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Response and Tolerance of Cyanobacterial Exopolysaccharides to Rice Field Herbicide 2,4-D

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ABSTRACT

This study aimed to check how herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) affects the production of EPS and its composition, growth, and biomass, as well as morphology in a cyanobacterial species isolated from a rice field in Meghalaya, India. Compared to the control cells, the growth of the organism measured in terms of chlorophyll concentration increased after being exposed to 10 and 20 ppm 2,4-D. However, cultures treated with 30 and 40 ppm experienced a decrease in their growth. Likewise, the biomass content of the organism experienced a minuscule increase in content upon exposure to 10 and 20 ppm 2,4-D but was compromised upon exposure to higher doses. When exposed to 10 ppm, the total EPS content, which includes the RPS and CPS content, showed a substantial increase. Maximum EPS production was seen at 20 ppm 2,4-D. However, exposure to 30 and 40 ppm 2,4-D, EPS production in the organism experienced a significant reduction, respectively. All components of EPS, such as uronic acid, neutral sugar, and proteins, individually showed an increase in 10 and 20 ppm 2, 4-D. A similar trend was seen in the organism's bio-flocculating activity, which increased when exposed to 10 and 20 ppm, respectively. However, this activity in cells exposed to 30 and 40 ppm 2,4-D was severely reduced. Not only the content of EPS but the rate of EPS production was also enhanced in lower concentrations of 2,4-D. Although exposure to 30 ppm 2,4-D, the rate of EPS production was not significantly compromised, 40 ppm exposure adversely affected the rate of EPS production. Furthermore, visualization using scanning electron microscopy revealed the morphological changes induced by the herbicide 2,4-D.

INTRODUCTION

The most ubiquitous photosynthetic microorganisms in nature are cyanobacteria (Frigaard 2018). They generate their energy from photosynthesis and avail their nitrogen requirement through nitrogen fixation. Thus, they have the most simple nutritional requirements from the environment. This allows them to adapt to a broad range of environments, from marine to terrestrial ecosystems (Zahra et al. 2020). These organisms have received a lot of attention in recent years due to their potential applications in biotechnology. They are employed in aquaculture, bioremediation, fertilizers, and synthesis of secondary metabolites, including exopolysaccharides, vitamins, toxins, enzymes, and medicines (Abed et al. 2009).

Cyanobacteria are known to produce significant amounts of extracellular polymeric substances or exopolysaccharides (EPS), comprised of polysaccharides, lipids, and proteins in the form of heteropolymer-like lipopolysaccharides or glycoproteins (Decho 1990, Mishra 2020, Singh et al. 2019). These EPS can either stay connected to the cell surface as sheaths, pellets, or slimes, or they can be discharged as released polysaccharides into the environment (Pereira et al. 2011). The synthesis and production of EPS by cyanobacteria are correlated to the ability of these organisms to survive in unfavorable and stressful conditions. Its synthesis helps create a microenvironment that is structurally stable and hydrated and provides chemical and physical defense from biotic and abiotic stress factors (Parwani et al. 2021). EPS produced by cyanobacteria is a type of biopolymer that favors the formation of complex microbial communities called biofilms, where they serve key structural and defensive roles (Rossi & De Philippis 2015). Also, the cyanobacterial EPSs produced promote the formation of microbial associations under stressed or hostile environments. Several important elements influence cyanobacterial EPS production. Two examples are energy availability and the C/N ratio (De Philippis & Vincenzini 1998, Rossi & Philippis 2015). Other key elements that influence cyanobacterial EPS synthesis include nutrient level as well as growth conditions like light intensity, salinity, and temperature (Rossi & De Philippis 2015).

Structurally, cyanobacterial EPS have a complex nature and are primarily due to the presence of proteins, uronic acids (glucuronic and galacturonic acid), pyruvic acid, and *O*-methyl, *O*-acetyl, and sulfate groups (Ozturk et al. 2010). The presence of a significant amount of uronic acids contributes to the anionic and sticky nature of the exopolysaccharides. Also, substantial amounts of neutral sugars such as xylose, arabinose, mannose, and glucose are found in EPS, where glucose is the most common sugar and accounts for a large amount of the EPS composition (Plude et al. 1991, Rossi & De Philippis 2016). Owing to their structural complexity, cyanobacterial EPSs have many possible potential applications, including bioflocculants, bioadhesives, soil conditioners, biopolymers, biofilms formation, food supplements, in bioremediation, and useful as medicinally important bioactive compounds (Singh et al. 2019).

Bacteria are the main removal/degradation agents for environmental toxins such as herbicides, pesticides, insecticides, and different petroleum hydrocarbons, among which some of these chemicals can be used as the sole source of carbon and energy (Fuller & Scow 1997). Several studies have looked at the impact of pesticides, especially herbicides, on exopolysaccharide synthesis (Ahemad & Khan 2012, Sultan et al. 2019, Shahid & Khan 2022). In the literature, it has been seen that herbicides like 2,4-D affect the generation of EPS in several species. However, there is very little information in the literature about anthropogenic variables affecting EPS production in cyanobacteria, such as excessive herbicide use in agricultural fields. As a result, the goal of this study is to see how the herbicide 2,4-D (10, 20, 30, and 40 ppm) affects exopolysaccharide (EPS) synthesis in the cyanobacterium *Nostoc muscorum* Meg 1.

MATERIALS AND METHODS

Growth Conditions

Nostoc muscorum Meg 1, a cyanobacterium used in the present study, was previously isolated by Ahad et al. (2017). The culture was grown in BG-11_0 media (pH 7.5) and maintained in a culture room under continuous light at a photon fluence rate of 50 $\mu mol.m^{-2}.s^{-1}$ and a temperature of 25 ± 2°C (Rippka et al. 1979).

2,4-D Treatment

Different concentrations of 2,4-D, such as 10, 20, 30, and 40 ppm, were prepared from a 2,4-D stock solution (300 ppm). To evaluate the effect of the herbicide 2,4-D on various parameters, the cultures were incubated with the herbicide for 7 days.

Chlorophyll *a* Content

The organism's growth was checked by a rise in chlorophyll-*a* concentration, as described by Mackinney (1941). Three mL of culture was centrifuged for three min at 2500 g. The supernatant was discarded, and 3 mL of methanol was added to the pellet and left overnight for chlorophyll extraction. After the incubation period, the solution was centrifuged at 2500 g. A spectrophotometer was used to measure the absorbance of the supernatant at 663 nm. The concentration of chlorophyll-*a* was determined using the formula:

Chlorophyll-*a* ($\mu g.mL^{-1}$) = Absorbance at 663 nm × 12.63

Biomass Content

The biomass content was determined by placing 3 mL of culture in pre-weighed filter sheets and drying them in a hot air oven at 45°C until they reached a constant weight. The filter papers were weighed again with a weighing balance, and the difference was calculated as the biomass's true weight. The dry weight of the whole biomass content was expressed in grams (g).

Extraction of Exopolysaccharide

The EPS was extracted using the procedures of Cérantola et al. (2000) and Chi et al. (2007). Cell cultures were centrifuged at 10,000 g for 10 min at 4°C after 30 days of incubation. The supernatant, which contains the released polysaccharide (RPS), was collected for extraction. For Capsular or cell-bound polysaccharides (CPS) extraction, 5 mL of 0.05% NaCl, pre-heated at 60°C, was added to the pellet and incubated in a hot water bath at 60°C for 30 min. The mixture was then centrifuged for 30 min at 10,000 rpm, and the supernatant was collected. An equivalent amount of 96% ethanol was added to the supernatant separately and incubated at -20°C for 72 h. After incubation, the precipitate was centrifuged at 10,000 rpm for 30 min at 4°C and rinsed thrice with 96% ethanol. The final precipitate was dissolved in 1 mL of distilled water and stored at – 20°C for further experiments.

Determination of the Polysaccharide Content

Using glucose as a reference standard, the carbohydrate content of extracellular polysaccharides was calculated using the Phenol-Sulphuric acid method described by Dubois et al. (1956).

Released polysaccharide (RPS) content: 200 µL of an extract solution was mixed with 200 µL of 5% phenol and 1000 µL of concentrated sulphuric acid. The tubes were vigorously agitated before being immersed in a boiling water bath for 5 min and then cooled before being left at room temperature for 30

min. The absorbance was spectrophotometrically measured at 492 nm.

Capsular polysaccharide (CPS) content: An extract solution (200 µL) was mixed with 5% phenol (200 µL) and concentrated sulphuric acid (500 µL), and the tubes were vigorously agitated before being immersed in a boiling water bath for 5 min and cooled before being left at room temperature for 30 min. The absorbance was spectrophotometrically measured at 492 nm.

Total polysaccharide content: Total polysaccharide content was obtained by calculating the sum of RPS and CPS content for its control and treated cultures.

Exopolysaccharide Components

Uronic acid content: 1.2 mL sodium tetraborate was added to 200 µL of the sample, and the mixture was vortexed before being treated in a hot water bath for 5 min at 100°C. It was then chilled in an ice bath. After that, 20 µL of m-hydroxy diphenyl reagent was added, mixed thoroughly, and allowed to stand for 5 min (Blumenkrantz & Asboe-Hansen 1973) before reading absorbance at 520 nm. D-glucuronic acid was used as standard (5-50 µg/mL).

Neutral sugar content: The neutral sugar content was estimated using Roe's (1955) method. 4 mL of anthrone reagent was added to 200 µL of extracted solution and carefully mixed. After 10 min of incubation in a boiling water bath, the mixture was chilled, and absorbance was measured at 630 nm. D-glucose was used as standard (10-100 µg/mL).

Protein content: Bradford's (1976) technique was used to estimate protein levels. 200 µL of an extracted solution was mixed with 3 mL of Bradford reagent. It was then incubated in the dark for 5 min, and the absorbance of the solution was read at 595 nm. BSA solution was used as standard (10-100 $\mu g.mL^{-1}$).

Bioflocculating Activity

Cell culture was centrifuged for 5 min at 2900 x g. 0.5 mL of the EPS-containing supernatant was then diluted with 4.25 mL of 0.5 N acetic acid and 0.25 mL of Alcian Blue dye preparation (Alcian Blue 8 GX (1 $mg.mL^{-1}$) in 0.5 N acetic acid). It was then incubated for 30 min at room temperature before being centrifuged for 10 min at 2900 x g. The supernatant was spectrophotometrically read at 610 nm. Alcian Blue and acetic acid are used in the reference assays with no EPS added.

$$\text{Flocculating activity} = \{(B - A)/B\} \times 100\%$$

where A and B are the absorbance values at 610 nm of sample and control, respectively (Kurane et al. 1994).

Rate of EPS Production

To check the rate of the production of EPS of the organism, the increase or decrease in EPS during that period was divided by the increase or decrease in biomass content during that period.

Scanning Electron Microscopy (SEM)

To check the morphological changes in the organism, control, and treated cultures were first centrifuged, and 4% glutaraldehyde in phosphate buffer was added to the pellet, followed by incubation at 4°C for 24 h. Next, the samples were washed in 0.1 % sodium cacodylate buffer three times (15 min each) and then dehydrated in acetone (20% – 100%) at 4°C at an interval of 15 min each. The dehydrated samples were mounted on brass stubs, followed by gold coating before viewing under SEM (JSM, 6360, JEOL, Japan).

Statistical Analysis

All experiments were performed in triplicate, and the data were presented as mean ± standard deviation. GraphPad Prism 5 Software was used to do a one-way analysis of variance (ANOVA) to check the significant differences between the control and treated cultures (Dunnett test).

RESULTS AND DISCUSSION

Effect of 2,4-D on Growth (Chlorophyll Content and Biomass)

Fig. 1a shows the chlorophyll content of *Nostoc muscorum* Meg1 after exposure to 2,4-D (10, 20, 30, and 40 ppm). Exposure to 10 and 20 ppm 2,4-D, the chlorophyll content was increased by 20% ($p < 0.01$) and 1.7%, respectively, compared to their control cells. However, cultures treated with 30 and 40 ppm 2,4-D, 27%, and 84.46% ($p < 0.01$) reduction were observed. Similarly, biomass content was significantly enhanced by 12.1% upon 10 ppm exposure ($p < 0.05$). The increase in biomass output was just 4.3%, with no significant differences observed in cultures exposed to 20 ppm 2,4-D. Upon exposure to 30 and 40 ppm, there was a drastic reduction in biomass production by 21.5% and 42.13%, respectively, with a significant value of $p < 0.001$, as shown in Fig. 1b.

Effect of 2,4-D on Exopolysaccharide (EPS) Content and Rate of EPS Production

In the control culture, the total EPS content of the organism was found to be 114.6 µg/mL, out of which 90.6 $\mu g.mL^{-1}$ is the released polysaccharide (RPS) and 23.9 $\mu g.mL^{-1}$ is the capsular/bound polysaccharide (CPS). These values for control were taken as 100% to check the organism's reaction

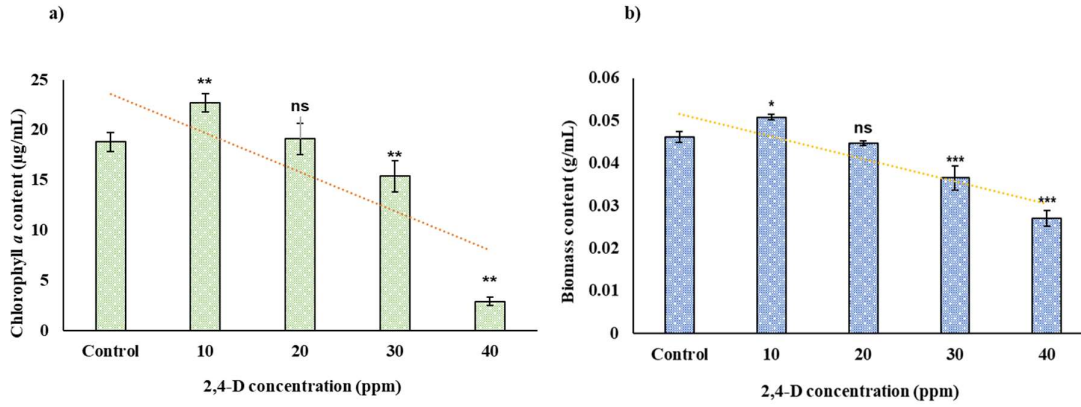

Fig. 1: Effect of 2,4-D herbicide on (a) chlorophyll content and (b) biomass content. All values are in mean ± standard deviation (N = 3), with asterisks (*$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$) above the histogram bars indicating significance in differences between control and 2,4-D treated cells.

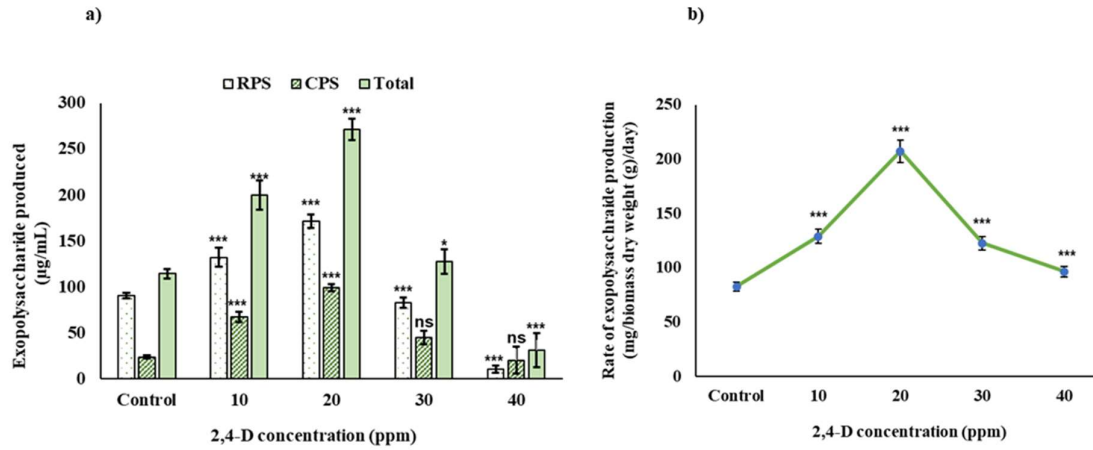

Fig. 2: Effect of 2,4-D on (a) EPS content and (b) rate of exopolysaccharide production. All values are in mean ± standard deviation (N = 3), with asterisks (*$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$) above the histogram bars indicating significance in differences between control and 2,4-D treated cells.

to 2,4-D at various concentrations in terms of percentage outcomes. At 10 ppm, the total EPS content was increased by 74.17%, in RPS by 45.6%, and in CPS by 182.5%. At 20 ppm, the concentration was increased by 136% (total), 89.16% (RPS), and 315.8% (CPS). At 30 ppm, there is a reduction in the total EPS and also in RPS content by 28%. Nevertheless, the CPS content was still inclined by as much as 42%. The effect on EPS content upon exposure to 40 ppm 2,4-D was drastically affected. The total EPS content was reduced by 64%. In addition, 66% and 56% reductions were detected for RPS and CPS (Fig. 2a). Furthermore, the rate of EPS production observed an increase of 58% and 144% upon exposure to 10 and 20 ppm 2,4-D. The increase in EPS production was still evident in 30 ppm 2,4-D (48%) and 16% in 40 ppm as compared to control (Fig. 2b).

Effect of 2,4-D on Uronic Acid, Neutral Sugar, and Protein Content

Cultures exposed to 10 and 20 ppm 2,4-D showed an increase in the total uronic acid content by 46% and 98.3%, respectively, as compared to the control (Fig. 3a). Although there was an increase of 11.8% in the content of the uronic acid even at 30 ppm 2,4-D exposure, the concentration of uronic acid was reduced by 22% in the presence of 40 ppm 2,4-D ($p < 0.05$).

There was an increase in the neutral sugar content by 10.3%, 41.7%, and 15.1% at 10, 20, and 30 ppm 2,4-D, respectively. However, a significant ($p < 0.05$) decrease of 29.4% has been recorded at exposure to 40 ppm 2,4-D in comparison to the control (Fig. 3b). Furthermore, the protein content of the extracted EPS treated with 10 ppm 2,4-D

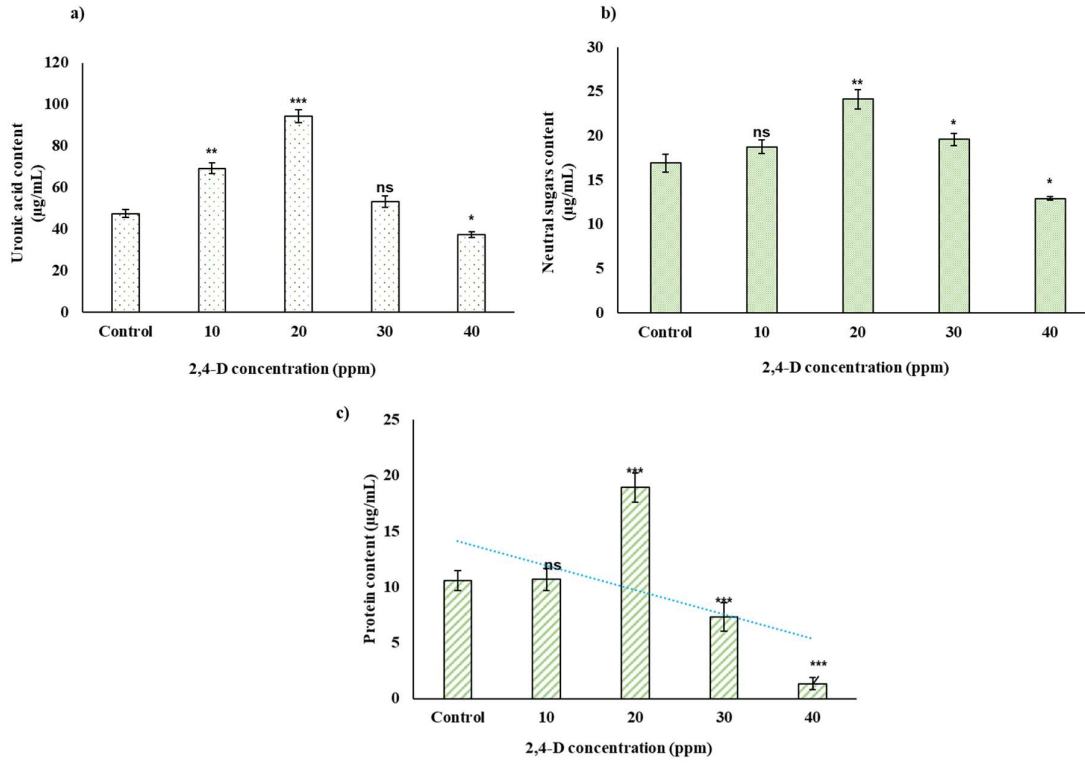

Fig. 3: Effect of 2,4-D on (a) Uronic acid content, (b) neutral sugar content, and (c) protein content. All values are in mean ± standard deviation (N = 3), with asterisks (*$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$) above the histogram bars indicating significance in differences between control and 2,4-D treated cells.

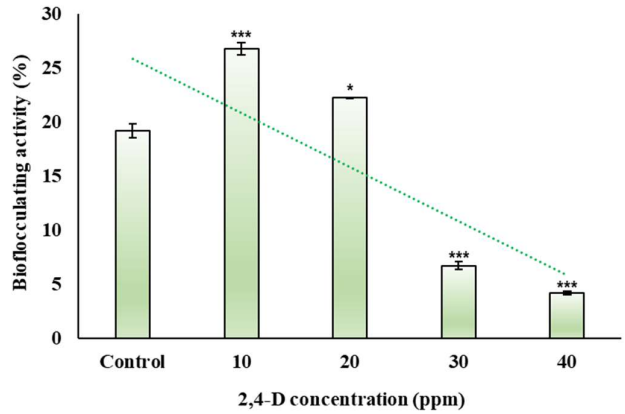

Fig. 4: Effect of 2,4-D on the bio-flocculating activity of *Nostoc muscorum* Meg 1. All values are in mean ± standard deviation (N = 3), with asterisks (*$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$) above the histogram bars indicating significance in differences between control and 2,4-D treated cells.

observed an insignificant increase by just < 1%, but extreme rise in the protein content was observed by as much as > 78% in culture treated with 20 ppm 2,4-D. At 30 and 40 ppm, the protein content showed a significant reduction ($p < 0.001$) by ~ 30 and 87%, respectively (Fig. 3c).

Effect of 2,4-D on Bio-flocculating Activity

There was an increase in the bio-flocculating activity compared to control upon exposure to 10 and 20 ppm 2,4-D by 39.5% ($p < 0.001$) and 15.8% ($p < 0.05$). In contrast, the bio-flocculating activity was significantly reduced by 65.1% and 78.2% ($p < 0.001$) upon exposure to 30 and 40 ppm (Fig. 4).

Effect of 2,4-D Stress on the Morphology of the Organism

The morphological changes of *Nostoc muscorum* Meg, 1

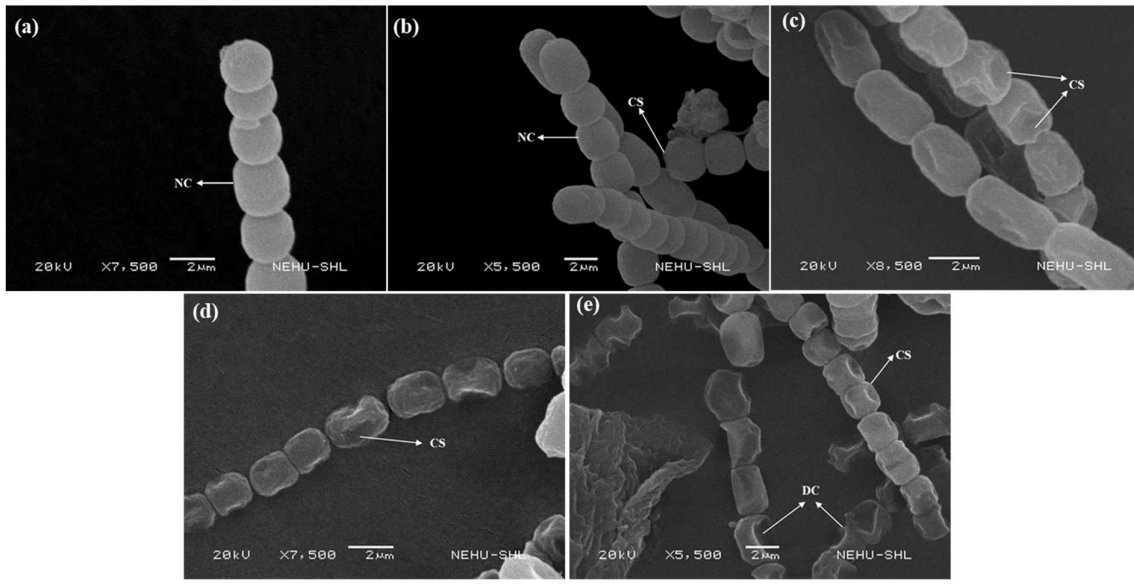

Fig. 5: Effect of 2,4-D on the morphology of *Nostoc muscorum* Meg 1. (a) Control and under-treatment of 2,4-D (b) 10 ppm (c) 20 ppm (d) 30 ppm (d) 40 ppm. NC: Normal cell; CS: Cell shrinkage; DC: Deformed cell.

under 2,4-D stress, were visualized under SEM. Control cultures showed healthy cells with intact filaments (Fig. 5a). At 10 ppm 2,4-D, the cells were intact, but minor shrinkages of the cells were seen (Fig. 5b). A much higher percentage of cell shrinkage was observed with increased 2,4-D doses (20 and 30 ppm) (Fig. 5c and 5d). However, at 40 ppm, cells were distorted and severely deformed (Fig. 5e).

Discussion

Environmental contamination and its remediation have recently gained a lot of worldwide attention (Ali et al. 2019). Agrochemical contaminations such as pesticide/herbicide from pesticide manufacturing plants, wastewater recharge facilities (wells or basins), and waste disposal sites are some substantial sources of environmental pollution (Pérez-Lucas et al. 2018). As a result, it is critical to find ways to reduce these agrochemicals from polluted areas to ensure that environmental pollution is regulated (Pavlidis & Tsihrintzis 2018). On the other hand, chemical and physical procedures for eliminating toxic agrochemical compounds are non-specific, costly, and environmentally unfriendly (Bala et al. 2022). As a result, finding suitable bioremediation processes and technologies based on microbial sources becomes critical. In this context, the usage of exopolysaccharides as bio-flocculants is one of the probable bioremediation possibilities in wastewater treatment since they are eco-friendly, non-toxic, and biodegradable.

Even though cyanobacteria can be used as an excellent tool for bioremediation of agrochemical pollution due to its bio-flocculating activity, an excessive and non-standardized amount of polluting chemicals such as pesticides and herbicides may impede the organism's ability to bio-remediate (Gouveia et al. 2019). Furthermore, pesticides have been found to harm cyanobacterial growth, production, and biomass when applied in a continuous and unstandardized dose (Sachu et al. 2021).

In the present study, the herbicide 2,4-D was seen to negatively affect the growth of the cyanobacterium *Nostoc muscorum* Meg 1 at concentrations above 30 ppm (Fig. 1). The effect of the herbicide on growth could be due to changes in pigment synthesizing enzymes or by bringing changes such as photochemical activity and/or disruption of the light-harvesting complexes (Kalaji et al. 2016, Székács 2021). The prominent change seen when cultures were exposed to lesser doses of 2,4-D (< 20 ppm) was an increase in growth compared to control cells. This observation finds its explanation in the publication by Bellinaso et al. (2003), where it was suggested that physiological changes that occur in the organisms following exposure to lower limits of stress cause microbial metabolism to develop an alternative metabolic pathway to avoid a biochemical reaction that is hindered by stressors such as herbicides or due to the hormetic effect where the organism increases its metabolic activities to overcome such immediate adverse exposure (Sachu et al. 2021).

Excess synthesis of exopolysaccharides could be one of the reasons which provide protection against environmental stress like herbicides in soils (Ahemad & Khan 2012). In the present study, the total EPS content, as well as the RPS

and CPS contents, experienced a significant upsurge ($p < 0.001$) in their contents upon exposure to 10 and 20 ppm, respectively, where the highest EPS production was observed at 20 ppm {136% (total), 89.14% (RPS) and 315.8% (CPS)}. An increase in the contents of EPS may be because these extracellular polysaccharides are thought to present barriers to compounds such as xenobiotics and prevent their access to the organism by forming biofilms and inhibiting membrane fusion (Rossi & De Phillippis 2015, Tiwari et al. 2019). However, with an increase in the concentration of 2,4-D (30 and 40 ppm), the synthesis of EPS was affected. It was found to be more severe at 40 ppm, where the total EPS content was cut by 72.7%, with reductions of 88.2% and 31.5% for RPS and CPS, respectively, in comparison to control (Fig. 2a). Its reduction might be because herbicide-membrane contact causes the outer protective polysaccharide layer to be destroyed, which results in a decrease in EPS content (Tiwari & Prasad 2022). Following this, it was found that the rate of production of exopolysaccharide showed a similar trend in which maximum production of EPS was found at 20 ppm. This can be attributed to the fact that the herbicide at this concentration may have been perceived as a toxic threat that, in turn, resulted in the induction of EPS synthesis in the cyanobacterium. This is an example of a hormetic response by the organism when lower concentrations of a toxic substance like 2,4-D were present in its surroundings. Ahemad and Khan (2010) have already explained this phenomenon that excess EPS generation is most likely to provide the microorganisms with additional protection while they exist in stressful environments. The reduction in the rate of EPS production at higher doses of exposure seen in the study reiterated that the metabolic activities leading to the production of EPS were severely compromised during chronic exposure to the herbicide.

The compositions of EPS, such as neutral sugars, uronic acids, and proteins (glycoproteins) produced by the organism, were also affected in a similar fashion under herbicide stress. The presence of uronic acids in EPS as one of its components is significant as the negatively charged uronic acids give EPS its anionic property. Due to this anionic nature, it gives the whole macromolecule a sticky texture. Additionally, it gives EPS its ability to bind positively charged ions. This distinguishing property is advantageous in bioremediation (Bender et al. 1994, Pereira et al. 2011). Similar conclusions were reported by Redmile-Gordon et al. (2014).

An upsurge was seen in the neutral sugar concentration up to an exposure to 30 ppm 2,4-D and above, which it declined (Fig. 3b). Costa et al. (2018) and Liu et al. (2019) pointed out that neutral sugars play an essential role in the formation of microbial aggregates. Further, neutral sugars represent intracellular carbon flux that is critical for the generation of cell energy and other molecular composites required for microbial growth. As a result, changes in this carbohydrate storage may have an impact on a variety of cell activities, including carbon fixation (Mesquita et al. 2021).

It has been pointed out that the presence of soluble proteins in the exopolysaccharide might be because of the trapped exoenzymes in the EPS matrix (Frølund et al. 1996, Kaplan Can et al. 2019). Several researchers have also reported the presence of tightly-linked peptide-sugar moieties in cyanobacterial exopolysaccharides (Panoff et al. 1988, Tiwari et al. 2020). Its presence provides varying degrees of hydrophobicity, which significantly contributes to the emulsifying property of the EPS (Alvarez et al. 2021). Protein content present in EPS helps to bind to surfaces where EPS and protein adhesins are involved in the primary steps of microbial adhesions to surfaces (Costa et al. 2018). The changes in EPS composition under 2,4-D stress may have been due to alterations in the protein content observed in the present study. This explanation has also been put forward by Donot et al. in 2012.

The increase in the exopolysaccharide's bio-flocculating activity in lower concentrations of 2,4-D (39.5% ↑ in 10 ppm) indicated that the increase in uronic acid content positively aided the flocculation mechanism (Khangembam et al. 2016).

The compromised level in all the parameters studied under higher doses of 2,4-D exposure was reflected in the biomass content of the organism. This finding was similar to the reports of Galhano et al. (2009) and Shen et al. (2009), who showed that excessive doses of herbicide could result in lessened biomass content of cyanobacteria. The inference of the present study is that the reduced EPS content registered in the study could be because of (1) compromised synthesis of EPS itself and/or (2) due to a reduction in biomass production that indirectly affected EPS production. The entire morphology of the cells was affected by the presence of 2,4-D was visible under SEM. Further, with the progressive increase in the concentration of 2,4-D, these effects were seen to be concurrently amplified, indicating that the higher doses of 2,4-D had severe consequences on the cells themselves, thus compromising their ability to mount any defense against such exposures. The schematic representation of the effect of herbicide on *Nostoc muscorum* Meg 1 is shown in Fig. 6

CONCLUSION

The outcome of this study undoubtedly established the toxic nature of unstandardized doses of 2,4-D. That 2,4-D at concentrations greater than 20 ppm can impair the production of various EPS components such as neutral sugars, uronic acids, and proteins, thereby severely restricting viable EPS synthesis was also established. The toxicity of 2,4-D was

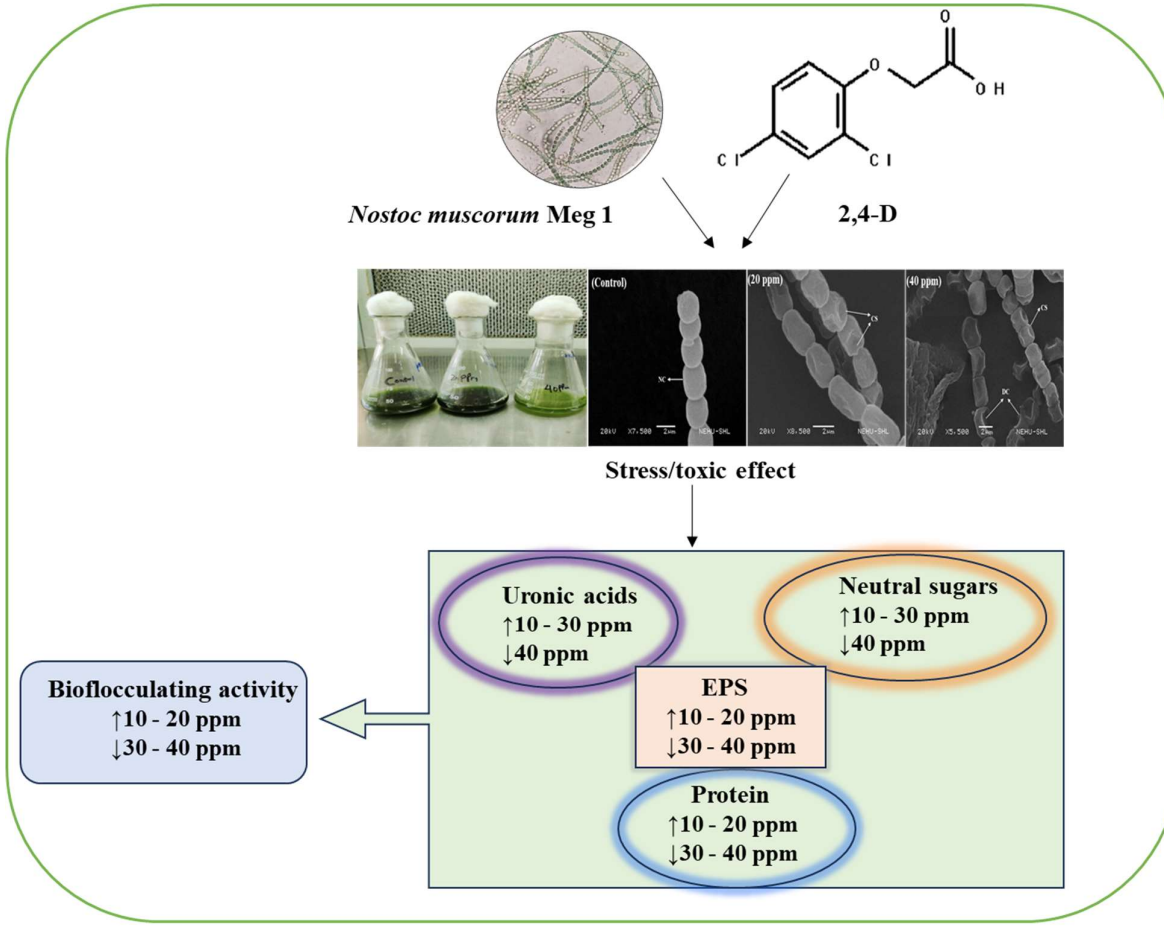

Fig. 6: Schematic representation of the effect of herbicide on *Nostoc muscorum* Meg 1.

such that at higher concentrations, it could penetrate the EPS barrier and affect the cellular metabolism, leading to reduced biomass production. However, looking further in the better part of this study, we found that when 2,4-D is present at low concentrations, the EPS production is stimulated, and the organism could mitigate the threat of 2,4-D at the EPS level itself without exposing the cells to this harmful herbicide.

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REFERENCES

Abed, R.M., Dobretsov, S. and Sudesh, K. 2009. Applications of cyanobacteria in biotechnology. J. Appl. Microbiol., 106(1): 1-12.

Ahad, R.I.A., Goswami, S. and Syiem, M. B. 2017. Biosorption and equilibrium isotherms study of cadmium removal by Nostoc muscorum Meg 1: morphological, physiological and biochemical alterations. 3 Biotech., 7: 1-12.

Ahemad, M. and Khan, M.S. 2010. Growth promotion and protection of lentils (*Lens esculenta*) against herbicide stress by *Rhizobium* species. Ann. Microbiol., 60: 735-745.

Ahemad, M. and Khan, M.S. 2012. Evaluation of plant-growth-promoting activities of rhizobacterium *Pseudomonas putida* under herbicide stress. Ann. Microbiol., 62(4): 1531-1540.

Ali, H., Khan, E. and Ilahi, I. 2019. Environmental chemistry and ecotoxicology of hazardous heavy metals: environmental persistence, toxicity, and bioaccumulation. J. Chem., 2: 1-14.

Alvarez, X., Alves, A., Ribeiro, M.P., Lazzari, M., Coutinho, P. and Otero, A. 2021. Biochemical characterization of *Nostoc* sp. exopolysaccharides and evaluation of potential use in wound healing. Carbohydr. Polym., 254: 117303.

Bala, S., Garg, D., Thirumalesh, B.V., Sharma, M., Sridhar, K., Inbaraj, B.S. and Tripathi, M. 2022. Recent strategies for bioremediation of emerging pollutants: A review for a green and sustainable environment. Toxics, 10(8): 484.

Bellinaso, M.D.L., Greer, C.W., Peralba, M.D.C., Henriques, J.A.P. and Gaylarde, C.C. 2003. Biodegradation of the herbicide trifluralin by bacteria isolated from soil. FEMS Microbiol. Ecol., 43(2): 191-194.

Bender, J., Rodriguez-Eaton, S., Ekanemesang, U.M. and Phillips, P. 1994.

Characterization of metal-binding bioflocculants produced by the cyanobacterial component of mixed microbial mats. Appl. Environ. Microbiol., 60(7): 2311-2315.

Blumenkrantz, N. and Asboe-Hansen, G. 1973. A new method for the quantitative determination of uronic acids. Anal Biochem., 54(2): 484-489.

Bradford, M.M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem., 72: 248-254.

Cérantola, S., Bounéry, J.D., Segonds, C., Marty, N. and Montrozier, H. 2000. Exopolysaccharide production by mucoid and non-mucoid strains of *Burkholderia cepacia*. FEMS Microbiol Lett., 185(2): 243-246.

Chi, Z., Su, C.D. and Lu, W.D. 2007. A new exopolysaccharide produced by marine *Cyanothece* sp. 113. Bioresour. Technol., 98(6): 1329-1332.

Costa, O.Y., Raaijmakers, J.M. and Kuramae, E.E. 2018. Microbial extracellular polymeric substances: ecological function and impact on soil aggregation. Front Microbiol., 9: 1636.

De Philippis, R. and Vincenzini, M. 1998. Exocellular polysaccharides from cyanobacteria and their possible applications. FEMS Microbiol. Rev., 22(3): 151-175.

Decho, A.W. 1990. Microbial exopolymer secretions in ocean environments: Their role(s) in food webs and marine processes. Oceanogr. Mar. Biol., 28: 73-153.

Donot, F., Fontana, A., Baccou, J. C. and Schorr-Galindo, S. 2012. Microbial exopolysaccharides: main examples of synthesis, excretion, genetics, and extraction. Carbohydr. Polym., 87(2): 951-962.

DuBois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.T. and Smith, F. 1956. Colorimetric method for determination of sugars and related substances. Anal. Chem., 28(3): 350-356.

Frigaard, N.U. 2018. Sugar and Sugar Alcohol Production in Genetically Modified Cyanobacteria. In Genetically Engineered Foods. Academic Press, Cambridge, MA, pp. 31-47.

Frølund, B., Palmgren, R., Keiding, K. and Nielsen, P.H. 1996. Extraction of extracellular polymers from activated sludge using a cation exchange resin. Water Res., 30(8): 1749-1758.

Fuller, M.E. and Scow, K.M. 1997. Lack of capsular exopolymer effects on the biodegradation of organic compounds by *Pseudomonas* sp. strains JS1 and JS150. Microb. Ecol., 34: 248-253.

Galhano, V., Peixoto, F., Gomes-Laranjo, J. and Fernandez-Valiente, E. 2009. Differential effects of bentazon and molinate on *Anabaena cylindrica*, an autochthonous cyanobacterium of Portuguese rice field agro-ecosystems. Water Air Soil Pollut., 197: 211-222.

Gouveia, D., Almunia, C., Cogne, Y., Pible, O., Degli-Esposti, D., Salvador, A. and Armengaud, J. 2019. Ecotoxicoproteomics: A decade of progress in our understanding of anthropogenic impact on the environment. J. Proteomics, 198: 66-77.

Kalaji, H.M., Jajoo, A., Oukarroum, A., Brestic, M., Zivcak, M., Samborska, I.A. and Ladle, R.J. 2016. Chlorophyll fluorescence is a tool to monitor the physiological status of plants under abiotic stress conditions. Acta Physiol. Plant, 38: 1-11.

Kaplan Can, H., Gurbuz, F. and Odabaşı, M. 2019. Partial characterization of cyanobacterial extracellular polymeric substances for aquatic ecosystems. Aquat. Ecol., 53: 431- 440.

Khangembam, R., Tiwari, O.N. and Kalita, M.C. 2016. Production of exopolysaccharides by the cyanobacterium *Anabaena* sp. BTA992 and application as bioflocculants. J. Appl. Biol. Biotechnol., 4(1): 008-011.

Kurane, R., Hatamochi, K., Kakuno, T., Kiyohara, M., Kawaguchi, K., Mizuno, Y. and Taniguchi, Y. 1994. Purification and characterization of lipid bioflocculant produced by *Rhodococcus erythropolis*. Biosci. Biotechnol. Biochem., 58(11): 1977-1982.

Liu, C., Jia, J., Ma, T. and Feng, X. 2019. Origin and distribution of neutral sugars in soils. Chin. J. Plant Ecol., 43(4): 284-295.

Mackinney, G. 1941. Absorption of light by chlorophyll solutions. J. Biol. Chem., 140(2): 315-322.

Mesquita, A.F., Gonçalves, F.J., Rocha, C.P., Marques, J.C. and Gonçalves, A.M. 2021. Biochemical effects of two pesticides in three different temperature scenarios on the diatom *Thalassiosira weissflogii*. Processes, 1247 :(7)9.

Mishra, P. 2020. Cyanobacterial Exopolysaccharide as Natural Sources for Food Packaging Applications. Springer, Singapore, pp. 171-184

Ozturk, S., Aslim, B. and Suludere, Z. 2010. Cadmium (II) sequestration characteristics by two isolates of *Synechocystis* sp. in terms of exopolysaccharide (EPS) production and monomer composition. Bioresour. Technol., 101(24): 9742-9748.

Panoff, J. M., Priem, B., Morvan, H. and Joset, F. 1988. Sulphated exopolysaccharides are produced by two unicellular strains of cyanobacteria, *Synechocystis* PCC 6803 and 6714. Arch. Microbiol., 150: 558-563.

Parwani, L., Bhatt, M. and Singh, J. 2021. Potential biotechnological applications of cyanobacterial exopolysaccharides. Braz. Arch. Biol. Technol., 64: e21200401.

Pavlidis, G. and Tsihrintzis, V.A. 2018. Environmental benefits and control of pollution to surface water and groundwater by agroforestry systems: a review. Water Resour. Manag., 32: 1-29.

Pereira, S., Micheletti, E., Zille, A., Santos, A., Moradas-Ferreira, P., Tamagnini, P. and De Philippis, R. 2011. Using extracellular polymeric substances (EPS)-producing cyanobacteria for the bioremediation of heavy metals: Do cations compete for the EPS functional groups and also accumulate inside the cell? Microbiology, 157(2): 451-458.

Pérez-Lucas, G., Vela, N., El Aatik, A. and Navarro, S. 2019. Environmental Risk of Groundwater Pollution by Pesticide Leaching Through the Soil Profile: Pesticides-Use and Misuse and Their Impact in the Environment. IntechOpen Publishers, NY, pp. 1-28.

Plude, J.L., Parker, D.L., Schommer, O.J., Timmerman, R.J., Hagstrom, S.A., Joers, J.M. and Hnasko, R. 1991. Chemical characterization of polysaccharide from the slime layer of the cyanobacterium *Microcystis flos-aquae* C3-40. Appl. Environ. Microbiol., 57(6): 1696-1700.

Redmile-Gordon, M.A., Brookes, P.C., Evershed, R.P., Goulding, K.W.T. and Hirsch, P.R. 2014. Measuring the soil-microbial interface: extraction of extracellular polymeric substances (EPS) from soil biofilms. Soil Biol. Biochem., 72: 163-171.

Rippka, R., Deruelles, J., Waterbury, J.B., Herdman, M. and Stanier, R.Y. 1979. Generic assignments, strain histories, and properties of pure cultures of cyanobacteria. Microbiology, 111(1): 1-61.

Roe, J.H. 1955. The determination of sugar in the blood and spinal fluid with anthrone reagent. J. Biol. Chem., 212: 335-343.

Rossi, F. and De Philippis, R. 2015. Role of cyanobacterial exopolysaccharides in phototrophic biofilms and complex microbial mats. Life, 5(2): 1218-1238.

Rossi, F. and De Philippis, R. 2016. Exocellular Polysaccharides in Microalgae and Cyanobacteria: Chemical Features, Role, and Enzymes and Genes Involved in Their Biosynthesis: The Physiology of Microalgae. Springer, Singapore, pp. 565-590.

Sachu, M., Kynshi, B.L., and Syiem, M.B. 2021. Herbicide monuron mediated alterations in carbon and nitrogen fixation in the cyanobacterium *Nostoc muscorum* Meg 1. J. Appl. Phycol., 33(4): 2209-2220.

Shahid, M. and Khan, M.S. 2022. Ecotoxicological implications of residual pesticides to beneficial soil bacteria: A review. Pestic. Biochem. Physiol., 188: 105272.

Shen, J., DiTommaso, A., Shen, M., Lu, W. and Li, Z. 2009. Molecular basis for differential metabolic responses to monosulfuron in three nitrogen-fixing cyanobacteria. Weed Sci., 57(2): 133-141.

Singh, S., Kant, C., Yadav, R.K., Reddy, Y.P. and Abraham, G. 2019. Cyanobacterial Exopolysaccharides: Composition, Biosynthesis, and Biotechnological Applications. Academic Press, Cambridge, MA, pp. 347-358.

Sultan, M., Waheed, S., Ali, U., Sweetman, A.J., Jones, K.C. and Malik, R.N. 2019. Insight into the occurrence, profile and spatial distribution

of organochlorine pesticides in soils of solid waste dumping sites of Pakistan: Influence of soil properties and implications for environmental fate. Ecotoxicol. Environ. Saf., 170: 195-204.

Székács, A. 2021. Herbicide Mode of Action. Elsevier, The Netherlands, pp. 41-86.

Tiwari, O.N., Muthuraj, M., Bhunia, B., Bandyopadhyay, T.K., Annapurna, K., Sahu, M. and Indrama, T. 2020. Biosynthesis, purification, and structure-property relationships of new cyanobacterial exopolysaccharides. Polym. Test, 89: 106592.

Tiwari, S. and Prasad, S.M. 2022. Exogenous application of phytohormones modulates pesticide-induced injury in two rice field cyanobacteria: Toxicity alleviation by up-regulation of the ascorbate-glutathione cycle. Plant Stress, 3: 100046.

Tiwari, S., Parihar, P., Patel, A., Singh, R. and Prasad, S.M. 2019. Metals in Cyanobacteria: Physiological and Molecular Regulation. Academic Press, Cambridge, MA, pp. 261-276.

Zahra, Z., Choo, D.H., Lee, H. and Parveen, A. 2020. Cyanobacteria: Review of current potentials and applications. Environments, 7(2): 13.

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