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Promoting Wheat Growth by Priming Grains with Aqueous Extracts of *Nostoc muscorum* and *Arthrospira platensis*

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THIS STUDY was conducted to evaluate different priming treatments for wheat grains, including *Nostoc muscorum* and *Arthrospira platensis* aqueous extracts, for different periods (2, 6, and 12h). Priming treatments of 12 h promoted growth of the emerging wheat seedlings (shoot and root length and fresh and dry weight). Pigment, carbohydrate, and protein contents also improved in response to different priming treatments. The highest estimates of all measured parameters were recorded with *A. platensis* aqueous extract, followed by *N. muscorum* aqueous extract and finally for tryptophan (as a synthetic growth stimulator). Biochemical analyses of *A. platensis* aqueous extract showed the presence of secondary metabolites, such as phenolic and flavonoid compounds. Such compounds may be responsible for the antioxidant activity of *A. platensis* aqueous extract, which was determined using 2,2-diphenyl-1-picrylhydrazyl, hydrogen peroxide, and phosphomolybdenum radical scavenging assays. Furthermore, *A. platensis* aqueous extract was subjected to gas chromatography–mass spectroscopy (GC-MS) analyses, which indicated the presence of different bioactive compounds in this extract, mainly 2-pentanone, 4-hydroxy-4-methyl-, 3,7,11,15-tetramethyl-2-hexadecane-1-ol, and 3-amino-2,3-dihydrobenzoic acid. These components, in addition to others detected, may act synergistically to promote the growth of wheat seedlings.

Keywords: Antioxidant activity, Cyanobacterial species, GC-MS analyses, Growth parameters, Phenolics.

Introduction

Wheat (*Triticum aestivum* L.) is a major commercial food crop, accounting for nearly twenty percent of human food requirements. Wheat is cultivated on approximately 2.15 million ha worldwide. There are, however, challenges that restrict wheat productivity, such as unsuitable seedbed preparation, low seed quality, poor sowing, insufficient soil moisture, and adverse soil conditions (Muñoz-Rojas et al., 2018). Seed priming is a dynamic seed enhancement technique that has been used successfully for several crops. The primary purpose of this practice is to accelerate germination and seedling growth under normal and stress conditions (Khan et al., 2017). Seed priming can be performed using different techniques, for example, hydro-priming, halo-priming, osmo-priming, bio-stimulant inducers, and solid-matrix priming (Rehman et al., 2015). Among the natural biostimulants are plant growth-promoting rhizobacteria, seaweeds, and cyanobacterial extracts (Ruttanaruangboworn et al., 2017). Synthetic substances, such as ascorbic acid, tryptophan, methionine, and phenylalanine, can also be applied to seeds, plants, or soil to stimulate growth (Yan et al., 2020). These substances cause changes in vital and structural environmental processes to induce tolerance to abiotic stresses, improve plant growth, and enhance seed yield and quality (Reche et al., 2018; Gaafar et al., 2020). Cyanobacteria are a diverse group of microorganisms that can live in almost all habitats and can adapt to varied environmental

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conditions in water and soil (Abinandan et al., 2019). They can synthesize numerous beneficial bioactive compounds, such as polysaccharides, glycerol, polyphenols, amino acids, growth-promoting substances, and carotenoids (Shao et al., 2018). These compounds are known to have important functions in plant growth regulation, metabolism, and development (Singh et al., 2017). Additionally, cyanobacteria can produce and accumulate various antioxidant molecules, such as vitamins C and E, fatty acids, phenolic and flavonoid compounds (Jerez-Martel et al., 2016). The latter are the most important secondary metabolites produced by plants and algae as natural products, ranging from simple structural molecules (phenolic acids) to highly polymerized molecules (flavonoids and tannins). These compounds are capable of scavenging free superoxide radicals, thereby protecting biological systems against the harmful effects of oxidative processes of vital macromolecules, such as carbohydrates, proteins, lipids, and DNA (Kaurinovic & Vastag, 2019). For cultivated plants, these molecules can contribute to antioxidation defense by alleviating the damage caused by many environmental stresses, e.g., soil salinity, drought, heavy metals, etc., as well as contributing to the adaptation and pigmentation processes in plants (Ismail et al., 2021; Gonçalves, 2021). Thus, cyanobacteria are recommended by many studies as natural and eco-friendly biofertilizers for sustainable agricultural development. Their availability and low cost renders their culture, biomass, filtrate, and/or extract suitable for seed or grain priming (Singh et al., 2016; Win et al., 2018).

The current study aimed to investigate the effects of priming wheat grains in aqueous extracts of *Nostoc muscorum* and *Arthrospira platensis* cyanobacteria compared with tryptophan as a synthetic priming solution. The study also evaluated the biochemical components and antioxidant activity of *N. muscorum* and *A. platensis* aqueous extracts to elucidate their plant-promoting mechanisms.

Materials and Methods

Cyanobacteria species and culture conditions

Two cyanobacteria (blue-green algae) species were used in this study: *N. muscorum* and *A. platensis*. The cyanobacteria were isolated from water and soil samples and purified according to standard microbiological methods described by Stein (1973). Next, the two axenic species were identified according to Desikachary (1959) using the morphological and taxonomical features of the purified isolates. Identification of the two species was further confirmed as *N. muscorum* (C. Agardh ex Bornet and Flahault) and *A. platensis* (Gomont) using the AlgaeBase website (http://www.algaebase.org). *Nostoc muscorum* was cultivated on BG11 medium (Rippka, 1988), while *A. platensis* was cultivated on modified Zarrouk's medium (Aiba & Ogawa, 1977). The axenic culture flasks were incubated at 27±3°C and light intensity of 45µmol photon m^{-2} s^{-1}, and a mixture of 97% dry filtered air and 3% CO_2 was supplied to the cultures to accelerate their growth. Each culture biomass was determined at the end of the exponential growth phase corresponding to each species (14 d for *N. muscorum* and 12 d for *A. platensis*). The cyanobacterial cultures were harvested by centrifugation at 4000 rpm (Fisher Centerific™ Centrifuge) for 15 min, washed twice with distilled water (Rogers & Burns, 1994), and dried in a freeze dryer for 12h. The lyophilized powder was stored at −20°C until use.

Preparation of Nostoc muscorum and Arthrospira platensis aqueous extracts

The freshly washed biomass (10g) of *N. muscorum* and *A. platensis* was suspended in 100 mL distilled water and shaken (VS-8480) for 48h at 25°C. After extraction, the supernatants were collected by centrifugation as previously mentioned and dried in a freeze dryer for 12–15h. The lyophilized powder was stored at −20°C until use.

Wheat grain treatments and pot experiments

Wheat grains (*Triticum aestivum* L., Giza 171) were obtained from Main Crops Improvement Station in Kafr El-Sheikh, Egypt. To determine the most effective priming treatment and its concentration, a primary experiment was carried out in which *N. muscorum* and *A. platensis*, either as biomass or as aqueous extracts, were used, individually, as a priming treatment. To prepare soaking solutions, different weights (0.25, 0.5, 0.75, and 1g) of dried biomass or aqueous extract powders of both species were homogenized in 100 mL distilled water, followed by soaking wheat grains in these solutions for 2h at room temperature (27±2°C) in dark conditions (Abo-Shady et al., 2018). After priming, the grains were sown in pots (11× 8cm, diameter × depth; 10 grains per pot), which contained equal amounts

of loamy soil [clay: sand, 2:1 w/w; pH= 7.3; EC (0.8m Mohs cm^{-1}); N and P contents of 2.6 and 2.4mg g^{-1} dry weight (DW), respectively, and field capacity of 57%] for 21 d until the seedlings were established. The pots were kept in a greenhouse (Faculty of Science-Tanta University) under normal conditions of daylight and temperature. Pots were irrigated with tap water every 48h. Ammonium nitrate and superphosphate chemical fertilizers were added according to the doses and timing recommended by the Ministry of Agriculture of Egypt. Various growth parameters of plant seedlings (the lengths and fresh and DWs of roots and shoots) were measured.

To determine the optimum period for priming wheat grains, as well as the most effective cyanobacterial aqueous extract, a second experiment was conducted in which grains were soaked in *N. muscorum* and *A. platensis* aqueous extract solutions at 1% concentration (determined from the primary experiment as the optimal treatment, Supplementary material file). The priming periods were 2, 6, or 12h. After priming, the grains were sown in soil and the experiment was conducted as previously described. In this experiment, wheat grains were also primed in a tryptophan standard solution (at 100ppm= 0.01g in 100mL of distilled water), as a standard synthetic growth stimulator (El Awadi et al., 2011), and in water solution as a negative control. Different morphological growth criteria of the germinated wheat seedlings were measured. In addition, the contents of photosynthetic pigment, total soluble carbohydrates, and total soluble proteins were assessed in the resulting wheat seedlings. The germination percentage and rate were also evaluated according to the following formulas **(**Tiquia et al., 1996; Bangarwa et al., 2012):

Relative grain germination percentage= (no. of grains germinated with treatment/ no. of grains germinated with control) * 100

Germination rate per day= (no. of grains sprouted/ no. of total grains sprouted) * 100

Photosynthetic pigment content

To estimate the photosynthetic pigment content (chlorophylls a and b, and carotene) of the seedlings leaves, the spectrophotometric method of Metzner et al. (1965) was applied. The following equations were applied for calculating each pigment concentration in the experimental plant leaf extracts in terms of µg mL^{-1}:

Chlorophyll a= 10.3 E663nm − 0.918 E644nm

Chlorophyll b= 19.7 E644nm − 3.8 E663nm

Carotenoids= 4.2 E452nm− (0.0264 Chl. a + 0.426 Chl. b)

where E denotes absorbance at a defined wavelengths.

The calculated pigment content was then expressed in mg g^{-1} DW as follows: (reading × dilution/ sample volume × dry wt) × 1000

Total soluble carbohydrate content

Total carbohydrates were determined using the phenol-sulfuric acid method (Dubois et al., 1956). The absorbance was measured against a blank at 490 nm wavelength. The carbohydrate concentration (mg mL^{-1}) in the unknown solution was estimated after the preparation of a calibration curve using glucose as a standard carbohydrate.

Total protein content

Total proteins were determined using the methods of Bradford (1976). The absorbance was measured using a spectrophotometer at 595 nm. The protein content was calculated as mg mL^{-1} using a prepared calibration curve of bovine serum albumin protein as a standard.

Qualitative phytochemical screening of Nostoc muscorum and Arthrospira platensis aqueous extracts

The presence or absence of bioactive phytochemicals, including phenolics, flavonoids, steroids, terpenoids, alkaloids, tannins, and cardiac glycosides, in the aqueous extracts of *N. muscorum* and *A. platensis* samples, was assessed according to Andima et al. (2014).

Antioxidant activity and total phenolic and flavonoid contents of Nostoc muscorum and Arthrospira platensis aqueous extracts

The antioxidant activity of *N. muscorum* and *A. platensis* aqueous extracts was assessed by three different methods of 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Yen & Chen, 1995), hydrogen peroxide (Ruch et al., 1989), and phosphomolybdenum (Prieto et al., 1999) radical scavenging assays. The total phenolic content was estimated using the method described by Jindal

& Singh (1975), while the flavonoid content was determined according to the methods of Zhishen et al. (1999).

Gas chromatography−mass spectroscopy

Arthrospira platensis aqueous extract (1%) was subjected to gas chromatography–mass spectroscopy (GC-MS; Perkin Elmer: Clarus 580/560S) to identify its different components. GC-MS analysis was performed by injecting 1µL of sample into the column (Elite-5MS, 30m× 0.25mm ID × 0.25µm df) with helium as a carrier gas. The oven was programmed as follows: initial temp 50°C for 4min, ramp 8°C min^{-1} to 180°C, hold 5 min, ramp 10°C min^{-1} to 280°C, hold 2min, inj= 280°C, split = 20:1, solvent delay= 3.50min, transfer temperature= 280°C, source temperature= 200°C, and scan 50 to 600Da. Components were identified by comparing their mass spectra with those in the database of the National Institute Standard and Technology. Mainlib and replib software libraries were applied to the GC-MS.

Statistical analysis

All results were evaluated as means ± standard deviation (SD) of five replicates (n=5). GraphPad Prism 6.01 (GraphPad Software, San Diego) was used for statistical analysis of the resulting data. One-way ANOVA was used to assess the variation in treatments at a significance level of P≤ 0.05. Data comparison was carried out using the t-test (unpaired), and significant differences were accepted at P≤ 0.05 for the algal culture.

Results

Wheat growth parameters and biochemical composition

The results of the primary experiment indicated that aqueous extracts of both *N. muscorum* and *A. platensis* at a concentration of 1% were the best solutions for priming wheat grains, followed by the biomass solutions of both species at the same concentration (Supplementary material file: Tables 1S and 2S; Figs. 1S and 2S). Wheat grains primed in *A. platensis* aqueous extract at 1% concentration for 2 h increased values of the estimated growth parameters as compared with those of the control seedlings, including the lengths of plant shoots (16%) and plant roots (21.2%), the fresh weights of shoots (30.1%) and roots (1 fold), as well as the DWs of shoots (58.8%) and roots (1.3 fold). These values were significantly different from those obtained for grains primed in *N. muscorum*, tryptophan, or the control solutions (Fig. 1a, b). Similar results were recorded for a 6 h priming period with *A. platensis* aqueous extract (1%) (Fig. 2a, b). The highest values were recorded after priming grains for 12h with all tested treatments. Seedlings from grains primed in *A. platensis* aqueous extract for 12h showed the greatest increase in growth morphological parameters for the lengths of shoots (75%) and roots (52.4%), fresh weights of shoots (78.9%) and roots (1.3 fold), and DWs of shoots (1 fold) and roots (2.3 fold) (Fig. 3a, b). Moreover, grains primed in *A. platensis* aqueous extract for 12 h showed the highest values of Chl a (2.4 fold), Chl. b (2.5 fold), and carotenoids (6 fold) as shown in Fig. 4a–c. In the same manner, the estimated values for carbohydrate and protein contents were 88.8% and 26.3%, respectively, for *A. platensis* aqueous extract, as compared with 60% and 21%, respectively, for grains primed in *N. muscorum* aqueous extract, and 48.9% and 14.5%, respectively, for grains primed with tryptophan (Fig. 5a, b). Although germination was at its maximum (100%) for all the tested treatments, germination was faster with the cyanobacterial treatments (Table 4S). For both *A. platensis* and *N. muscorum* treatments, germination of total grains was achieved within 7 days of sowing, while it continued for 10 days with both the control and tryptophan treatments.

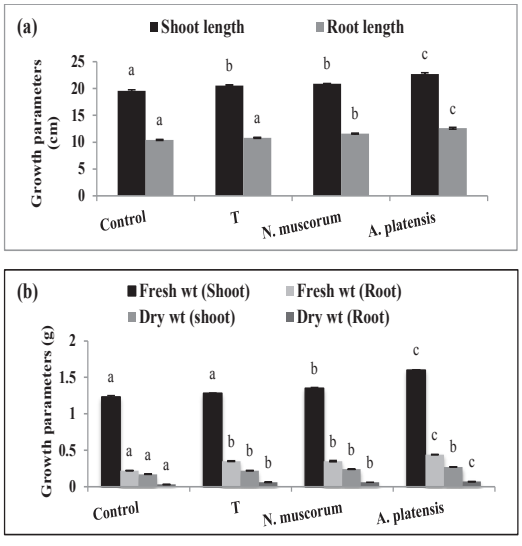

Fig. 1. Growth parameters of wheat seedlings primed for 2h in water (control), tryptophan (T, 100ppm), *N. moscorum*, and *A. platensis* aqueous extract (1%) treatments: (a) length of shoots and roots and (b) fresh and dry weight of shoots and roots [Different lowercase letters for the same parameter in different

treatments indicate a significant difference at P< 0.05]

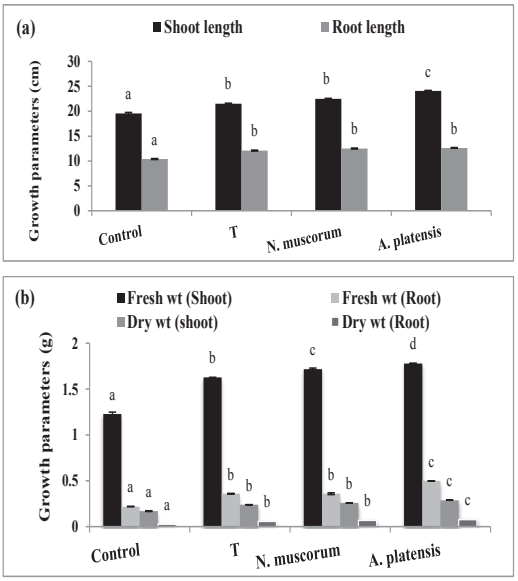

Fig. 2. Growth parameters of wheat seedlings primed for 6h in water (control), tryptophan (T, 100ppm), *N. muscorum*, and *A. platensis* aqueous extract (1%) treatments: (a) length of shoots and roots and (b) fresh and dry weight of shoots and roots [Different lowercase letters for the same parameter in different treatments indicate a significant difference at P< 0.05]

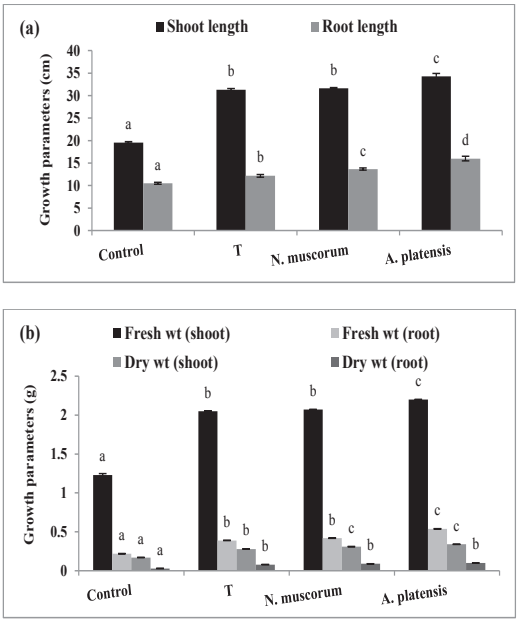

Fig. 3. Growth parameters of wheat seedlings primed for 12 h in water (control), tryptophan (T, 100 ppm), *N. muscorum,* and *A. platensis* aqueous extract (1%) treatments: (a) length of shoots and roots and (b) fresh and dry weight of shoots and roots. [Different lowercase letters for the same parameter in different treatments indicate a significant difference at P< 0.05]

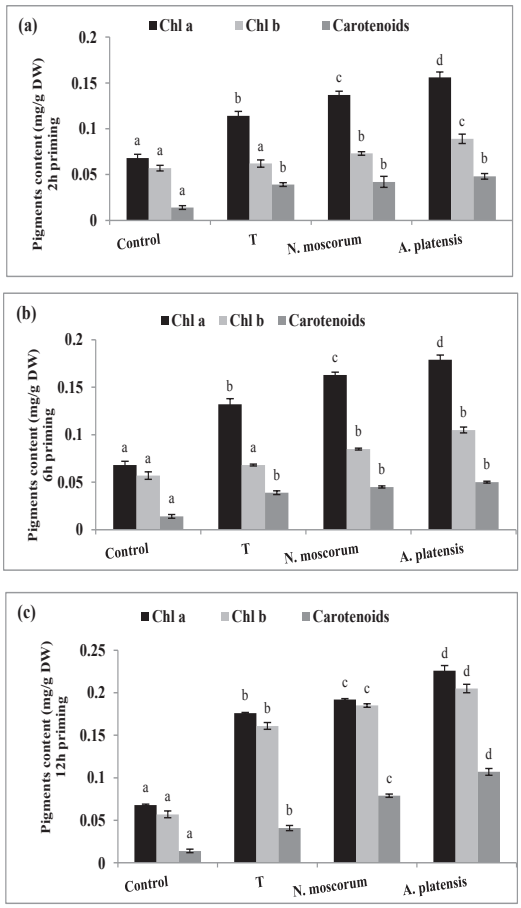

Fig. 4. Effect of priming period on the pigment content (mg g^{-1} DW) of wheat seedlings primed in water (control), tryptophan, *N. muscorum,* and *A. platensis* aqueous extract (1%) treatments for (a) 2h, (b) 6h, and (c) 12h [Different lowercase letters for the same parameter indicate a significant difference at P< 0.05]

Antioxidant activity of N. muscorum and A. platensis aqueous extracts

As shown in Table 1, *A. platensis* aqueous extract (1%) showed the highest scavenging activities of 64.8%, 75.8%, and 2.4 mg g^{-1}, for the three examined assays, namely, DPPH, hydrogen peroxide, and phosphomolybdenum radical scavenging assays, respectively. *N. muscorum* extract, at the same concentration, showed scavenging activities of 46.5%, 48%, and 1.34mg g^{-1} for DPPH, hydrogen peroxide, and phosphomolybdenum radical scavenging assays, respectively.

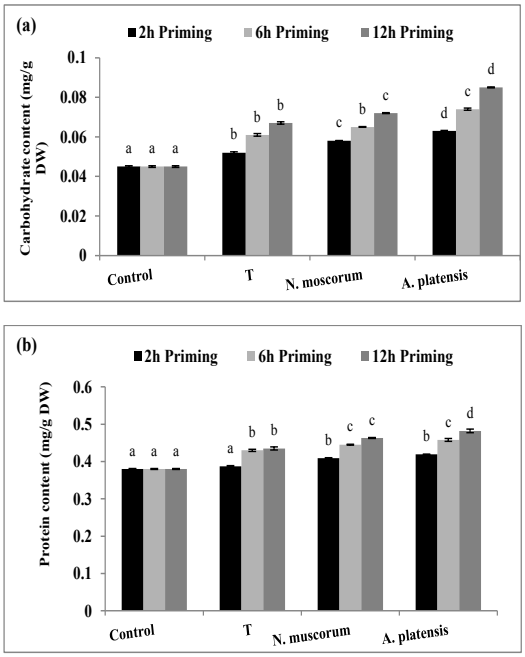

Fig. 5. Effect of priming period on the (a) carbohydrate and (b) protein contents (mg g^{-1} DW) of wheat seedlings primed for 2, 6, and 12h in water (control), tryptophan, *N. muscorum*, and *A. platensis* aqueous extract (1%) treatments [Different lowercase letters for the same parameter indicate a significant difference at P< 0.05]

TABLE 1. Antioxidant activity of *N. muscorum* and *A. platensis* water extracts (1%) **

Antioxidant assay	*N. muscorum*	*A. platensis*	*t* value
DPPH (%)	46.5±1.6	64.8 ±1.3	NA
Hydrogen peroxide (%)	48.0±1.7	75.8 ± 1.5	NA
Phosphomolybdenum (mg/g DW)	1.3±0.4	2.4± 0.1	18.44*

** Values are mean of replicates ± SD (*n*=5), NA= *t*-test is not applied for percentages estimations.

*= *t* value indicates a significant difference at P< 0.05.

Total phenolic and flavonoid content of N. muscorum and A. platensis aqueous extracts

The total phenolic (0.058mg g^{-1} DW) and flavonoid (0.087 mg g^{-1} DW) contents of *A. platensis* aqueous extract were significantly higher than those of *N. muscorum* aqueous extract (0.045 and 0.061 mg g^{-1} DW, respectively) at 1% concentration, as illustrated in Table 2. In general, the content of phenolic compounds was higher than that of flavonoids in both extracts.

TABLE 2. Total phenolic and flavonoid contents (mg/g DW) of *N. muscorum* and *A. platensis* water extracts (1%) **

Content (mg/g DW)	*N. muscorum*	*A. platensis*	*t* value
Total phenolics	0.058±0.02	0.087±0.02	61.52*
Total flavonoids	0.045±0.01	0.061±0.01	33.94*

**Values are mean of replicates ± SD (n=5)

*= *t* value indicates a significant difference at P< 0.05.

GC-MS analysis of Arthrospira platensis aqueous extract

According to the previous results, *A. platensis* aqueous extract (1%) improved growth of cultivated wheat seedlings, and it was, therefore, subjected to GC-MS analysis. The GC-MS chromatogram (Fig. 6) displayed different bioactive compounds, including 2-pentanone, 4-hydroxy-4-methyl (24.693%), 3,7,11,15-tetramethyl-2-hexadecen-1-ol (phytol alcohol)(20.571%),3-amino-2,3-dihydrobenzoic acid (13.143%), 2-hexanol, and 2-methyl-(3.064%). In addition, other components of a phenolic nature were found as 4-ethylbenzoic acid, cyclohexyl ester (5.765%), phenyl-pentamethyl-disiloxane (2.022%), benzoic acid, 2-formyl-4,6-dimethoxy, 8,8-dimethoxyoct-2-yl ester (2.286%), and benzaldehyde, 2,5 bis[(trimethylsilyl)oxy] (1.365%)], and also their bioactive properties were listed in Table 3.

Discussion

Priming allows some of the metabolic processes necessary for germination to occur without germination taking place. In priming, seeds are soaked in different solutions with low molecular weight and high osmotic potential, thus preventing the seeds from absorbing enough water for radicle protrusion and suspending the seeds in the lag phase (Shah et al., 2017). It is a simple, inexpensive, and easy treatment that increases the germination percentage and reduces the seedling emergence time, especially under unfavorable environmental conditions (Muñoz-Rojas et al., 2018). Seed priming has an important role in increasing the yield of vital crops such as wheat, barley, rice, maize, sorghum, and pearl millet (Reche et al., 2018).

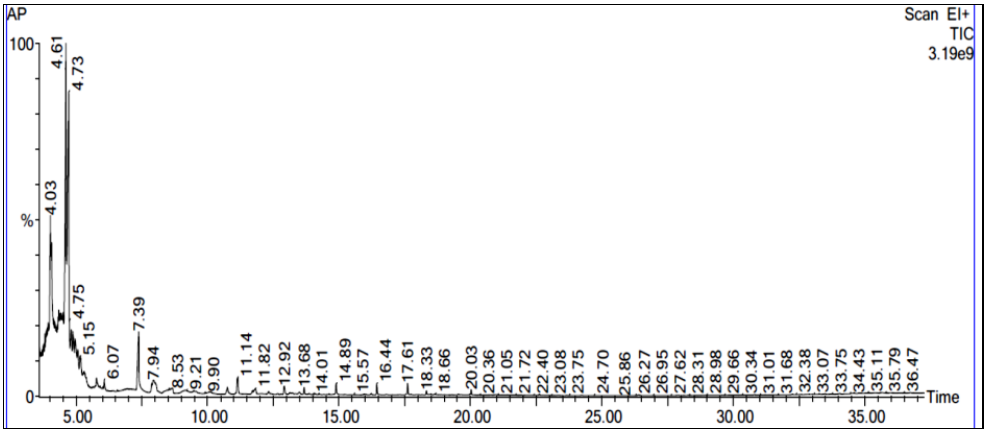

Fig. 6. GC-MS chromatogram of *Arthrospira platensis* aqueous extract

TABLE 3. GC-MS analysis of *A. platensis* water extract*

RT	Compound name	PA %	MF	Biological activity
3.819	3-Amino-2,3-dihydrobenzoic acid	13.143	$C_7H_{10}ClNO_2$	phenolic compound, antioxidant activity, antimicrobial
3.904	3-Hexen-2-one	0.764	$C_6H_{10}O$	Plant metabolite
3.934	2,3-Dibromo-Thiopropionic acid, S-ethyl ester	1.057	$C_5H_8Br_2OS$	Antibacterial, Fungicide, Herbicide, Pesticide
4.349	2-Pentanone, 4-hydroxy-4-methyl-	1.404	$C_6H_{12}O_2$	Food additives, Flavoring Agents, Antimicrobial, plant metabolites, Pesticide
4.379	Formamide, N-formyl-N-methyl-	0.625	$C_3H_5NO_2$	pharmaceuticals, herbicides, pesticides
4.424	2-Hexanol, 2-methyl-	1.101	$C_7H_{16}O$	Food additives, Flavoring, fatty acyls
4.449	2-Pentanone, 4-hydroxy-4-methyl-	1.170	$C_5H_{10}O_2$	As mentioned
4.514	2-Hexanol, 2-methyl-	1.963	$C_7H_{16}O$	Food additives, cosmetics
4.624	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	17.114	$C_{20}H_{40}O$	Anti-inflammatory, antioxidant, antimicrobial
4.729	2-Pentanone, 4-hydroxy-4-methyl-	22.119	$C_6H_{12}O_2$	As mentioned
4.819	2,7,11,15-Tetramethyl-2-hexadecen-1-ol	1.651	$C_{20}H_{40}O$	As mentioned
4.894	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	1.806	$C_{20}H_{40}O$	As mentioned
4.964	Phenyl-pentamethyl-disiloxane	2.022	$C_{11}H_{20}OSi_2$	Phenolics aldehyde
5.069	4-Hydroxybutanoic acid	1.180	$C_4H_8O_3$	pharmaceuticals, fatty acyls
5.169	Methylamine	1.310	CH_3NH_2	herbicides, biocides, insecticides
5.779	Propionic acid, 2-hydroxy-2-methyl-	0.995	$C_4H_8O_3$	Pesticide, antimicrobial, active ingredient
7.085	Oxime-, methoxy-phenyl-	0.873	$C_8H_9NO_2$	Antimicrobials, Antioxidant
7.390	4-Ethylbenzoic acid, cyclohexyl ester	5.765	$C_{15}H_{20}O_2$	Food additives, Bactericides, Fungicides, Insecticides, Antimicrobials
7.950	Benzoic acid, 2-formyl-4,6-dimethoxy-, 8,8- dimethoxyoct-2-yl ester	2.286	$C_{14}H_{14}N_2O_5$	Food additives, Bactericides, Fungicides, Herbicide, Insecticides, Antimicrobials
8.665	Cyclotetrasiloxane, octamethyl-	0.744	$C_8H_{24}O_4Si_4$	Pesticide, Antimicrobial, antioxidant
9.151	N-Methylvaleramide	1.189	$C_6H_{13}NO$	Pesticide
9.481	3,4-Hexanedione, 2,5-dimethyl-	0.662	$C_8H_{14}O_2$	Food additive, flavor
10.756	Cyclotrisiloxane, hexamethyl-	0.645	$C_6H_{18}O_3Si_3$	Pesticide, cosmetics, Antimicrobial, antioxidant
11.156	Benzaldehyde, 2,5 bis[(trimethylsilyl) oxy]-	1.365	$C_{13}H_{22}O_3Si_2$	Food additive, flavor, Pesticide

*RT: Retention Time; PA: Peak Area; MF: Molecular Formula. Biological activities of different compounds were driven from the Pubchem database website (https://pubchem.ncbi.nlm.nih.gov/)

According to the results presented here, different morphological and biochemical growth parameters of wheat seedlings, as well as the germination rate, increased in response to priming grains in different cyanobacterial solutions with varying concentrations and times. Wheat grains primed in *A. platensis* aqueous extract for 12 h recorded higher values for the seedling growth parameters (i.e., shoot, root fresh weight, DW, and length) than those values obtained when *N. muscorum* aqueous extract, tryptophan, or the control solutions were used. This result appears to be linked to improvements in photosynthetic activity through stimulation of pigment biosynthesis, as represented by the increases in chlorophyll (a and b) and carotenoid contents using the same extract. In this context, our findings are in accordance with those of many previous studies. Essa et al. (2015) reported significant enhancement of seed germination, shoot length, lateral roots, spike length, grain weight, protein content, micronutrients, and the endogenous phytohormone pool of *sorghum durra* var. *aegyptiacum* and *Helianthus annuus* L. var Giza 102 plants after pre-priming their grains in *Anabaena oryzae, Nostoc ellipsosporum*, and *Synechococcus* sp. filtrates before cultivation. In another study, Ismail & Abo-Hamad (2017) reported that grains of *Hordeum vulgare* and *Trigonella foenum-graecum* L. primed in *Anabaena variabilis* 1% fresh cyanobacterial extract showed improvements in germination percentage, shoot length, and fresh and dry weights of the emergent seedlings; greater amounts of photosynthetic pigments and protein content were also observed. Dineshkumar et al. (2017) reported that the application of *Chlorella vulgaris* and *Spirulina platensis* to maize increased the germination rate, yield, fresh and dry weights of shoot and root, and the number of leaves. Likewise, filtrates of *A. platensis*, applied via seed soaking, via foliar spray, or as a homogenate used for seed coating, were stimulative for radish plants and showed high potential for application in horticultural and agricultural practices (Godlewska et al., 2019). Furthermore, Zarezadeh et al. (2020) applied cyanobacterial suspensions of *N. muscorum*, *Wolle avaginicola*, and *Nostoc punctiforme* as biofertilizers on *Matricaria chamomilla* L. (chamomile). The authors reported an increased content of nitrogen and growth hormones; roots and shoots were also longer under these conditions.

Several mechanisms have been suggested for the established plant growth-promoting action of cyanobacteria. Cyanobacteria (as either biomass or extracts) produce various biologically active substances, such as plant growth hormones, vitamins, amino acids, polypeptides, and exopolysaccharides. These secondary metabolites are directly available in the soil, enhancing its fertility and nourishing the cultivated plants as plant growth promotors (Shao et al., 2018). Moreover, cyanobacteria can mitigate nitrogen deficiency in plants and soil because of their ability to fix free atmospheric nitrogen, maintain the soil structure and improve its aeration, as well as manage nutrients in the soil system (Chittora et al., 2020). As a result of their ability to produce biologically active molecules, studies have recommended microalgae and cyanobacteria for agricultural use as biofertilizers, biostimulants, or biopesticides (Gonçalves, 2021).

Phenolic compounds (aromatic hydrocarbons bonded to one or more hydroxyl groups) are released by plants and algae as secondary metabolites, depending on the physiological status and environmental conditions. According to Cotelle (2001), the antioxidant properties of phenolic compounds are governed by the following mechanisms: scavenging radical oxygen species (ROS) or radical nitrogen species (RNS); inhibiting ROS or RNS creation by suppressing the activities of certain enzymes or chelating trace metals; encouraging free radical production, and up-regulation and protection of antioxidant defense systems. In cyanobacteria and microalgae, phenolic compounds are linked to their antioxidant properties, growth, reproduction, and protection against stress conditions (Azim et al., 2018). The results of the present study indicated higher antioxidant activity and phenolic and flavonoid contents for *A. platensis* aqueous extract as compared with those of *N. muscorum* aqueous extract. In addition, after priming wheat grains, *A. platensis* aqueous extract showed the greatest stimulation of the morphological and biochemical parameters of the emerging wheat seedlings compared with those grains primed in *N. muscorum* aqueous extract, tryptophan, or control solutions. This finding suggests a possible growth-promoting role exerted by the biochemical components of *A. platensis* and *N. muscorum* aqueous extracts on the primed wheat grains. In addition to the presence of phenolics and flavonoids in both *A. platensis* and *N. muscorum*

aqueous extract. The results also showed the presence of other phytochemical constituents of alkaloids, steroids, and terpenoids, while tannin and glycoside components were absent (Table 3S, Supplementary material). Many studies reported the relationship between the antioxidant activity of algal species and the presence of different bioactive compounds. Babu et al. (2014) found that using *Anabaena torulosa*, *Anabaena laxa*, *Anabaena azollae*, *Anabaena oscillarioides*, and *Calothrix* sp. increased the content of nitrogen, activity of hydrolytic and defense enzymes (endoglucanase, peroxidase, polyphenol oxidase, and phenylalanine ammonia-lyase), and the fresh and dry weights of wheat plants. Depending on the species, priming may change the mobilization of inorganic and organic nutrients from storage cells to the emerging embryo, which can trigger several metabolic responses in plants, such as respiration, photosynthesis, nucleic acid synthesis, chlorophyll production, and ion uptake (Górka, 2018). These processes, in turn, promote antioxidant activity to alleviate the complex stresses that may be imposed by different environmental conditions (Hussain et al., 2016).

GC-MS analysis of *A. platensis* aqueous extract showed the presence of different bioactive compounds, mainly 3,7,11,15-tetramethyl-2-hexadecane-1-ol, and 2-pentanone, 4-hydroxy-4-methyl-, which could be responsible for its antioxidant activity (Alves et al., 2013). The cyclic diterpene 3,7,11,15-tetramethyl-2-hexadecane-1-ol, known as phytol alcohol, is a constituent of the chlorophyll molecule. It has also been known to act as a preventative for reactive oxygen species and a precursor for vitamin E and vitamin K1 (Ganesh & Mohankumar, 2017). 3-Amino-2,3-dihydrobenzoic acid is a phenolic compound with antioxidant and antimicrobial activities. Furthermore, cyclic unsaturated cyclopentasiloxane, octamethyl, and cyclotrisiloxane, hexamethyl have been reported as antimicrobial agents (Musini et al., 2013), with antioxidant activity (Prakash & Vuppu, 2014). Oxime-, methoxy-phenyl- has been reported for its antioxidant properties (Özen & Taş, 2009). Numerous other compounds were identified in the tested aqueous extract with validated bio-functions. All of these compounds may act synergistically to exert their influence on wheat grain protection and growth-promoting activity. Moreover, the GC-MS results confirmed the presence of many phenolic and flavonoid compounds, which have already been estimated quantitatively in *A. platensis* aqueous extract. For agricultural purposes, phenolic compounds can provide crop protection against pathogens or other biotic and abiotic stress conditions, owing to their antimicrobial, fungicidal, and antioxidant properties (Pan et al., 2019). Furthermore, cyanobacterial biomass, filtrates, and/or extracts contain a wide diversity of bioactive secondary metabolites that exhibit herbicidal, insecticidal, antioxidant, and immunosuppressive functions (Chittora et al., 2020; Gonçalves, 2021), which is in agreement with the present study results.

Conclusions

According to the results obtained in this study, the priming technique used is beneficial to wheat grain germination and for stimulating growth of the emergent seedlings. In particular, priming in *A. platensis* aqueous extract (1%) for 12 h improved various morphological growth parameters and enriched the biochemical content of pigments, carbohydrates, and proteins in the wheat seedlings, implying better photosynthetic performance. High contents of phenolic and flavonoid components in *A. platensis* aqueous extract triggered elevated antioxidant activity, which benefits the establishment and development of seedlings with superior germination rates. A GC-MS chromatogram of *A. platensis* aqueous extract confirmed the presence of bioactive compounds responsible for antioxidant and other biological activities, which could act cooperatively to enhance the performance of the sown wheat grains. Therefore, we can recommend priming wheat grains in cyanobacteria aqueous extracts before cultivation in the soil as a natural and simple technique for using cyanobacteria in agricultural systems.

Conflict of interest: The authors declare that they have no conflict of interest.

Authors contribution: 1) Project conceptualization: Osman M E H, El-Nagar M M F. 2) Methodology, data creation, and analysis: Ismail GA, El-Nagar MM F. 3) Writing- first manuscript draft and editing: Ismail GA, El-Nagar MM F. 4) Writing-final manuscript and approval: Osman M E H, Abo-shady AM, Gafaar RM, Ismail GA, El-Nagar MM F. 5) Project Supervision: Osman M E H, Abo-shady AM, Gafaar RM, Ismail GA.

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تحفيز نمو القمح عن طريق نقع البذورفي المستخلصات المائية لكل من النوستوك موسكورم والارثروسبيرابلاتنسيس

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وفقًا للنتائج التي تم الحصول عليها في هذه الدراسة، يمكن استنتاج أن تقنية نقع حبوب القمح في المستخلصات المائية لكل من النوستوك موسكورم والارثروسبيرابلاتنسيس مفيدة لإنبات حبوب القمح وتحفيز نمو الشتلات الناشئة. وبشكل خاص، فقد وجدت الدراسة أن غمرالحبوب في المستخلص المائي (1%) لارثروسبيرا بلاتنسيس لمدة 12 ساعة أدى إلى تحسين معايير النمو المختلفة، وإثراء المحتوى الكيميائي الحيوي للأصباغ، والكربوهيدرات، والبروتينات في شتلات القمح الناتجة. وايضا فقد أدى وجود محتويات عالية من مكونات الفينول والفلافونويد في المستخلص المائي (1%) لارثروسبيرا بلاتنسيس إلى زيادة نشاط مضادات الأكسدة، بعد نقع الحبوب، وبذلك تفيد في تكوين وتنمية شتلات فائقة الجودة مقارنة بالكنترول الغير معامل بالمستخلص.

وبالاضافة إلى ذلك، أكدت نتائج تحليل كروماتوجرافي الغاز الكتلي (GC-MS) لمستخلص لارثروسبيرابلاتنسيس المائي وجود مركبات نشطة بيولوجيًا مسئولة عن مضادات الأكسدة والأنشطة البيولوجية الأخرى للمستخلص ويمكن أن تعمل بشكل مشترك عند نقع الحبوب لتعزيز أداء بادرات القمح المزروعة. لذلك توصي نتائج هذه الدراسة بنقع حبوب نبات القمح في المستخلصات المائية للبكتيريا الخضراء المزرقة قبل زراعتها في التربة كأسلوب طبيعي آمن وبسيط لاستخدام البكتيريا الزرقاء في النظم الزراعية.