar per ton/feddadan was positively correlated with the root yield per ton/feddadan and the percentage of sucrose. Data in Table (4) clear that *S. platensis* had the highest effect on rising sucrose% and total soluble sugar TSS% among all treatments followed by *N. muscorum* during the two seasons at Sakha Experimental Farm, followed by the fungicide. Finally, the least effective was *A. oryzae*. It should be noted that the ethanol extract had higher effect on sugar contents than cell culture during the two grown seasons in the field, and the obtained results are in harmony with those published by Aly *et al.* (2008) and Ghazy *et al.* (2021).



**Fig (4):** Micrograph of scanning electron microscopy (SEM) findings that promising foliar application on sugar beet, (A) Control, (B) *N. muscorum*, (C) *S. platensis*, and (D) *A. oryzae*.



**Table (3): Effect of *N. muscorum*, *S. platensis*, *A. oryzae*, or Tetraconazole on yield of sugar beet grown under field conditions for two growing seasons 2020/2021 and 2021/2022.**

Treatment	Forms	100root/ Kg						Root yield Ton/ Fed.					
		1 <sup>st</sup> season	2 <sup>nd</sup> season	Mean	1 <sup>st</sup> season Increase rat %	2 <sup>nd</sup> season Increase rat %	Mean	1 <sup>st</sup> season	2 <sup>nd</sup> season	Mean	1 <sup>st</sup> season Increase rat %	2 <sup>nd</sup> season Increase rat %	Mean
<i>S. platensis</i>	Biomass extract	150.00	152.00	151.00	60.14	58.88	59.51	32.90	34.80	33.85	75.65	81.90	78.77
	cell culture	142.80	145.00	143.9	52.45	51.56	52.01	29.40	30.00	29.70	56.97	56.80	56.88
<i>N. muscorum</i>	biomass extract	147.50	148.30	147.9	57.47	55.01	56.24	31.30	32.40	31.85	67.11	69.37	68.24
	cell culture	136.70	137.00	136.85	45.94	43.20	44.57	26.20	27.90	27.05	39.88	45.80	42.84
<i>A. oryzae</i>	biomass extract	119.90	123.20	121.55	28.00	28.78	28.39	22.10	23.00	22.55	17.99	20.23	19.11
	cell culture	113.40	116.70	115.05	21.06	21.98	21.52	20.30	21.00	20.65	8.38	9.78	9.08
Fungicide	100cm <sup>3</sup> \100L.	140.30	142.40	141.35	49.78	48.80	49.29	28.80	29.47	29.13	53.76	54.05	53.90
Control	---	93.67	95.67	94.67	---	---	---	18.73	19.13	18.93	---	---	---
LSD at 0.05													
Treatments(A)				11.89			2.62						
Forms (F)				7.52			1.61						
Seasons (S)				7.59			1.66						
A×F				16.81			3.71						
A×S				16.86			3.75						
F×S				10.63			2.351						
A×F×S				23.78			5.257						

**Table (4): Effect of *N. muscorum*, *S. platensis*, *A. oryza*, and Tetraconazole on, Sucrose%, TSS%, Purity% and Sugar yield ton/ Fed. of sugar beet roots grown under field conditions for two growing seasons 2020/2021 and 2021/2022.**

Treatment	Forms	Sucrose%			TSS%			Purity%			Sugar yield Ton/ Fed.				
		1 <sup>st</sup> season	2 <sup>nd</sup> season	Mean	1 <sup>st</sup> season	2 <sup>nd</sup> season	Mean	1 <sup>st</sup> season	2 <sup>nd</sup> season	Mean	1 <sup>st</sup> season	2 <sup>nd</sup> season	Mean		
<i>S. platensis</i>	biomass extract	21	21.9	21.45	24.78	25	24.89	84.73	87.78	86.25	6.92	7.63	7.27		
	cell culture	19.8	20.1	19.95	23.3	23.4	23.35	83.64	85.94	84.79	5.7	6.02	5.86		
<i>N. muscorum</i>	biomass extract	20	21	20.5	23.98	24	23.99	83.62	87.49	85.55	6.24	6.81	6.52		
	cell culture	18.3	18.8	18.55	22	22.9	22.45	83.48	82.18	82.83	5.39	5.25	5.32		
<i>A. oryzae</i>	biomass extract	15.8	16.1	15.95	19.2	20.3	19.75	82.42	79.39	80.91	3.49	3.7	3.59		
	cell culture	15.5	15.9	15.7	18.8	19.93	19.36	82.49	79.99	81.24	3.15	3.35	3.25		
fungicide	100cm <sup>3</sup> \10 OL.	18	19	18.5	21.7	22.6	22.15	83.07	83.64	83.35	5.17	5.61	5.39		
Control	.....	14.21	14.68	14.44	18.36	19.68	19.02	78.1	74.65	76.37	2.67	2.81	2.74		
LSD at 0.05															
Treatments (A)				1.66					2.21					7.87	0.57
Forms (F)				1.02					1.4					4.97	0.34
Seasons (S)				1.05					1.11					4.79	0.36
A×F				2.35					3.11					11.14	0.81
A×S				2.33					3.13					11.13	0.85
F×S				1.48					1.98					7.04	0.51
A×F×S				3.32					4.42					15.74	1.15

## CONCLUSION

This study provided evidence that ethanolic extract and culture media mainly those of *Anabaena oryzae*, *Nostoc muscorum*, and *Spirulina platensis* showed a good potential to control powdery mildew of sugar beet and improve their productivity as well as root yield quality under field conditions. Therefore, the authors recommend using for soaking seeds for 24 hrs and foliar spraying with algal cell culture media or biomass extract as safe biocontrol agents compared with the fungicide. Biomass ethanolic extract is suggested to be attributed to intracellular production. Moreover, it is more effective in increasing the activity of defense enzymes, phenolic compounds, and root yield. Finally, the most important observation in this study is unexpected behavior of cell culture of *N. muscorum* in management of the disease. It could be that, when antifungal substances are induced by Cyanobacteria in the presence of fungus, such as induced by *N. muscorum* against *E. betae*. The chemical nature of these allopathic constitutive needs more studies.

## CONFLICTS OF INTEREST

The author(s) declare no conflict of interest.

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