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ORIGINAL ARTICLE

Evaluating the potential of exopolysaccharide extracted from the spent cultivation media of *Spirulina* sp. as plant biostimulant

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Abstract
Overall growth in per capita income (along with the population), increased health awareness, and lifestyle changes have raised the global demand for fruits and vegetables. Meeting such rise in food demand through a sustainable agricultural practice is one of the major challenges faced by the scientific community today. The use of microalgal extracts as plant biostimulant has lately gained the attention of researchers due to its multifaceted biostimulant properties, low production cost, and ease of maintenance. However, the potential of microalgal exocellular polysaccharide (EPS), particularly its released (non-capsulated) fraction, as biostimulant has remained largely untapped. Therefore, the current study aimed to evaluate the potential of this released fraction of microalgal EPS (extracted from the spent cultivation medium of *Spirulina* sp.) as a plant biostimulant. After cultivation for a period of 30 days, 0.75 ± 0.03 g/L EPS was recovered from the cultivation medium. The recovered EPS was characterized and its potential was evaluated by applying it as a biostimulant in the cultivation of two hydroponically grown plants (*Ocimum basilicum* and *Spinacia oleracea*) at various dosages ranging from 0.25 to 1.5 mg/ml. The study revealed that for both plants, the treatment with EPS could enhance the plant growth, biomass productivity, and chlorophyll content. Application of 1.5 mg/ml EPS showed a highest plant biomass productivity of 0.96 ± 0.03 g/m^2/day in *Ocimum basilicum* (18% higher compare to the plants without any EPS treatment). For *Spinacia oleracea*, the optimum dosage of EPS was found to be 0.5 mg/ml as it showed the 27% higher plant biomass productivity (1.13 ± 0.01 g/m^2/day). The study also revealed that the recovery of released EPS from spent cultivation medium for the purpose of increasing crop production is a practicable proposition, which at the same time brightens the possibility of taking a bio-refinery like approach to *Spirulina* sp. cultivation.

Keywords Biostimulant · Microalgal exopolysaccharide · *Spirulina* sp. · Microalgal biorefinery · Sustainable agricultural practice

1 Introduction

Microalgae, a diverse group of photosynthetic organisms that survive in harsh environmental conditions and grow rapidly, use sunlight and CO_2 to synthesize a wide range of valuable products such as carbohydrates, lipids, proteins, and other bioactive metabolites including exopolysaccharides. These exopolysaccharides or EPS are complex carbohydrate compounds with various substituents like lipids, amino acids, or sulphates attached to their linear or branched backbones. They are produced and accumulated on the microalgal surface during the growth phase [1, 2]. Depending on the attachment style, this EPS can be of two types: cell-bound EPS (loosely or tightly attached on the outer surface of the microalgal cell) and EPS released in the culture medium [1, 3]. Microalgae produce EPS to bind with different surfaces [4], protect themselves from different abiotic stresses (such as extreme temperature, pH, or nutrient-limiting situations) [5], and also serve as a reserve for energy and carbon [6]. They have a wide range of applications in different fields including pharmaceuticals, food, cosmetics, and agriculture owing to their structural features that are associated with several unique biological (anti-inflammatory, antibacterial, antiviral, anticoagulant, anti-oxidative, and wound healing) and physicochemical properties [7, 8].

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Sustainable production of sufficient food to meet the increasing global demand for food is one of the biggest challenges in the agriculture sector. To address this issue of growing demand for food, the use of plant biostimulants to enhance plant growth and improve their tolerance to abiotic stress is becoming increasingly popular in agriculture, especially in sustainable and organic farming systems [9–11]. Novel, eco-friendly approaches such as the use of exopolysaccharides (EPS) as plant biostimulants for yield enhancement and yield stability (under different biotic and abiotic stresses) can be helpful in this regard.

Previous studies showed that microalgal EPS could stimulate plant growth and development by promoting root growth and nutrient uptake, increasing photosynthetic efficiency, and enhancing the crop's resilience against unfavourable environmental conditions (i.e., different biotic and abiotic stressors) [12–14]. For example, microalgal EPS extracted from three different microalgal strains: *Dunaliella salina*, *Porphyridium* sp., and *Arthrospira platensis* showed increased plant growth along with higher photosynthetic activity when applied to tomato plants at a concentration of 1 mg/ml [15]. Drira et al. [16] reported that exopolysaccharide recovered from the culture medium of *Porphyridium sordidum* was able to act as an elicitor to induce a defense mechanism in *Arabidopsis thaliana* against a fungal infection**.** EPS extracted from other microalgal strains like *Dunaliella salina* and *Lessonia nigrescens* showed their potential for increasing the plant's tolerance to salt stress [12, 13, 17]. Moreover, when applied to soil, EPS showed improvements in both soil structure and its water retention capacity [18, 19]. The above studies pointed to the great potential of microalgal EPS to act as a sustainable source of plant biostimulants, as they are produced from renewable sources without any adverse impact on the environment or human health. However, it was observed that the composition and structure of microalgal EPS could be highly diverse in terms of their composition and structure depending on the microalgal strain and growth circumstances. It was also noted that the majority of these studies (that investigated the potential of microalgal EPS as a plant biostimulant) focused on the cell-bound EPS (mentioned previously), whereas the recovery of the EPS released into the spent culture media remained underexplored. Hence, an attempt was made to explore the extraction and recovery of EPS from the spent cultivation medium (after microalgal harvesting) need to be explored and finally evaluate the potential of EPS as a plant biostimulant, which is likely to contribute to the development of a sustainable bioeconomy.

Spirulina sp. is known to be one of the most commonly cultivated microalgae at a commercial scale. The global production of this strain is estimated at 70,000 tonnes per year [20]. Its high market demand (as a nutritional supplement and also as a source of natural blue pigment called phycocyanin), easy maintenance of the contamination-free culture (i.e., being an extremophile prokaryote, it grows in a highly alkaline environment, which is toxic to other organisms), and simple harvesting technique have contributed to its rise into the lead in the microalgal industry. Polysaccharides obtained from *Spirulina* sp. strain is reported to have biostimulant properties due to the presence of several bioactive compounds. The production of polysaccharides was found to occur during the growth phase and reached the maximum level during the stationary phase [21–25]. Trabelsi et al. [22] reported that the concentration of the polysaccharides released into the culture media further increased with recycling of the culture media as these polysaccharides went on accumulating in the culture media with each harvesting cycle. However, it was observed that accumulated EPS or organic matter not only made the harvesting process difficult by increasing the viscosity of the cultivation media but also reduced the microalgal biomass productivity [21]. Therefore, separation or extraction of this EPS from the cultivation medium would be advantageous for maintaining the culture's productivity and utilizing the EPS as a valuable product such as plant biostimulant to generate additional economic benefits and promote the circular economy.

The literature survey presented above indicates that, until date, only a limited amount of research work has been carried out evaluating the potential of EPS (derived from different microalgal strains including *Spirulina* sp.) as a biostimulant. Furthermore, there are even less reports available focusing on the extraction of free EPS released into the culture medium and its application for promoting plant growth in a hydroponic cultivation system. Therefore, recovering the released EPS from the culture media of *Spirulina* sp. and using it for promoting plant growth in a hydroponic system by adding it to the nutrient solution will be a novel approach. If this simple approach turns out to be successful in stimulating plant growth, it could widen the scope for establishing a sustainable microalgal biorefinery, where the cultivated microalgal biomass can be used for extracting other valuable components such as bio-pigment (Phycocyanin), and biofertilizer in addition to microalgal EPS in a sequential manner. With this perspective in mind, the present work aimed to achieve two main objectives: (i) extracting microalgal EPS from the spent cultivation media and comprehending its basic composition, and (ii) assessing its potential as a plant biostimulant on different leafy vegetables grown in a hydroponic system.

To carry out the study, *Spirulina* sp. was cultivated in Zarrouk's medium for 30 days until it reached the stationary phase, and the microalgal EPS released into the culture medium was extracted after harvesting the microalgal biomass. The recovered EPS was then analysed and applied at various dosages (ranging from 0.25 to 1.5 mg/ml) to grow

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two different plant species: *Ocimum basilicum* (basil) and *Spinacia oleracea* (spinach), in a hydroponic system of agriculture. Finally, after a 5-week cultivation period, the plants were evaluated in terms of different growth parameters to find out how effective the recovered EPS is augmenting the crop production.

2 Methodology

2.1 Microalgal cultivation and growth condition

Spirulina sp. was collected from Dr. M.G.R. Fisheries College and Research Institute, Ponneri, Tamil Nadu (India), and cultivated in the modified Zarrouk medium as mentioned by Rajasekaran et al. [26] in a 500-ml Erlenmeyer flask under a controlled environment with an artificial light source of 180 µmol photons m^2 s^1 photo intensity and a 12-h photoperiod for 30 days. The average ambient temperature throughout the experiment was 26.3 ± 2 °C. The pH of the culture medium was kept between 8.8 and 11.0 during the cultivation period to avoid any kind of contamination.

For checking the microalgal growth, an absorbance reading was taken at 680 nm on every 3rd day and a growth curve was plotted. In order to evaluate the microalgal concentration, 2 ml of the culture media was withdrawn after every 5 days and centrifuged at 7000 rpm for 10 min [26]. After the centrifugation, microalgal pallet was dried and biomass concentration was calculated. For calculating the specific growth rate or μ_{max} (d^{-1}) of the *Spirulina* sp., Eq. 1 was used, where x_1 and x_2 are the microalgal concentration at the beginning and at the end of the exponential growth phase and Δt is the time span of the exponential growth phase.

$$\mu_{\max}\left(d^{-1}\right) = \frac{\ln x_2 - \ln x_1}{\Delta t} \quad (1)$$

2.2 Extraction of microalgal exopolysaccharides (EPS) and characterization

2.2.1 Extraction of EPS produced from *Spirulina* sp.

After 30 days of cultivation, the microalgal biomass was harvested using Whatman filter paper (grade 1). For the extraction of EPS from the spent culture medium, the method described by Wang et al. [27] was followed. Briefly, the spent culture medium was concentrated by reducing its volume to one tenth of the original by a using a vacuum oven (Model-126, SSSIW, India) at 30 °C and the dissolved EPS was separated from the concentrated medium by using ice-cold ethanol (95% $_{v/v}$) three times the volume of the medium. The mixture was then kept in a refrigerator overnight and the precipitated EPS was recovered by centrifugation at 8000 rpm for 15 min at 4 °C. The precipitate was redissolved in ultrapure Milli Q water followed by dialysis using 14 kDa membrane (for desalting) and lyophilisation. The crude EPS thus obtained was stored in the refrigerator for future use.

2.2.2 Quantitative and qualitative analysis of the microalgal EPS

EPS yield from the culture media was calculated with respect to the produced microalgal biomass using Eq. 2

$$\text{EPS yield}\left(g/g_{(\text{dry biomass})}\right) = \frac{\text{Microalgal biomass concentration (g/L)}}{\text{EPS dry weight (g)/Culture medium (L)}} \quad (2)$$

In order to find out the composition of the extracted EPS, different colorimetric assays were performed. In this study, phenol–sulfuric acid method and Lowry method were used for estimating the total carbohydrate and total protein content, respectively [28, 29]. The uronic acid content was estimated using the method described by Blumenkrantz and Asboe-Hansen [30], while the sulphate content of the extracted EPS was estimated using the barium chloride-gelatin method as described by Llyod et al. [31].

The major functional groups present in the microalgal EPS were identified using FTIR analysis. The lyophilized EPS samples were mixed with potassium bromide (KBr) and converted into pellets for the FTIR analysis. The FTIR spectrum of the microalgal EPS was obtained in the frequency range of 400–4000 cm^{-1} with a resolution of 4 cm^{-1}.

The surface morphology of the extracted polysaccharide was examined using a scanning electron microscope (SEM). SEM was linked to an energy dispersive X-ray analyzer (EDX Unit) energised at 15 kV for elemental mapping.

2.3 Comparison of the growth of the plants treated with different dosage of EPS

For this study, two leafy vegetables such as *Ocimum basilicum* and *Spinacia oleracea* were chosen due to their fast-growing nature. The seeds of both the plants were purchased from City Greens (India). After the sterilization of the surface (i.e., cleaning the seed surface with 1% NaOCl solution for 20 min followed by rinsing with water), the seed were dried and sowed in wet coco peat trays for germination. After the germination, the seedlings were kept

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in the grow bed for 15 more days until they reached the 4-leaf stage. At 4-leaf stage, seedlings were transferred to the experimental setup, where plants were cultivated in a hydroponic way using 500 ml beakers with plastic cups and thick polystyrene sheets were used to fix on them on the beaker. While coco peat was used as supporting media, modified Hoagland solution (composition as mentioned in Zhang et al. [32]) was utilised as substrate. Plants were grown with an artificial light source of 205 µmol photons $m^{-2}s^{-1}$, with a 12-h photoperiod for 35 days. The average ambient temperature throughout the experiment was 26.3 ± 3 °C. All the experimental plants were irrigated with 30 ml of the EPS solution at three different concentrations (0.25, 0.5 and 1.5 mg/mL) once a week against a control where 30 ml of distilled water was added instead of the EPS solution. The plant experiment for each of the three selected concentrations of EPS was carried out in triplicate.

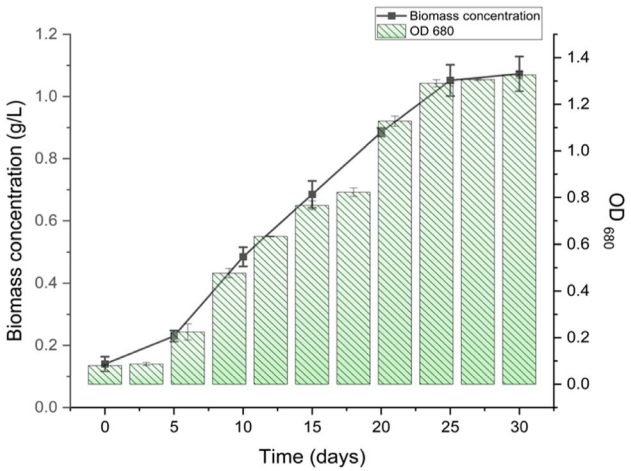

Fig. 1 Growth curve of *Spirulina* sp. cultivated in Zarrouk's medium

2.4 Plant growth biochemical parameter analysis

The plants were harvested after 35 days of cultivation in a hydroponic set up. At first, the number of leaves in each plant was counted and the numbers were noted down. Then, the roots were separated from the shoots and they were weighed separately. These weights were noted as fresh weight. The shoot heights were then measured and noted. For measuring the root volume, segregated roots of each plant were immersed in water in a measuring cylinder and the root volume was then estimated by measuring the volume of the water it replaced [33]. For estimating the dry weight, the segregated parts of the plant biomass were dried overnight in a hot air oven at 80 °C till a constant dry weight was obtained [34].

$$\text{Plant biomass productivity}\left(\frac{g}{(m^2)(day)}\right)=\frac{\text{Dry weight of the plant biomass }(g)}{\text{Area of the culture medium }(\text{m}^2)\times\text{ time (day)}} \quad (3)$$

Plant biomass productivity was estimated using Eq. 3 [34].

Chlorophyll, a pigment that is essential for photosynthesis is also estimated for evaluating the photosynthetic activity of the plants. Along with chlorophyll-a and chlorophyll-b, the presence of carotenoid was also measured following the protocol described by Sumanta et al. [35].

2.5 Statistical analysis

All the experiments in the batch study were performed in triplicate and the results were expressed as means ± standard deviation. Sigmastat software was used to perform one-way ANOVA along with the Tukey test for comparing the means. The differences were considered as significant at probability value $p < 0.05$.

3 Results and discussion

3.1 Growth kinetics of *Spirulina* sp.

Spirulina sp. was cultivated in modified Zarrouk's media for 30 days and the microalgal growth was estimated from the increase in algal concentration over time. For measuring the raise in the algal concentration, optical density (OD) was measured, as it is directly proportional to the algal concentration. By measuring the OD of the culture at 680 nm on every 3rd day and plotting the values against time, a 3-day lag phase, followed by a log phase was observed (Fig. 1). Again, after cultivation for 24 days, a stationary growth phase was observed. The microalgal biomass harvested by filtering the culture media after cultivation for 30 days gave a biomass yield of 1.07 ± 0.06 g/L and a growth rate of 0.08 d^{-1}. The filtrate containing the spent media was used later for the EPS extraction experiment.

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3.2 Quantitive and qualitative analysis of the EPS

After a cultivation period of 30 days, *Spirulina* sp. biomass was separated by filtration and the spent cultivation media obtained as the filtrate was analysed for EPS. The results shown that the filtrate contained EPS at a level of 0.75 ± 0.03 g/L which, when expressed on dry mass basis, worked out to around 0.70 ± 0.01 $g/g_{(dry\ biomass)}$. The yield of EPS observed here is slightly higher than the value reported by Chentir et al. [36], where 0.62 ± 0.08 g of EPS yield was obtained from 1 g of *Spirulina* sp. biomass (dry). However, it is noteworthy to mention that the EPS yield reported by the author was obtained from a 5-day culture. Published reports indicate that it is possible to enhance the EPS yield further by subjecting the *Spirulina* sp. strain under different stressed conditions [36] or by repeating the cultivation in the same medium [22]. Therefore, the obtained EPS yield from the present study should not be considered fixed as there are strategies available to enhance the yield further.

The qualitative analysis of the extracted crude polysaccharide revealed a carbohydrate content of 14.5 ± 0.41%, accompanied by protein (6.04 ± 1.36%), sulphate (3.06 ± 1.58%), and uronic acid (3.26 ± 1.34%). These results were found to be similar with the values reported by Rachidi et al. [15] where cell bound EPS was extracted from the dry biomass of *A. platensis*. The authors also reported a higher content of protein, sulphate, and uronic acid in EPS extracted from *Dunaliella salina*. The presence of sulphate and uronic acid along with the carbohydrate in the crude polysaccharide indicates its potential for functioning as a plant biostimulant as suggested in published reports [37, 38].

3.3 Characterization of the extracted EPS using FTIR analysis and SEM-EDX

The FTIR spectrum of the extracted EPS shows a weak absorption band around frequency 2576 cm^{-1} due to the weak S-H stretching (Fig. 2a), which represents the presence of thiol or sulfhydryl group [39, 40]. Apart, there are two sharp absorption bands at 1379 cm^{-1} and 850 cm^{-1} representing a sulfonylchloride group and the bending vibration of C-O-S of sulfate group at a C-4 axial position, respectively [24, 41]. All these results point to the presence sulphate group in the backbone structure of EPS. Other absorption band observed around 1635 cm^{-1} (assigned to the stretching vibration of C=O) and 1416.9 cm^{-1} indicates the presence of uronic acid, another component commonly found in microalgal EPS, which plays an important role as a biostimulant [42, 43]. The absorption band around 1121 cm^{-1} assigned to C-O stretching [40]. Finally, a very broad absorption band around the frequency range from 3400 to 3450 cm^{-1} and an exceedingly small absorption close to frequency 2927 cm^{-1} might be due to hydroxyl and methylene groups commonly found in microalgal exopolysaccharides [36, 44].

The surface morphology of the EPS was analysed using SEM and it showed slightly rough surface with conglomerates as uneven flakes (Fig. 2b). The elemental analysis of the EPS surface showed that it contained 52.5 $\%_{(w/w)}$ carbon (C), along with 35.3 $\%_{(w/w)}$, 3% $_{(w/w)}$ and 2 $\%_{(w/w)}$ of oxygen, nitrogen, and sulphur, respectively. The higher proportion of (C) along with a small proportion of sulphur (S) indicates that the EPS is comprised of carbohydrate along with a small amount of sulphate groups. Phosphate and sodium were also found in small quantity (2.20% $_{(w/w)}$ and 5.00% $_{(w/w)}$, respectively). However, they may be considered to be an impurity arising from salt precipitated by alcohol during extraction.

3.4 Effect of extracted EPS on plant growth

In order to facilitate a comparison of the magnitudes of the growth observed in various plants, the images of a complete set of plants grown with and without EPS treatment are given in Fig. 3. The images make it clear that in both kinds of plants the treatment with EPS extracted from spent medium gives better growth compared to control. The results of the plant growth analysis are presented in

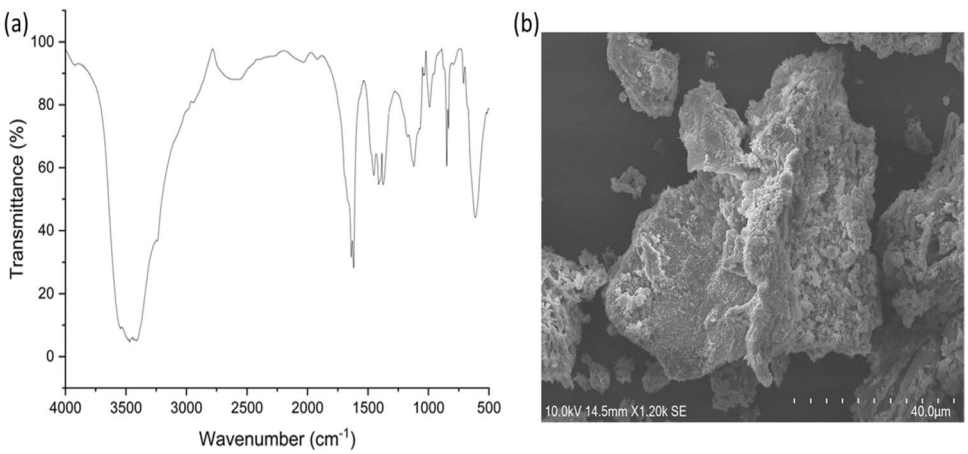

Fig. 2 FTIR analysis (**a**) and SEM image (**b**) of the extracted EPS from *Spirulina* sp.

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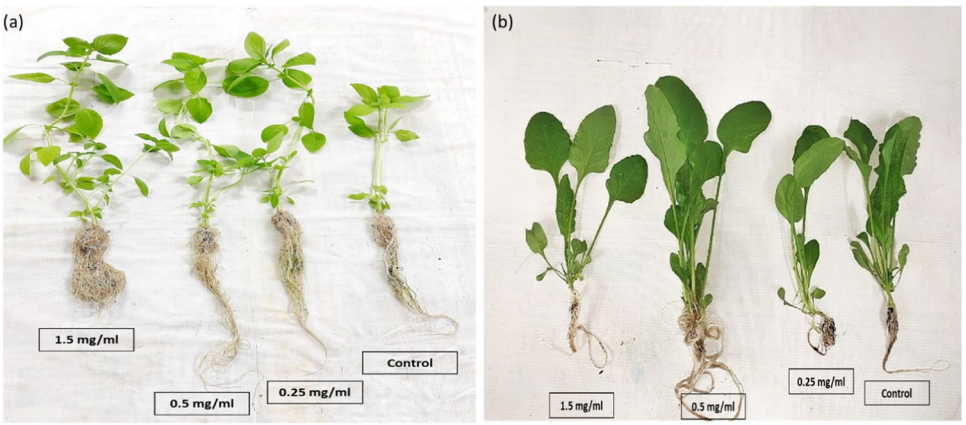

Fig. 3 Comparison between plant growth in basil (**a**) and spinach (**b**) treated with different concentration of EPS solution

Table 1. The results given in this table objectively demonstrate that the overall plant growth figures in terms of productivity for all the experimental plants are higher than those for the control irrespective of the plant species and dosage employed. This is in perfect agreement with the visual observation made in this regard. The reported increase in plant growth stimulated by EPS [14, 15] also corroborates this view, though the cited studies differ from the present one in respect of EPS source, plant species, and mode of application.

Further examination of the data given in Table 1 shows that for basil (*Ocimum basilicum*), the highest fresh plant biomass (20.53 ± 0.63 g) and biomass productivity (0.96 ± 0.03 g/m^2/d) are observed in the plants treated with 1.5 mg/ml EPS for 35 days. Compared to the control, the plants treated with 1.5 mg/ml EPS show around 18% higher plant growth with 53% higher root volume and 36% higher stem length. So, 1.5 mg/ml EPS is the optimum dosage for basil. As for spinach (*Spinacia oleracea)*, the optimum dosage of EPS for maximum plant growth is 0.5 mg/ml of EPS, as it shows the highest fresh plant biomass of 21.42 ± 0.52 g and plant biomass productivity of 1.13 ± 0.01g/m^2/day, which is 27% higher than the control (Table 1). So, the optimum dosage of EPS is different for two different plant species.

When comparing the overall productivity figures of basil and spinach at different dosages of EPS, it becomes evident that the responses of the two plant species are different. In the case of basil, productivity shows an almost steady increase as the EPS dosage is raised. Whereas for spinach, the productivity does not exhibit the same consistent growth pattern with increasing EPS dosage. While an EPS dosage of 0.5 mg/ml led to increased biomass productivity compared to the control, raising the EPS dosage further to 1.5 mg/ml did not result in any additional growth in overall productivity in spinach. At 1.5 mg/ml EPS dosage, the biomass productivity in spinach declined slightly (from 1.13 ± 0.01 to 1.07 ± 0.02 g/m^2/day). Upon closer examination of the individual components contributing to overall productivity, such as shoot height, shoot weight, root volume, root weight, and number of leaves reveals that the decline observed in the overall productivity is also true of these components individually. Interestingly, the EPS dosage of 1.5 mg/ml induces no further growth in any of these individual components in spinach. While the reasons for this behavior are not clear at this stage, one thing is evident—the optimal dosage of EPS for maximum plant growth is species-dependent. Such variability in the response to EPS biostimulation is also reported by Elarroussi et al. [23]. These authors observed greater amenability of *Solanum lycopersium* to biostimulation compared to *Capsicum annum* when both were treated with 3 mg/ml EPS solution extracted from *Spirulina* sp. under identical environmental conditions.

For basil, an almost steady growth of overall productivity or total fresh weight with increasing dosage of EPS from 0.25 to 1.5 mg/ml is apparent, but a closer scrutiny of the growth figures of the various components reveals that the promotion of plant growth at the level of 1.5 mg/ml EPS has not happened in all these components in a very consistent manner. The data show that at this level, the positive effect on shoot growth is slightly lower than the positive effect on root growth. In case of plant growth expressed in terms of the number of leaves, the figure for the number of leaves actually shows a small decline (from 42.67 ± 2.25 to 32.67 ± 9.14) when the EPS dosage is raised from 0.5 to 1.5 mg/ml. The differential stimulation exerted by EPS on various plant-parts such as roots and leaves could be the probable reason, though there is some uncertainty associated with this presumption as the difference between the two values for the number of leaves (42.67 ± 2.25 and 32.67 ± 9.14) is not statistically significant. So, it may be inferred that the EPS treatment stimulates the growth of different parts of a plant, though the effect on different parts may vary.

As mentioned in Section 3.2, the crude EPS used in this study contains uronic acid, sulphate, carbohydrate, and proteins. Since the first two components of polysaccharides

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Table 1 Growth analysis of basil and spinach plants treated with varying concentration of EPS extracted from the spent cultivation media of *Spirulina* sp.

Treatment	Fresh weight		Dry weight		Plant root volume (cm^3)	Plant shoot height (cm)	Number of leaves	Total fresh weight (g)	Total dry weight (g)	Biomass productivity (g/m^2/day)
	Root (g)	Shoot (g)	Root (g)	Shoot (g)						
Basil										
Control	5.05 ± 0.20	10.33 ± 0.23	0.54 ± 0.01	0.72 ± 0.01	5.07 ± 0.31	29.77 ± 2.16	39.67 ± 2.25	15.38 ± 0.43	1.26 ± 0.02	0.82 ± 0.01
0.25 mg/ml	5.87 ± 0.26*	11.11 ± 0.11*	0.57 ± 0.02	0.80 ± 0.03*	5.17 ± 0.23	26.99 ± 0.57	39.33 ± 3.72	16.98 ± 0.37*	1.37 ± 0.05	0.89 ± 0.03
0.5 mg/ml	6.18 ± 0.13*	11.59 ± 0.34*	0.62 ± 0.01*	0.84 ± 0.00*	7.17 ± 0.78*	39.47 ± 2.33*	42.67 ± 2.25	17.76 ± 0.47*	1.46 ± 0.02*	0.95 ± 0.01*
1.5 mg/ml	8.60 ± 0.31*	11.93 ± 0.32	0.68 ± 0.01*	0.81 ± 0.04*	7.77 ± 0.83	40.73 ± 1.22*	32.67 ± 9.14	20.53 ± 0.63*	1.48 ± 0.05*	0.96 ± 0.03*
Spinach										
Control	3.03 ± 0.75	13.39 ± 0.26	0.30 ± 0.03	1.07 ± 0.03	3.50 ± 0.32	25.10 ± 1.03	9.67 ± 2.25	16.92 ± 1.00	1.37 ± 0.04	0.89 ± 0.03
0.25 mg/ml	3.25 ± 0.39	14.27 ± 0.74	0.34 ± 0.04*	1.04 ± 0.04	3.57 ± 0.47	28.00 ± 2.05	10.00 ± 18.02	17.52 ± 0.73	1.48 ± 0.05	0.96 ± 0.03
0.5 mg/ml	5.22 ± 0.26*	16.20 ± 0.35*	0.51 ± 0.02*	1.23 ± 0.01*	5.33± 1.37	30.33 ± 1.81*	15.33 ± 0.52*	21.42 ± 0.52*	1.75 ± 0.01*	1.13 ± 0.01*
1.5 mg/ml	4.77 ± 0.21*	15.98 ± 0.49*	0.44 ± 0.02*	1.21 ± 0.01*	4.34 ± 0.46	28.40 ± 0.50*	14.67 ± 2.73	20.75 ± 0.66*	1.65 ± 0.03*	1.07 ± 0.02*

Data represents average of 3 replicates ± standard deviation

*Represents a significant difference compared to the control treatment using ANOVA ($p < 0.05$)

have been found to correlate with the plant growth [15], their presence in the EPS used in this study corroborates the stimulating influence of EPS on plant growth seen in Table 1. Although no attempts have been made in the present study to evaluate the relative importance of these components in respect of plant growth, the close similarity of the levels of these components observed in the present study to those observed in the cited study [15] suggests that the trend is likely to be very similar.

Chlorophyll and carotenoids are two photosynthetic pigments that are found in chloroplast. They play an important role in harvesting photons from sunlight or artificial light source in order to carry out photochemical redox reactions.

In the study reported here, the level of chlorophyll and carotenoid present in the leaves of both experimental and control plants were estimated and the values obtained are graphically presented in Fig. 4. From the graphics presented in Fig. 4, it can be seen that overall, in all experimental plants, regardless of their species, the level of chlorophyll and carotenoid shows a rising trend with increasing level of treatment. However, it is important to note that the trend is not consistent at the highest treatment level (1.5 mg/ml of EPS), especially in spinach, where a small downfall in the production of plant pigment has occurred.

In spinach, all the three EPS treatment showed significant increase in chlorophyll and the treatment with 0.5 mg/ml EPS solution gives the highest production of various pigments (chlorophyll a: 6.99 ± 0.09 µg/ml, chlorophyll b: 1.50 ± 0.05 µg/ml, and carotenoid: 1.26 ± 0.02 µg/ml). On the contrary, the results for basil paint a slightly different picture. As the EPS dosage exceeded 0.25 mg/ml, a notable and statistically significant increase in both chlorophyll and carotenoid levels was observed. The treatment with 1.5 mg/ml EPS solution led to the highest level of pigments (chlorophyll a: 5.37 ± 0.50 µg/ml, chlorophyll b: 1.97 ± 0.29 µg/ml, and carotenoid: 1.42 ± 0.13 µg/ml) roughly two times greater than that of the control.

The overall pattern that emerges from these data closely resembles the pattern observed earlier in the case of plant growth. As chlorophyll and carotenoids are closely related to photosynthesis, the increase in photosynthetic pigment levels is expected to result in greater plant growth. The strong correlation between pigment production and plant growth further validates the earlier results obtained in the plant growth analysis. Therefore, it may be inferred that the controlled use of EPS recovered from spent cultivation medium has the potential to stimulate the production of photosynthetic pigments in plants like basil and spinach, thereby promoting their overall growth. Furthermore, the EPS treatment has been shown to be capable of inducing the production of carotenoids, which plays a vital role in protecting the photosynthetic apparatus and securing plant yield under oxidative stress. This finding suggests

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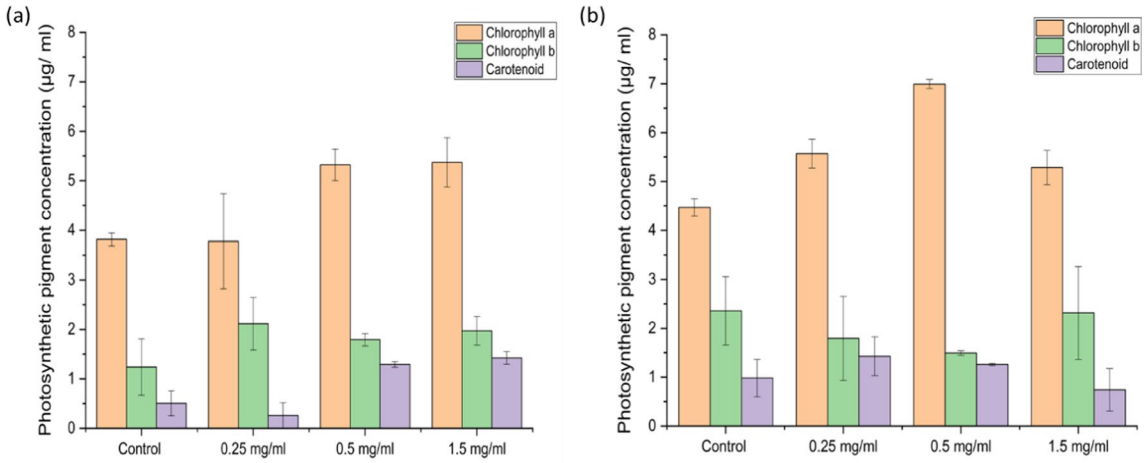

Fig. 4 Photosynthetic pigment content in (**a**) basil and (**b**) spinach leaves treated with varying concentration of EPS extracted from the spent cultivation media of *Spirulina* sp.

the potential for utilizing EPS treatment as an intriguing stress management strategy, for securing plant yield, even under adverse environmental condition.

Finally, considering all the results discussed earlier in this section, it may be stated that this study has made it possible to simultaneously cultivate *Spirulina* sp., recover EPS from the spent cultivation medium, and use the recovered EPS for promoting the growth of leafy vegetables in a hydroponic set-up covering the entire path from beginning to end without generating any additional waste. Therefore, even though this work has not included an economic feasibility study, it has brought the *Spirulina* sp. cultivation a step closer to an ideal microalgal biorefinery-like production system (as shown in Fig. 5) facilitating its implementation.

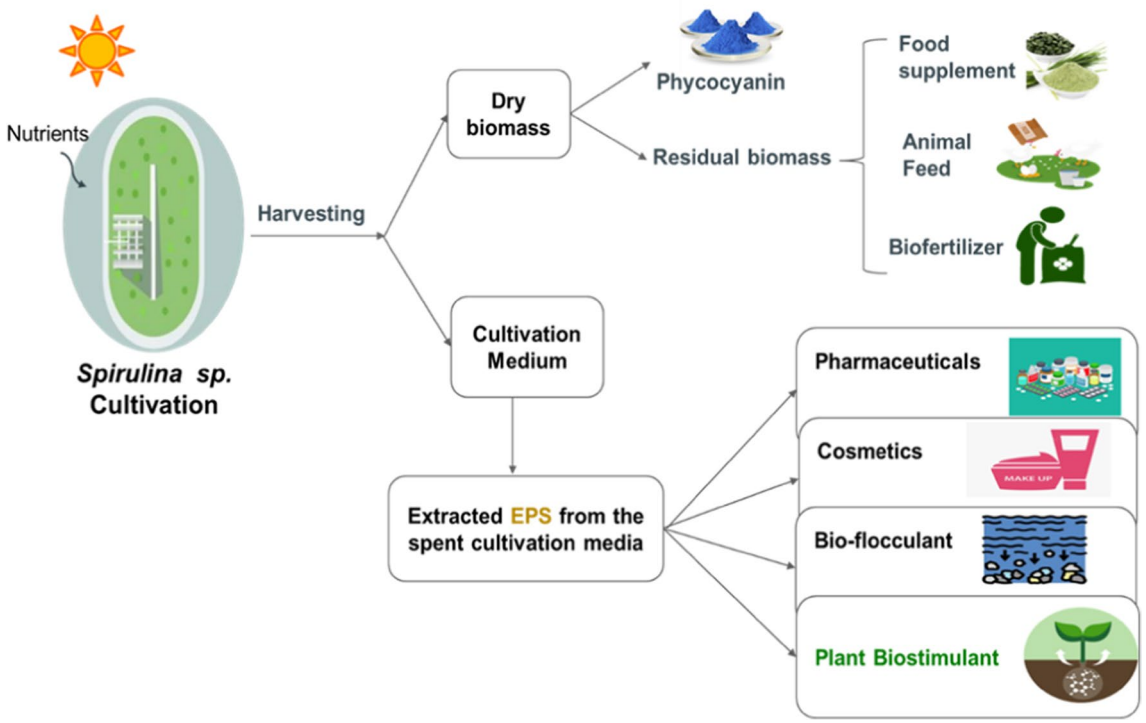

Fig. 5 Suggested pathway for simultaneous production of EPS, phycocyanin, and other valuable components from *Spirulina* sp. for establishing a biorefinery approach

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4 Conclusion

This study was concerned with the recovery of EPS from waste cultivation medium of *Spirulina* sp. and its application as a biostimulant for promoting plant growth in a hydroponic set-up. In the present study, after a cultivation period of 30 days, the EPS recovered from the spent cultivation medium of *Spirulina* sp. gave a yield of 0.75 ± 0.03 g/l, which was comparable with the existing literature results. The recovered EPS was found to contain uronic acid and sulphate, which are indicative of its potential as a biostimulant.

The results obtained from the plant growth study show that the EPS recovered from the spent cultivation medium is capable of stimulating the growth of both plants examined, though the maximum growth is achieved at a specific dosage of EPS depending on the plant species. The results also indicate that the application of EPS influences the production of chlorophyll along with the carotenoids which is known to play a protective role against UV radiation and oxidative stress in plants.

Overall, this study unveils a promising avenue to utilise the spent cultivation medium of *Spirulina* sp. for the production of EPS, which will help in meeting the rising demand for food by enhancing the biomass yield. The study also widens the scope for adopting a biorefinery-like approach to *Spirulina* sp. cultivation. However, since sustainability (economic as well as environmental) is an important issue about which the present study says very little, this aspect of the process needs to be studied further. A complete material balance of the whole system might also be useful to check the exclusivity of EPS's influence. Hence, it is crucial to investigate this aspect as well. Finally, it is critical to assess EPS's potential as a biostimulant for ensuring yield security in the face of changing climatic conditions. Therefore, studies similar to the present one must be conducted under various abiotic stress conditions (various temperature and salinity levels).

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Author contribution Rayanee Chaudhuri: conceptualisation, investigation, data analysis, writing—original draft; Paramasivan Balasubramanian: conceptualisation, investigation, funding acquisition, writing—review and editing

Data availability All the data provided in the manuscript has been added in the manuscript as well as in the supplementary file.

Declarations

Ethics approval Not applicable

Competing interests The authors declare no competing interests.

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