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Auxin (IAA) up regulates growth, photosynthetic pigment and antioxidant machinery in paddy field cyanobacteria *N.muscorum*

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Abstract:

The present study was conducted to assess the toxicity instigated by Chromium (Cr) stress and alleviating effect of IAA against Cr on biomass, status of photosynthetic pigments and PS II photochemistry of paddy field cyanobacterium. The Cr significantly declined growth and growth regulating processes and apparent increase in oxidative stress biomarkers and antioxidative enzymes. However, upon IAA implication an assuaging effect against Cr toxicity was noticed on growth, pigment content and photosynthesis. The overall study demonstrates that IAA implication significantly improved Cr induced toxicity by improving growth and important metabolic mechanisms like photosynthesis and enzymatic activity.

Introduction

Cyanobacteria are principal photosynthetic prokaryotes that are liable for the release of oxygen in the environment also being the integral part of ecosystem they act as primary producers and pluralistic in terrestrial as well as aquatic environment and contribute to more than half of the total primary producer on earth [1]. Cyanobacteria are dominant in wetlands particularly in paddy fields [2]. To one side they plays protagonist role as bio-fertilizer, also they are heady source of biochemical compound such as carbohydrates, lipids, vitamins, amino acids, phenolic, sugars as well as plant growth regulators that directly or indirectly enhance the crop yield [3]. Cyanobacteria also enhance the physio-chemical properties of soil and also increased the water holding capacity of soil [4]. They are natural bio-fertilizer that fulfills the nitrogen requirement of soil where nitrogen-containing chemical fertilizers are not used. In Asian countries, crop fields particularly rice fields are provided with the flooded condition prior to seedling transplantation that offers the natural environment for the growth of cyanobacteria (blue-green algae). But these crop fields are mainly irrigated with the contaminated water bodies (canal and river system) due to discharge of industrial waste directly in to water bodies, and increased industrialization and urbanization has also augmented this.

Industrial run off fundamentally comprise of heavy and toxic metals such as As, Cr, Pb, Ni, Co, etc., among them Cr is the toxic metal and considered as a human carcinogen [5]. In atmosphere Cr exist as Cr^{VI} chromate oxyanions (CrO_4^{-2} and $Cr_2O_7^{-2}$) and Cr^{III} in form of oxides [6].

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Hexavalent chromate (Cr^{VI}) is most toxic due to its solubility and mobility across the cell membranes which exerts sturdy destructive effects on biological systems [7] and also acts as strong mutagens and oxidizing agent [8]. In drinking water the maximum permissible limit for Cr^{VI} is 50 µg L^{-1} [9]. The major Cr contributing states are Andhra Pradesh, Chhattisgarh, Gujarat, Odisha, Uttar Pradesh and West Bengal. In Uttarpradesh Kanpur is the major Cr contributing i.e. around 1500 metric tons of chromium sulphate as waste [10] directly into open lands and rivers [11]. This contaminated water is used to irrigate the agricultural fields thus affecting the growth of cyanobacteria inhabiting the crop field and also productivity of plants. This alleviate level of Cr significantly affects the physiological and biochemical process of cyanobacteria such as photosynthetic pigments photosynthesis photosystem II electron transport [5], and chlorophyll *a* fluorescence parameters [12, 13]. Moreover, reducing site of PS II and water splitting complex is Cr inhibitory site [14]. The Cr^{VI} being reactive oxidant behaves as strong oxidizing agent thereby leads to generation of oxidants like $O_2^{\bullet -}$ and H_2O_2 [15]. The $O_2^{\bullet -}$ needs to be rapidly dismutate, either enzymatically or non-enzymatically, to yield H_2O_2 and O_2. Additionally, H_2O_2 interacts with metal ion to produce highly reactive $^{\bullet}OH$ [16]. The ROS disturb the metabolic processes leading to oxidative stress and damage membrane macromolecule and DNA, inhibition of photosynthesis [4]. To combat this stressful condition plants have antioxidant machinery involving enzymatic and nonenzymatic antioxidant, enzymatic antioxidant involve SOD, POD, CAT, GST through which plants are able to survive under stressful environment. Plants and microbes endure the metal contaminated areas by stowing growth stimulating substances like phytohormones that operates signalling responses and mediate the growth and development [17] even at low concentrations.

Thus sustaining the micro-flora of marine ecosystem or management of aquatic vegetation by the solicitation of natural or synthetic plant growth regulators will be a new step in understanding the role of plant hormones in cyanobacteria. The phytohormones facilitate various morphological, physiological, and developmental processes and regulate the growth and development. Thus the present work has been taken into consideration to evaluate the ameliorating role of auxin (Indole acetic acid; IAA) on growth and growth-substantiating attributes of *Nostoc* under Cr exposition.

2. Materials and methods

2.1. Growing condition and treatments designing

Cultures of *Nostoc* maintained in growth (BG-11) medium at temperature 25 ± 2 °C under the 75 µmol photons m−2 s−1 light by white fluorescent tubes under light: dark regimes of 15:9 h in well maintained culture room. With cultures in their exponential phase all the experiments were performed. The doses selected for the current study includes 100 and 150 µM of Cr^{VI} (heavy metal stress treatment that corresponds to LC_{10} and LC_{30}) and 300 nm of IAA (hormone treatments).

2.2. Biomass accretion

Measured in terms of dry weight.

2.3. Quantification of photosynthetic pigments

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Chlorophyll a (Chl *a*), carotenoids (Car) and phycocyanin (Phy) contents were estimated using the methods given by Porra et al. [18], Goodwin [19] and Blumwald and Tel-Or [20], respectively.

2.4. Photosynthesis and respiration rate

The photosynthesis and respiration rate was measured by using Clark type oxygen electrode.

2.5. Measurement of PS II photochemistry

The PS II photochemistry was measured in terms of chlorophyll a fluorescence and was determined in dark-adapted cultures by using AquaPen AP 100, fluorometer [21]. The fluorescence parameters involves kinetic and energy flux parameter.

2.6. Oxidative stress biomarkers and lipid peroxidation

Superoxide radicals (SOR; $O_2^{\bullet-}$), Hydrogen peroxide (H_2O_2) and Lipid peroxidation in terms of MDA equivalents content was determined by ensuing the method of Elstner and Heupel [22], Velikova et al. [23] and Heath and Packer [24], respectively.

2.7. Estimation of enzymatic antioxidant activity

Enzymatic antioxidant superoxide dismutase, peroxidase, catalase, glutathione-*S*-transferase activity was assessed by following the method of Giannopolitis and Reis [25, Gahagan et al. [26], Aebi [27] and Habig et al. [28], respectively.

2.8. Statistical analysis

Results were statistically analyzed using Duncan multiple range test (DMRT) in ANOVA (analysis of variance).

3. Experimental outcomes

3.1. Biomass accretion

The biomass accretion was measured in terms of dry weight that found to decline in dose dependent manner under Cr stress i.e. by 10 and 30% (Figure. 1). However upon exogenous supplementation of IAA showed significant ameliorative effect i.e. reduces the reduction in growth by 3 and 19% with the tested dose of Cr as compared to control. Furthermore, in Cr untreated sample, IAA significantly enhanced the biomass by 9% over control values.

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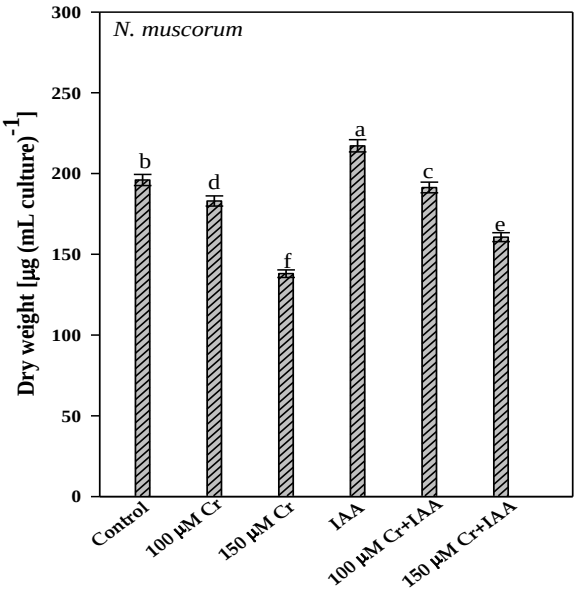

Figure 1. Effect of IAA on growth of *N. muscorum* after 96 h of experiments. Data are means ± standard error of three replicates (n=3). Bars followed by different letters show significant difference at $P < 0.05$ significance level according to Duncan multiple range test (DMRT).

3.2. Photosynthetic pigments

The Cr treated cells of *N. muscorum* showed a substantial reduction in photosynthetic pigments i.e. (Chl *a*, Car and Phy) as depicted in Figure 2. The Chl *a* content was declined by 12 and 34% on exposure to tested doses of Cr over the respective control. Parallel to the growth result supplementation of IAA produced assuaging effect i.e. along with Cr it showed reduction of only 6 and 25% however without Cr it stimulated the Chl *a* content by 10%. Similar declining trend was also noticed for Car and Phy, but values clearly points that Phy content was found to majorly affected compared to Chl *a* and Car respectively.

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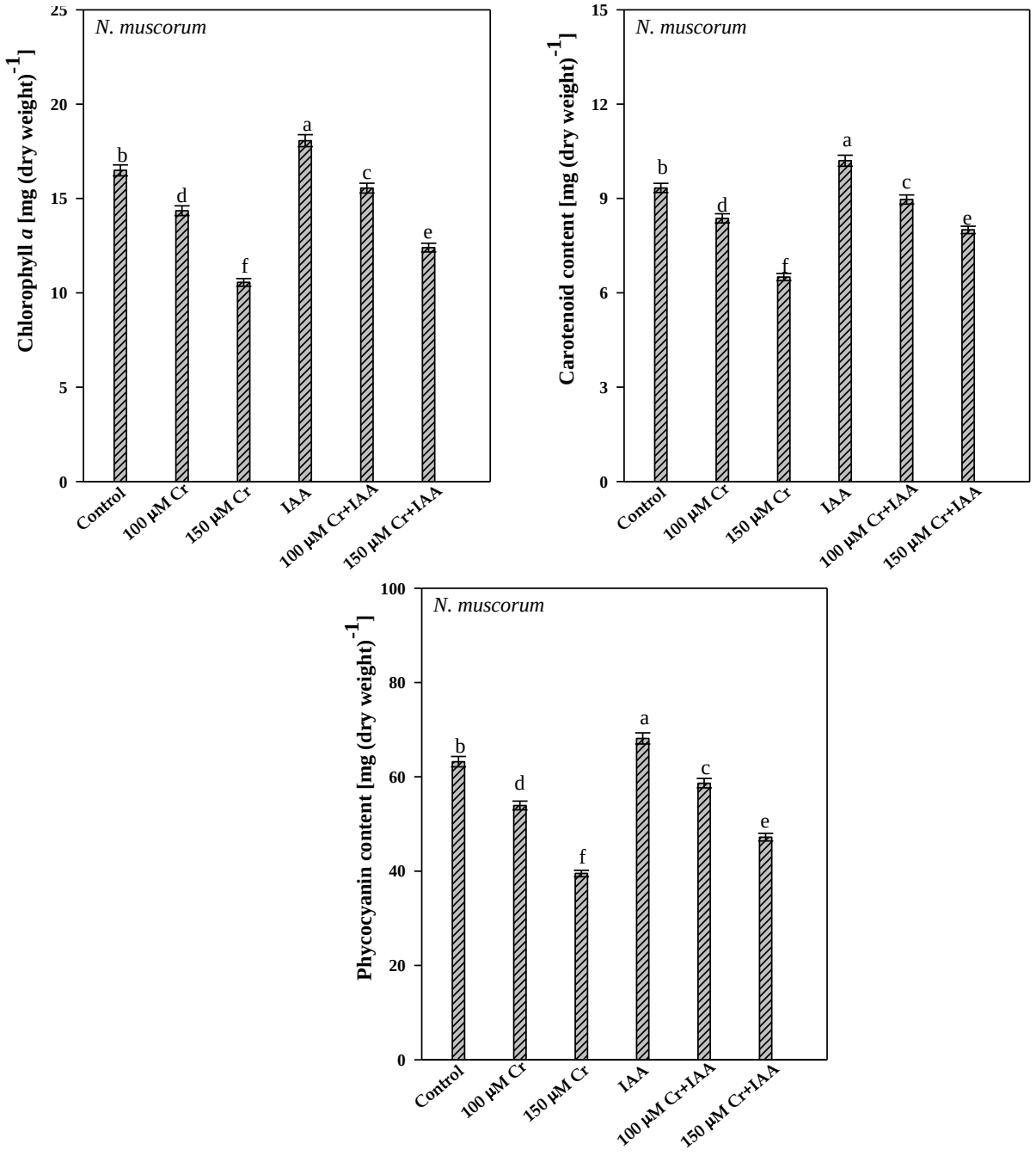

Figure 2. Effect of IAA on photosynthetic pigments of *N. muscorum* after 96 h of experiments. Data are means ± standard error of three replicates (n=3). Bars followed by different letters show significant difference at $P < 0.05$ significance level according to Duncan multiple range test (DMRT).

3.3. Photosynthesis and Respiration rate

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Under Cr stress the cyanobacterial cell exhibited a substantial damage to photosynthetic activity (Figure. 3). Photosynthetic rate dropped by of 12% and 33%, respectively. However IAA application reduces the Cr induced toxicity as it showed only 4 and 19% reduction with tested doses of Cr, moreover IAA without Cr treatment showed 6% enhancement in photosynthetic activity. Contrary to photosynthesis under similar condition, the respiration rate was found to be increased with Cr treatment. However, IAA supplementation declined the enhancement in respiratory rate in Cr treated cells.

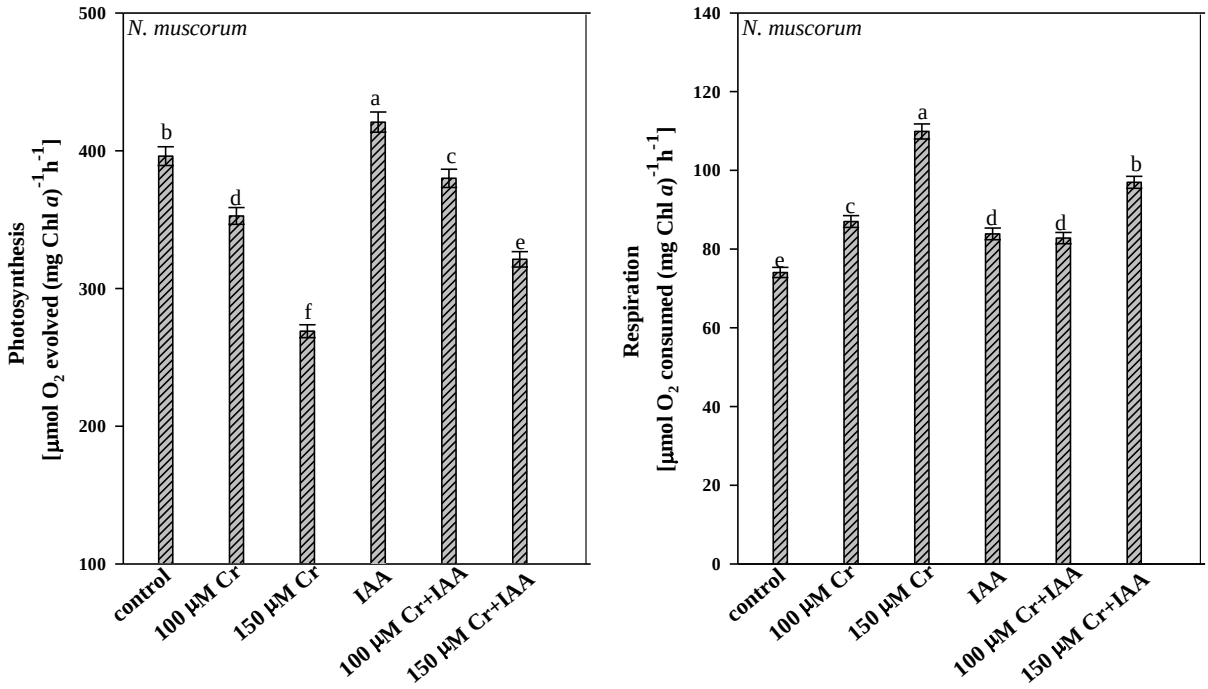

Figure 3. Effect of IAA on photosynthesis and respiration rate of *N. muscorum* after 96 h of experiments. Data are means ± standard error of three replicates (n=3). Bars followed by different letters show significant difference at $P < 0.05$ significance level according to Duncan multiple range test (DMRT).

3.4. PS II photochemistry

The PS II photochemistry was measured by analyzing kinetic as well as energy flux parameter. The tested doses of Cr significantly lowered all the tested the values of kinetics parameter viz., Fv/Fm or ϕP_0, ϕE_0, $\Psi 0$), PI_{ABS} of PS II, F_v/F_0 except F_0/F_v, Contrary to this, the energy fluxes parametres (ABS/RC, TR_0/RC, ET_0/RC and DI_0/RC) were increased under Cr stress. The exogenous supplementation of IAA improved the performance of PS II (Figure 4).

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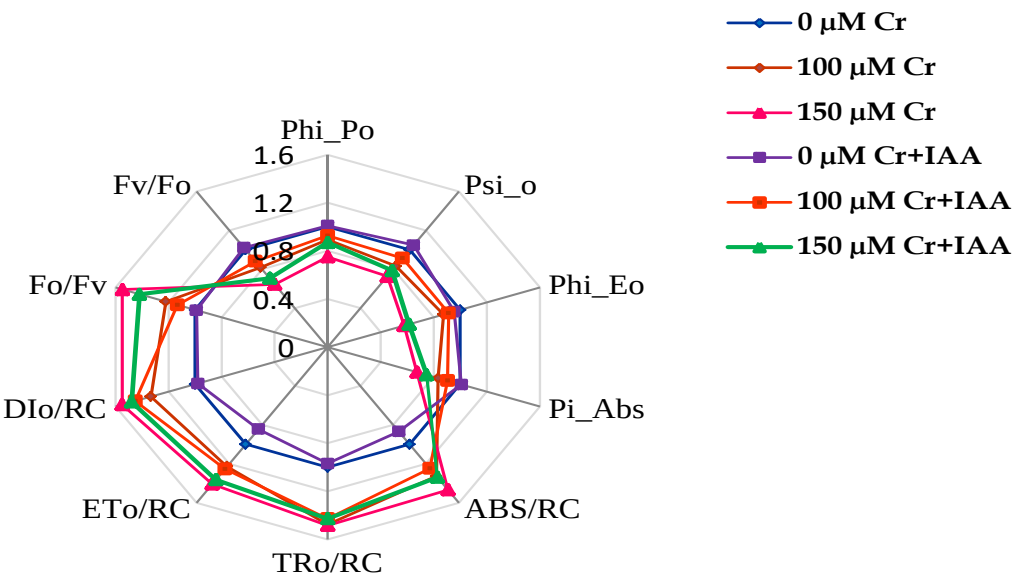

Figure 4. Effect of IAA on PS II photochemistry (fluorescence) of *N. muscorum* after 96 h of experiments. Data are means ± standard error of three replicates (n=3). Bars followed by different letters show significant difference at $P < 0.05$ significance level according to Duncan multiple range test (DMRT).

3.5. Oxidative stress biomarkers

The oxidative stress biomarkers: $O_2^{\bullet -}$, H_2O_2 and MDA equivalents content are portrayed in Figure 5. The SOR content was raised by 50% and 140% on exposition to Cr stress and upon IAA supplementation SOR content exhibited a declining trend as it showed only 65% and 112%, enhancement respectively over the value of control. Similar trend was also noticed for H_2O_2. The values for MDA equivalents induced lipid peroxidation was also found to enhance under the tested doses of Cr i.e. by 85 and 172% respectively, however implication of IAA significantly restores the values as with tested doses of Cr the values recorded were less than those recorded under Cr treatments.

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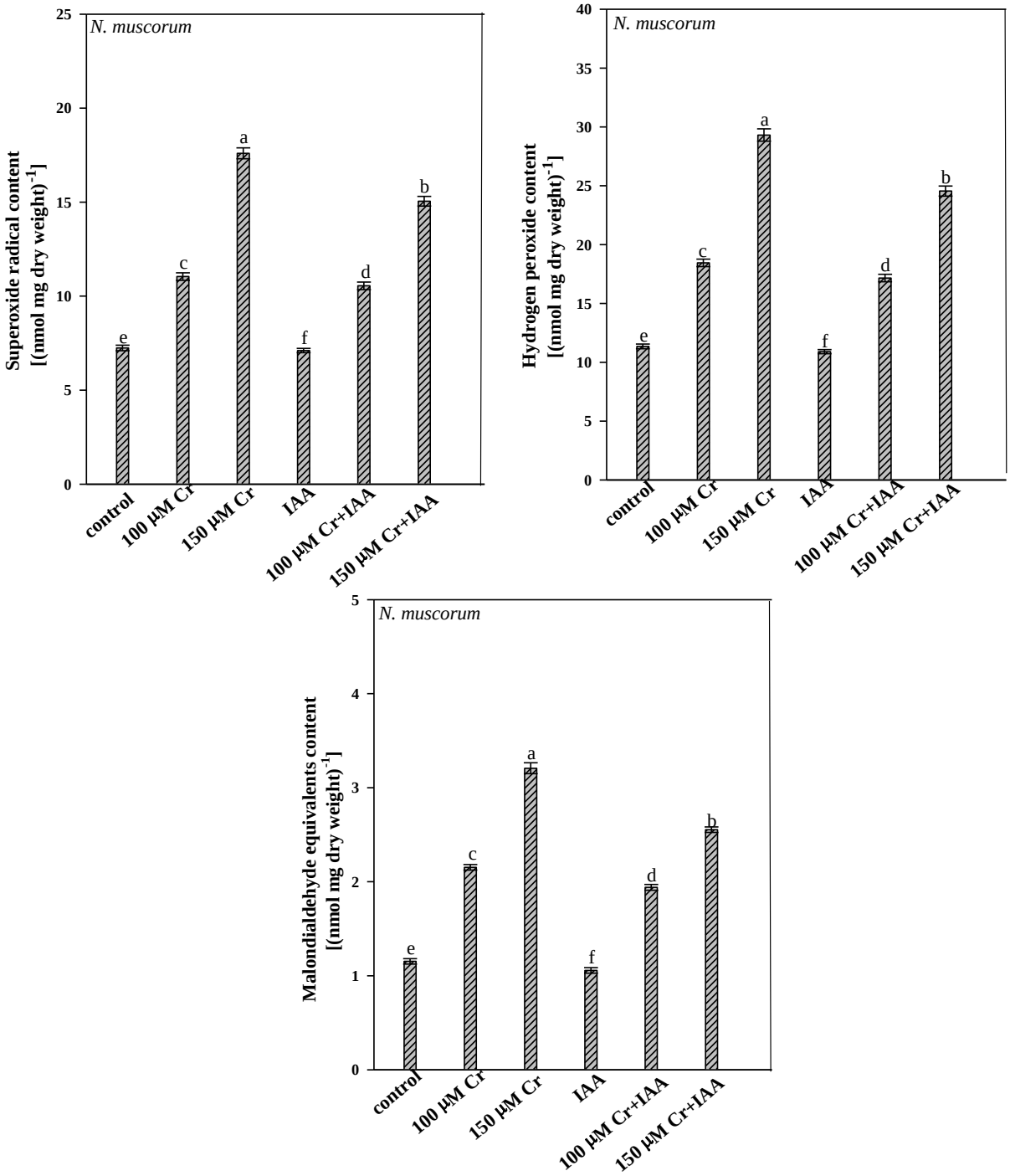

Figure 5. Effect of IAA on oxidative stress biomarkers (SOR, H_2O_2 and MDA equivalents content) of *N. muscorum* after 96 h of experiments. Data are means ± standard error of three replicates (n=3). Bars followed by different letters show significant difference at $P < 0.05$ significance level according to Duncan multiple range test (DMRT).

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3.6. Enzymatic antioxidant activity

The results for activity of enzymatic antioxidant are presented in Figure. 6. Under stress condition i.e. both the tested doses of Cr the SOD activity was enhanced by 43 and 120% over the control values. However, upon following IAA implication further the activity was raised by 56% and 145%. Similar results were noticed for CAT, POD and GST activities.

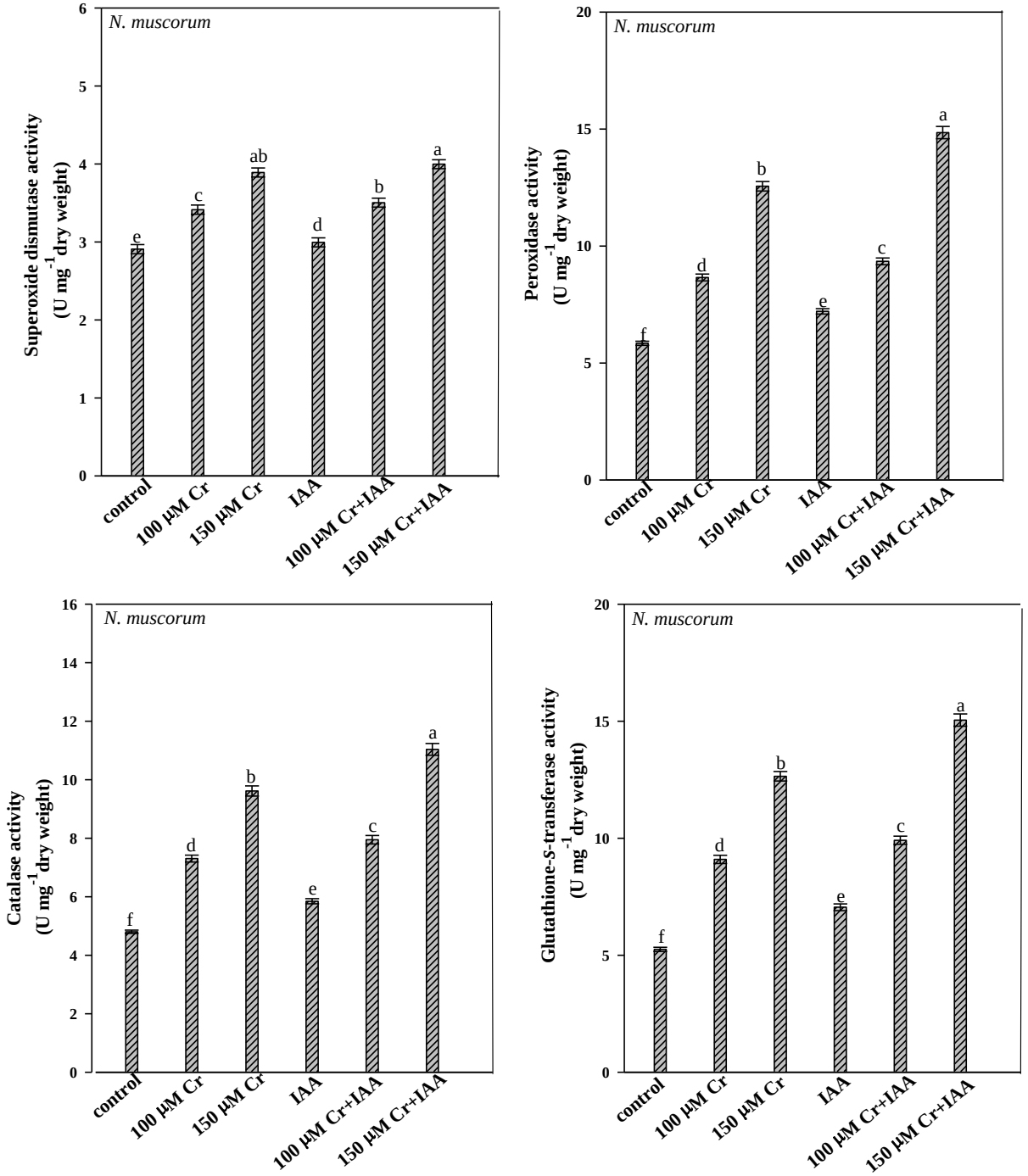

Figure 6. Effect of IAA on enzymatic antioxidants (SOD, POD, CAT and GST) of *N. muscorum* after 96 h of experiments. Data are means ± standard error of three replicates (n=3).

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Bars followed by different letters show significant difference at $P < 0.05$ significance level according to Duncan multiple range test (DMRT).

4. Discussion

The study carried out to scrutinize the sway of exogenously supplied IAA in the paddy field cyanobacterium *N. muscorum* under Cr exposition. The Cr treatment significantly declined the growth performance of *N. muscorum* (Figure 1) which is mainly correlated with the decrease in status of photosynthetic pigment (Figure 2) and photosynthesis and respiration and PS II photochemistry (Figure. 3 and 4) that might be due to intracellular accumulation of Cr. Further, it is apparent from our findings that implication of IAA alleviated Cr toxicity by stimulating growth (Figure. 1) which is associated with reduced Cr uptake. Cr has ambiguous imp act on pigments content (Chl a, Car and Phy), that led to further decline in photosynthesis and ultimately growth. The increased ROS, lipid peroxidation and protein oxidation is might be a reason for reduction in pigment and photosynthesis. Our results are consistent with earlier findings of di-Toppi et al [29], where Cr has been reported to affect the chlorophyll biosynthesis. Phycocyanin content is majorly affected under Cr or heavy metal stress because of its exterior localization on thylakoid membrane [30]. Apart from light harvesting pigment the accessory pigment i.e. carotenoid content also showed greater reduction under Cr toxicity, furthermore decrease in biomass accretion and pigment status under Cr exposition is also because the Cr interacts with the enzymes involved in cell division and interact with the cellular process. The photosynthesis rate was found to be inhibited under Cr stress in dose dependent manner due to hindrance of Cr on oxygen evolving complex of PS II and inhibits the electron flow and our results are in agreement with Tiwari et al., [5] that have suggested decline in performance of PS II due to interrupted electron flow.

The IAA implication produced an ameliorative effect on growth and growth regulating parameters i.e. significantly improve the status of the photosynthetic pigment and accessory pigment contents (figure 2) and thereby improved photosynthesis (Figure 3), and antioxidant defense system (Figure 6), Albeit the real mechanism of IAA in improving the growth is not clear but it has been proposed that the foremost role of IAA is to arbitrate the cell division as well as cell expansion that is related with the enhancement in growth [31]. Salama et al., [32] reported that IAA supplementation enhanced the fatty acid content in *Scendesmus* which is a prominent reason for better growth.

Supplementation of IAA caused appreciable improvement in growth and photosynthetic rate which could be correlated with the improved status of photosynthetic pigments Similarly, Singh and Prasad [33] have also reported that other phytohormone i.e. KN that caused improvement of photosynthetic apparatus structurally and functionally thereby a rise in the photosynthetic rate was witnessed in *Solanum melongena* exposed to Cr stress. The Cr doses with increasing concentrations significantly raised respiratory rate the reason is explained that to fulfill the demand of energy needed to combat the stress condition, similar result was reported by Patel et al. [30] in *N.muscorum* under As stress.

To locate the mechanism of Cr toxicity PS II photochemistry was analysed in terms of chlorophyll a fluorescence kinetics. The fallouts shows that photochemistry of PS II was adversely affected by exposition of Cr as apparent from kinetic parameters (Figure. 4) due to reduced electron flow [12]. Furthermore, increased value of F_0/F_V showed that PS II activity

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mainly affected at the site of oxygen evolving complex (OEC). Similar to our result, Singh et al. [34] also reported decline in activity of PS II and suggested the major reason to be the reduction in total number of active reaction centre. The Cr reduces the number of active reaction centre and increases load on the remaining centers thus results in the increase value of energy flux parameter i.e. ABS/RC, TR_0/RC, ET_0/RC and DI_0/RC. Moreover the overall performance of PS II photochemistry denoted by the values of PI_{ABS} was found to decrease under stress condition. Under similar condition the implication of IAA significantly alleviated the toxic effect of Cr by restoring the values of kinetics and energy flux parameter.

Additionally, generation of oxidants is a common point to stress condition, especially in aerobic organism the ROS are generated from the spilling out of electrons from transport chain to the oxygen evolving due to water splitting [16]. The Cr at both the tested doses substantially increases the SOR and H_2O_2 contents. SOR is more reactive than H_2O_2, but it is considered more toxic due to its permeability across the biological membrane and on reaction with Fe^{2+} and Cu^{2+} thus generates hydroxyl radicle which causes lipid peroxidation indicated by enhanced value for MDA equivalents content. Our results (figure 5) are in agreement with earlier findings [15, 35]. Upon supplementation of IAA cyanobacterial cells exhibited an appreciable decrease in oxidant contents that possibly be due to enhanced antioxidant system (Figure. 6). To combat the oxidative stress condition organism holds an array of antioxidant machinery to maintain the basal metabolism of oxidants and maintaining a balance between ROS and antioxidant [4]. Under tested doses of Cr a significant rise in the activity of tested antioxidants was noticed. SOD is a first barrier against oxidative stress and it dismutates SOR into H_2O_2 with then further breakdown in to H_2O and O_2 by the action of CAT and POD, apart from this GST is important enzyme that contributes in detoxification of xenobiotics via glutathione. [16]. Similar findings were also reported where appreciable rise in activities of tested antioxidants was observed in *N.muscorum* and *Anabaena* under pretilachlor stress [36], As stress [30] and Cr stress [5]. The IAA supplementation, further accelerate the activity of enzymatic antioxidants. Similar to our findings Bashri et al., [37] reported that the IAA cause enhancement in the enzymatic activity of *Trigonella foenum- graceum*.

5. Conclusion

Our findings points towards the substantial role of auxin IAA in assuaging Cr toxicity in paddy field cyanobacterium *N. muscorum*. The reduced and negative effect is due to the Cr induced toxicity. Exogenous supplementation of auxin (IAA) strengthened the antioxidant capability of tested organism, thereby controlling the level of xenobiotic and oxidants that substantiate the improvement in growth and its related parameters such as pigments and photosynthesis.

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