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A COMPARATIVE STUDY OF INDOLE-3-ACETIC ACID (IAA)AND KINETIN (KN) IN ALLEVIATING THE ARSENIC-INDUCED TOXICITY IN RICE SEEDLINGS: OXIDATIVE STRESS VS ANTIOXIDATIVE DEFENCE SYSTEM

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ABSTRACT

Among variousenvironmental stresses, heavy metals stress especially the arsenic (As) has long been viewed as the synonymous of toxicity. In the present piece of work hydroponically grown *Oryza sativa* L. seedlings were exposed to 50 and 100 mM of as alone and in combination with indole-3-acetic acid (IAA) and kinetin (KN) phytohormones. As at both the doses cause significant reduction in fresh and dry mass of test seedlings, photosynthetic pigments content which was also manifested in the accumulation of as contents in respective tissues. Moreover, the oxidative stress markers viz. SOR and H_2O_2 and thereby damage to lipid in terms of MDA equivalents content and electrolyte leakage were also increased against both the doses of As. Contrary to this supplementation of IAA and KN considerably attenuates the As toxicity in rice seedlings which was reflected in the growth attributes,pigments system as well as reduced oxidative stress markers and indices of damages. Further, both the phytohormones also check the accumulation of As in rice seedlings and empowers the antioxidative defense system by augmenting the SOD, POD, CAT and GST enzymes activity along with the studied non-enzymatic antioxidants viz. NP-SH, cysteine and proline contents. After comparing the responses of both hormones' treatment, it was found that KN always dominant over the IAA, as it much neutralizes the deteriorating impact of As and supports the growth of rice seedlings under stress conditions too.
***Keywords* :** Arsenic, indole-3-acetic acid, Kinetin, *Oryza sativa* L., Oxidative stress

Introduction

The rapid modernization and technological advancements necessary to sustain the growth of modern society have resulted in increased environmental stress and degradation. These environmental stresses pose a significant threat to global agriculture, resulting in an alarming average loss of 70% of crop plants worldwide (Vaughan *et al.*, 2018; Waqas *et al.*, 2019). Among various environmental stresses, heavy metals stress especially the arsenic (As) has long been viewed as the synonymous of toxicity, not only for human beings but for other animals and plants too. Conferring to the World Health Organization (WHO), the minimum permissible safe level of As in drinking water is 10 µg L^{-1} whereas, in soil it should be 24 mg kg^{-1} as per the report of US Environmental Protection Agency. In India, arsenic contamination in ground water is very severe in West Bengal, and now it has reached the Gangetic plain regions of Bihar and Uttar Pradesh covering the district Ballia, Varanasi and Gazipur (Ahamed *et al.*, 2006; Kumar *et al.*, 2016). As loaded groundwater used for agricultural purpose is considered as the key cause of As infiltration into the food chain (Adomako *et al*., 2009). As^{V} is dominant and stable in aerobic environments while As^{III} dominates in O_2 deficient or reducing environments, as in groundwater. Recently, in a study performed by Dahlawi *et al*. (2018) show that rice can accumulate 20 to 22 times more As than other staple crops owing to the existence of aquaporins and phosphate transporters, as well as use of water (As contaminated) for irrigation practices of rice fields. Kamiya *et al*. (2013) in his study explained that how As^{III} get transported into the rice plants through aquaporins since, in flooded paddy fields provide a favorable anaerobic condition and owing to the As^{III} and silicic acid of chemical analogs they are readily taken up by the rice plant.

As severely intoxicates plants by reducing their biomass, plant height, wilting and necrosis on leaves decrease in leaf area, photosynthetic pigments and photosynthesis culminating into decrease in plants productivity and total death of the plant may occur (Mishra *et al*., 2016; Ahmad *et al*., 2020). There is significant experimental evidence that the exposure of plants to As does result in the over-production of active oxygen species (like$^1O_2$; $O_2^{\bullet -}$; and H_2O_2) which is concerned with the valance change from As^{V} to As^{III} (Talukdar, 2013). Higher concentration of ROS than the certain threshold level breaks photosynthetic pigments, organic components of membrane system and nucleic acids. Therefore, usual cellular metabolism is disturbed (Talukdar, 2013; Gill *et al*., 2015). The equilibrium between the rate of ROS formation and their quenching decides the successful survival of life thus quenching of ROS is performed by a pervasive antioxidant system, having several enzymatic antioxidants such as superoxide dismutase (SOD), guaicol peroxidase (GPX),catalase (CAT), glutathione-S- transferase (GST), etc. as well as non-enzymatic antioxidants like Np-SH, cysteine, proline etc. and thus the redox status of the cells is retained (Shakeri *et al*., 2019; Singh *et al*., 2020). A certain amount of ROS is always needed for the cell signalling process but when they are present in greater amount, they damage various components of cells, the situation is termed as oxidative stress (Gill *et al*., 2015).

Cereals play a crucial role in meeting the nutritional demands of the expanding global population. Among numerous cereals, *Oryza sativa* L. (rice) is of great concern. With a history dating back around 10,000 years, rice was initially cultivated in the river valleys of South and Southeast Asia, and China, serving as a fundamental food crop for the region's inhabitants. Later on, the area of rice cultivation expand significantly and now it is being harvested in various other continents like Latin America, Europe, several other regions of Africa and USA too (See review’s by Bin Rahman and Zhang, 2023). Consequently, rice has become the second most widely consumed cereal worldwide, following closely behind wheat. Despite being a semi-aquatic annual grass plant, rice serves as the staple food for nearly two-thirds of the worldwide population. Notably, over 2 billion people in Asia rely heavily on rice, deriving approximately 80% of their daily energy needs from it. Rice has the great nutrient value too where it contains 80% carbohydrates, 7-8% protein, 3% fat, and 3% fiber (Juliano, 1985). Rice is revered as the "grain of life" in Asian cultures, where it is deeply intertwined with tradition, identity, and sustenance. The diverse ecological and agricultural conditions under which rice is grown render the crop more susceptible to abiotic stresses, ultimately affecting its productivity and resulting in yield losses.

In response to arrest in growth and productivity of plant under various environmental stress conditions the scientists have been evolved and introduced countless strategies for plant to cope up with these stresses. In this concern application of phytohormones like indole-3-acetic acid (IAA) and kinetin (KN) is now becoming a promising tool to manage the crop productivity against various abiotic stresses (Singh and Prasad, 2015; 2016; Singh *et al*., 2018; Khan and Qadir, 2021). IAA levels change when plants are treated with heavy metals, which can decrease cell wall elasticity and the uptake of heavy metals, enhancing plant resistance to heavy metals (Singh and Prasad, 2015). Exogenous KN application has been shown to mitigate the toxic effects of cadmium on growth, pigmentation, and photosynthesis (Al-Hakimi, 2007). Similarly, Gangwar *et al*. (2010) also revealed that the application of KN effectively ameliorated the deleterious effects of Mn on pea seedling growth, ostensibly due to enhanced NH^{4+} assimilating enzyme activities in roots and shoots along with the pronounced increase in the enzymatic and non-enzymatic antioxidantslevels.Application of IAA and kinetin helps improve the photosynthetic efficiency by maintaining chlorophyll content and stabilizing the photosynthetic machinery (Khan and Qadir, 2021; Asghari *et al*., 2023). Research conducted by Ouzounidou and Ilias (2005) underscored the pivotal role of IAA in conserving Chl and Car contents in plants subjected to copper stress. This phenomenon is attributed to the hormone's ability to modulate cellular processes, thereby mitigating the detrimental effects of Cu toxicity. Despite the surfeit of studies examining the impact of exogenous phytohormone applications on plant growth and development, the existing literature is marred by inconsistencies and contradictory findings. Surprisingly, there is a significant lacuna in research investigating the responses of rice seedlings to exogenous applications of IAA and KN during their formative growth stages under abiotic stress conditions. To bridge this knowledge gap, the present study undertakes a comprehensive investigation into the comparative effects of exogenous IAA and KN applications on growth, oxidative stress, and antioxidant systems in *Oryza sativa* L. seedlings during their early growth stages under Asphytotoxicity.

Material and Methods

Plant materials and culture conditions

Among different cultivars of *Oryza sativa* L. available and grown at the nearby places of Ayodhya (India), Bheem cultivar is known to be the best in terms of hydroponic environment. Certified seeds of Bheem cultivar were purchased from authentic supplier of Ayodhya, India. and were surface sanitized in 0.1% $HgCl_2$ solution, followed by washing them with distilled water and soaked in water for 24 h. The seeds were then transferred on a blotting sheet kept in a plastic tray (30 × 14 cm^2) and then placed in dark at 25 ± 2°C for germination. After the germination of seeds it was shifted in a controlled environment of the growth chamber with a 16:8 h light:dark photoperiod (350 µmol photons m^{-2} s^{-1} of photosynthetic active radiation) and at temperatures of 25 ± 2°C with 60% relative humidity. At 10^{th} day of growth 5 plants per pot were selected for the hydroponic system and transferred to PVC cups (4 × 5 cm^2) containing 1/3 strength Hewitt nutrient medium having 50 and 100 mM of As, and 10 µM of IAA and KN. The PVC cups were then organized in a simple randomized design and placed in natural environmental conditions, as outlined earlier, to simulate real-world growth conditions. Seedlings growing under only Hewitt nutrient medium regarded as control. At 7 days post-treatment, the seedlings were harvested, and a range of morphological, physiological, and biochemical parameters were assessed to determine treatment effects.

Determination of growth

The seedlings of each set were excised and the fresh weight of root and shoot was recorded instantly, while for the dry weight samples were left at 80 ºC for 48 h in microwave oven in order to squeeze the moisture content and thereby weighed using the single pan of electronic balance.

Spectrophotometric determination of photosynthetic pigments

Photosynthetic pigments *viz.* chlorophyll *a*, chlorophyll *b* and carotenoids content were extracted from fresh leaves (20 mg) of rice seedlings in 80% (v/v) acetone. The obtained supernatant was used to assay the amount of chlorophyll *a*, chlorophyll *b* and carotenoids by taking the absorbance at 663.2, 646.5 and 470 nm spectrophotometrically respectively and quantified as per the reference of Lichtenthaler (1987).

Arsenic (As) accumulation

Arsenic levels in individual plant samples were quantified using atomic absorption spectrophotometry (AAS), following the protocol outlined by Allen *et al*. (1986).

Quantification of ROS generation and oxidative damage markers

Superoxide radical ($O_2^{\bullet-}$) content was computed by monitoring the oxidation of hydroxylamine to nitrite, catalyzed by $O_2^{\bullet-}$, according to the protocol outlined by Elstner and Heupel (1976) whereas the hydrogen peroxide (H_2O_2) content in tested samples was determined using the protocol outlined by Velikova *et al.* (2000).

Assessment of the oxidative damage indices

As a result of over produced oxidative stress markers the damage conferred to lipid and membrane i.e. the extent of lipid peroxidation in the leaves of test seedlings was evaluated by assessing the amount of malondialdehyde (MDA) equivalents, which are formed during the peroxidation of polyunsaturated fatty acids. The MDA content was determined spectrophotometrically using the 2-thiobarbituric acid (TBA) assay. In this reaction, MDA equivalents content form pink coloured product under acidic environment as described by Heath and Packer (1968). In addition to this, electrolyte leakage which is an indicator of plasma membrane damage was also measured in test seedlings using the protocol outlined by Gong *et al*. (1998).

Estimation of enzymatic antioxidants

SOD activity was measured by assessing the enzyme's ability to inhibit the reduction of nitro blue tetrazolium chloride (NBT), using the assay protocol established by Giannopolitis and Ries (1977). One unit of SOD activity was defined as the amount of enzyme required to inhibit NBT reduction by 50%. The catalase (CAT) activity was quantified in tested rice seedlings according to the protocol outlined by Aebi (1984).One unit of CAT activity was defined as the amount of enzyme required to dissociate 1 nmol of H_2O_2 per minute. Guaiacol peroxidase activity (POD) in each set was calculated according to the protocol outlined by Zhang (1992).One unit of POD activity was defined as the amount of enzyme that oxidizes 1 μmol of guaiacol per minute at 25 °C. Glutathione-*S*-transferase (GST) activity was calculated according to the protocol outlined by Habig *et al*. (1974) using CDNB (1-chloro, 2, 4-dinitro benzene) as a substrate. GST activity was calculated using the extinction coefficient ($\varepsilon = 9.6\ mM^{-1}\ cm^{-1}$) and expressed as nmol conjugate formed per minute.

Estimation of non-enzymatic antioxidants

Non-protein thiols in each test seedlings were quantified as per the method of Ellman (1959). Fresh leaves (100 mg) were blend in 6.67 % of sulphosalicylic acid under ice-cool condition followed by the centrifugation at 10,000 g for 10 min. at 4 °C. The reaction mixture was prepared which contain the obtained supernatant, Ellman Reagent having EDTA and DTNB prepared in PPB. Spectrophotometric analysis of the prepared reaction mixture was performed at 412 nm, and the resulting absorbance value was quantified using a standard curve.

Cysteine content in tested rice seedlings was done by using the protocol developed by Gaitonde (1967).Fresh leaves (200 mg) were crushed in 5 % of perchloric acid and centrifuged at 10,000 g for 10 min. at 4 ºC. Later on, the obtained supernatant, 2 ml of glacial acetic acid and 2 ml of acid ninhydrin mixed well and kept at water bath for 3 min. In last the developed chromophores in the reaction were extracted with toluene (4 ml) and separated at 4 °C. The absorbance of these separated chromophores was read spectrophotometrically at 560 nm and the cysteine content was quantified by the standard curve of cysteine.

Proline content in test samples was quantified by following the protocol of Bates *et al*. (1973).Fresh leaf (200 mg) were blend in 3% aqueous sulfosalicylic acid and centrifuged at 10,000 g for 10 min at 4 °C. The supernatant was then used to prepare a reaction mixture containing glacial acetic acid and acid ninhydrin. The mixture was incubated at 95 °C for 1 hour, cooled, and then extracted with 4 ml of toluene followed by vortexing the samples for 15 seconds. The absorbance of the toluene layer was measured spectrophotometrically at 520 nm by serving the toluene as blank and proline content was calculated from a standard curve.

Statistical analysis

The data expressed in this study are of means ± standard error of three independent experiments, each with two replicates, to confirm the data reproducibility. Statistical analysis was performed using one-way ANOVA, followed by Duncan's Multiple Range Test (DMRT) to determine significant differences between control and treatment means at a significance level of $p < 0.05$.

Results and Discussion

Growth

This study undertakes a comprehensive examination of the efficacy of exogenous phytohormone supplementation, comprising indole-3-acetic acid (IAA), a naturally occurring auxin, and kinetin (KN), a synthetic cytokinin, in mitigating the adverse effects of arsenic (As) toxicity on the growth and development of rice (*Oryza sativa* L.) seedlings. As at both doses 50 and 100 mM ominously suppressed the growth of test seedlings (Fig. 1). The As induced reduction in growth parameters could have resulted from higher accumulation of As in root and shoot tissues (Fig. 2), reduced level of photosynthetic pigments content (Table 1) and elevated levels of ROS and thereby indices of membrane damage (Fig. 3). The As induced deleterious effects on growth attributes have also been reported in earlier studies on *Allium sativum* and rice seedlings (Anjum *et al*., 2017; Mishra *et al*., 2022). Further, exogenous application of IAA and KN at the tested concentrations alleviated As-induced toxicity in rice seedlings through the regulation of critical metabolic pathways, notably the antioxidant defense system, thereby enhancing stress tolerance in the seedlings. The supplementation of IAA and KN significantly counteracted As-induced toxicity in test seedlings, likely due to the decreased accumulation of As in root and shoot tissues (Fig. 2). Additionally, the growth-promoting effects of these phytohormones under As stress conditions can be ascribed to the enhanced levels of light-harvesting pigments (Table1), and reduced oxidative stress (Fig. 3), which were achieved through the up regulation of the antioxidant defense system (Figs. 4). Previous research has demonstrated that the exogenous application of IAA and KN can effectively mitigate the detrimental effects of Cd toxicity on the growth and development of *Solanummelanogena* seedlings (Singh and Prasad, 2014, 2015). Moreover, kinetin has been shown to enhance plant tolerance to heavy metal stress by modulating a plethora of physiological processes, including the accumulation of osmoprotectants such as proline, methionine, and γ-aminobutyrate (Pavlíková *et al*., 2014a), altering polyamine levels (Damyanova *et al*., 2014), augmenting photosynthetic and transpirational rates (Pavlíková *et al*., 2014b), and minimizing oxidative damage to cellular membranes (Singh and Prasad, 2014). After comparing the responses of both hormones treatment it was found that KN always dominant over the IAA, as it much neutralizes the deteriorating impact of As and supports the growth of rice seedlings under stress conditions too.

Photosynthetic pigments

Results pertaining the photosynthetic pigments was in accordance with the reduced plant growth (Table1) and biomass suggesting that as might have inhibited the activities of enzymes (sulfhydryl requiring) participating in Chl biosynthesis, impaired the uptake of mineral nutrients Fe, P, Zn etc. (Singh *et al*., 2018), required for the biosynthesis of Chl (Yadav *et al*., 2014), replaced phosphate which contributes in pigment biosynthesis (Mishra *et al*., 2016) and hindered the chlorophyll biosynthesis by decreasing the protoporphyrin IX, Mg-protoporphyrin, Mg-protoporphyrin methyl ester and divinylprotochlorophyllide precursor (Mishra *et al*., 2016). Conversely, the substantial enhancement of Chl and Car contents in response to IAA and KN supplementation, both individually and in conjunction with As, indicates a mitigating effect on the growth impairment of seedlings subjected to stress, implying a potential role for these phytohormones in enhancing stress tolerance. Consistent with these findings, Ouzounidou and Ilias (2005) observed that IAA helped preserve Chl and Car contents in plants grown under Cu stress. Moreover, cytokinins have been found to promote tetrapyrrole synthesis, which is essential for chloroplast function in barley seedlings (Yaronskaya *et al*., 2006).Our results align with the findings of Singh and Prasad (2015), who observed that IAA foliar spray counteracted the Cd-induced reduction in chlorophyll and carotenoid contents, as well as the ratios of chlorophyll a/b and chlorophyll/carotenoids. Furthermore, Behera *et al*. (2002) reported that KN supplementation enhanced carotenoid synthesis, thereby safeguarding the photosynthetic apparatus against oxidative damage by ROS.

Oxidative stress markers Vs. Antioxidative defense system

Arsenic is capable to induce oxidative stress by the excessive generation of ROS i.e. superoxideradicals ($O_2^{\bullet-}$), hydroxyl radical ($OH^{\bullet-}$), and hydrogen peroxide (H_2O_2), which can interact with biomolecules and modify their structure and function (Singh *et al*., 2015). In case of As reactive oxygen species are also produced through the conversion of As^V to As^{III} in plants (Mascher *et al*., 2002). In the present study too, the rice seedlings exposed to As showed a severe oxidative stress as evident by a sharp increase in the level of $O_2^{\bullet-}$ and $H_2O_2$and thereby MDA equivalents contents, which might have occurred as a result of electron leakage (Fig. 3) from overloaded ETC of chloroplasts and mitochondria, stimulated by As (Singh *et al*., 2020). Our results corroborate the findings of preceding investigations (Talukdar, 2013; Mishra *et al*., 2016; 2022), where ROS generation and stress markers were highlighted against the As stress. Conversely, the supplementation with IAA and KN, either alone or in combination, markedly check ROS levels, with a more pronounced effect observed in seedlings treated with KN. The reason behind this might be the induced activities of antioxidant enzymes (Fig. 4) and improved level of non-enzymatic antioxidants (Table 2), which is of an adaptive significance. They play a decisive role in detoxification of free radicals and tend to reduce oxidative damage to membranes (Wang *et al*., 2015). In this study, the possibility through which IAA and KN reduce ROS level and stress biomarkers might be reduced uptake and accumulation of As (Fig. 2).The phytohormones mediated limitation in ROS generation under heavy metal toxicity can be well elaborated by the preceding studies of Ahammed *et al*. (2013) and Singh *et al*. (2018). Notably, above research has demonstrated that phytohormones elicit a response characterized by the judicious production of ROS, which function as key signaling molecules. These ROS play a pivotal role in regulating root gravitropism in maize and conferring protection against Cd toxicity in various plant species, including *Cicer arietinum*, *Solanum lycopersicum*, and *Solanum melongena*. This protective mechanism is mediated by the stimulation of antioxidant defenses, which serves to mitigate the deleterious effects of Cd toxicity.

Rice seedlings treated with as showed a marked elevation in the activities of key antioxidant enzymes i.e. SOD, POD, CAT, and GST (Fig. 4). The arsenic induced enhanced SOD activity might be an exercise of cells to dismutase $O_2^{\bullet-}$ into lesser toxic H_2O_2 (Gill *et al*., 2015) as increased H_2O_2 production under arsenic stress was observed in the present study (Fig. 3). The highest SOD activity under supplementation of IAA and KN (Fig. 4) indicated successful dismutation of highly toxic $O_2^{\bullet-}$ or adequate expression of genes encoding SOD to combat the cell from oxidative stress.

The H_2O_2 produced by SOD activity is mostly detoxified by the enzymes: CAT (functions in peroxisomes and mitochondria) and POD (present in the cell wall, performs a function in the cytoplasm). The upregulation of POD and CAT activities in response to IAA and KN treatment facilitates the efficient scavenging of H_2O_2, converting it into H_2O and O_2, and thus maintaining cellular redox balance, as previously reported by Ahammed *et al*. (2013), and Wang *et al*. (2015).The elevated GST activity observed with additional IAA and KN supplementation suggests that the plant is attempting to counteract oxidative stress. This finding is consistent with the study by Singh and Prasad (2014 and 2016), which demonstrated that foliar application of plant growth regulators increased the activities of SOD, POD, CAT, and GST in *Solanum melongena* and *Solanum lycopersicum* seedlings.

Notably, the present study revealed a significant increase in cysteine and NPTs under as stress, with a further pronounced elevation in their contents following IAA and KN supplementation (Table 2). This increase can be attributed to the enhanced confiscation of arsenic in roots, thereby reducing the translocation of As to shoots and alleviating the toxic burden on the test seedlings (Duan *et al*., 2011), a phenomenon consistently observed in this study. Our data are in accordance with the results of previous studies conducted on *Cicer arietinum* and *Allium sativum* seedlings against as stress (AL-Huqail *et al*., 2017; Ruíz-Torres *et al*., 2017). The elevated Cys content observed under as exposure and/or following IAA and KN treatment suggests that these treatments may have triggered the upregulation of key enzymes involved in the sulfur-assimilating pathway, thereby facilitating an enhanced sulfur flux (Dhankher *et al*., 2002). Moreover, proline was also accumulated against both the doses of As (Table 2) which could have possible owing to its ability to protect the enzymes, maintain the water potential of plants (Fariduddin *et al*., 2009) and antioxidant property (Yadav *et al*., 2014). The combined supplementation of IAA and KN proved to be an effective strategy for further enhancing proline accumulation, thereby providing an additional layer of protection against As-induced stress (Table 2). Our results are corroborated with the earlier studies of Singh *et al*. (2015) in *Solanum melongena* L. plants treated with as stress. Our findings are corroborated by the research of Kaya *et al*. (2010), who observed a significant 1.5-fold increase in proline content in maize plants treated with kinetin and indole acetic acid, highlighting the potential of plant growth regulators to modulate proline accumulation and mitigate stress-induced damage.

Conclusion

This study reveal that as exposure at both 50- and 100-mM doses exerted a detrimental impact on the growth of rice seedlings. Specifically, as treatment led to a significant reduction in photosynthetic pigments, while concurrently triggering the production of ROS and inducing oxidative stress. Notably, despite the upregulation of enzymatic and non-enzymatic antioxidant defense systems, as exposure still inflicted considerable damage on the rice seedlings, underscoring the severity of As-induced stress in rice. In stark contrast, supplementation with IAA and KN significantly mitigated As-induced toxicity in rice seedlings. This amelioration was manifested through improved photosynthetic pigment status, enhanced activities and formation of antioxidant defense systems, and reduced arsenic accumulation in plant tissues. Notably, KN supplementation proved more efficacious than IAA in alleviating As-induced stress, highlighting the potential of KN as a more effective phytoprotectant against arsenic toxicity in rice. Overall results indicate that supplementation of both the hormones IAA and KN may improve the yield and productivity of rice seedlings but from comparative study it can be recommended that supplementation of KN will be more effective in As contaminated sites.

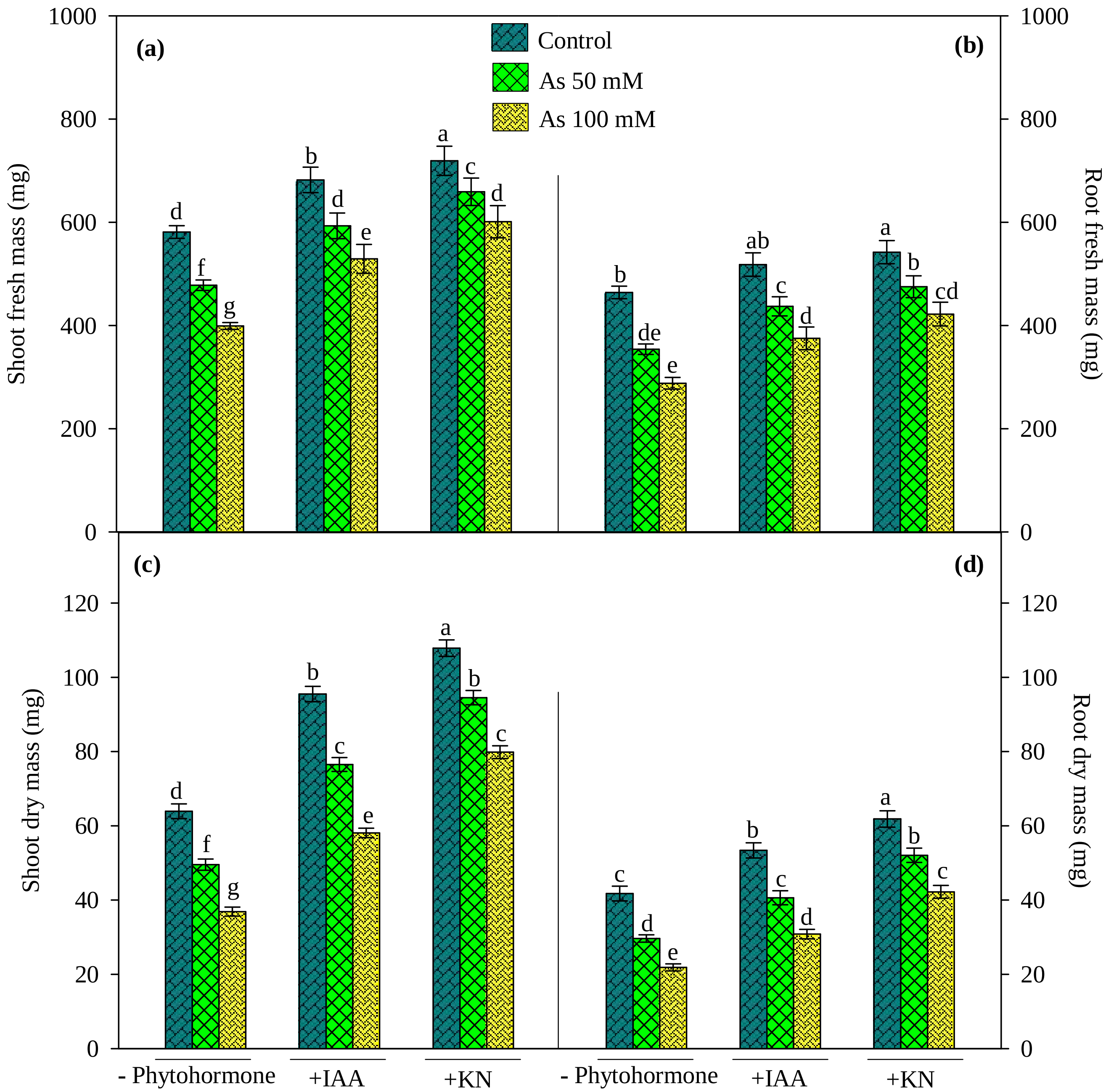

Fig. 1: Impact of IAA and KN on shoot (a) and root (b) fresh mass and shoot (c) and root (d) dry mass of rice seedlings under as toxicity. Data are mean ± standard error of three independent experiments. The bars followed by different letter show significance level of difference at P<0.05 between treatments according to Duncan's multiple range test.

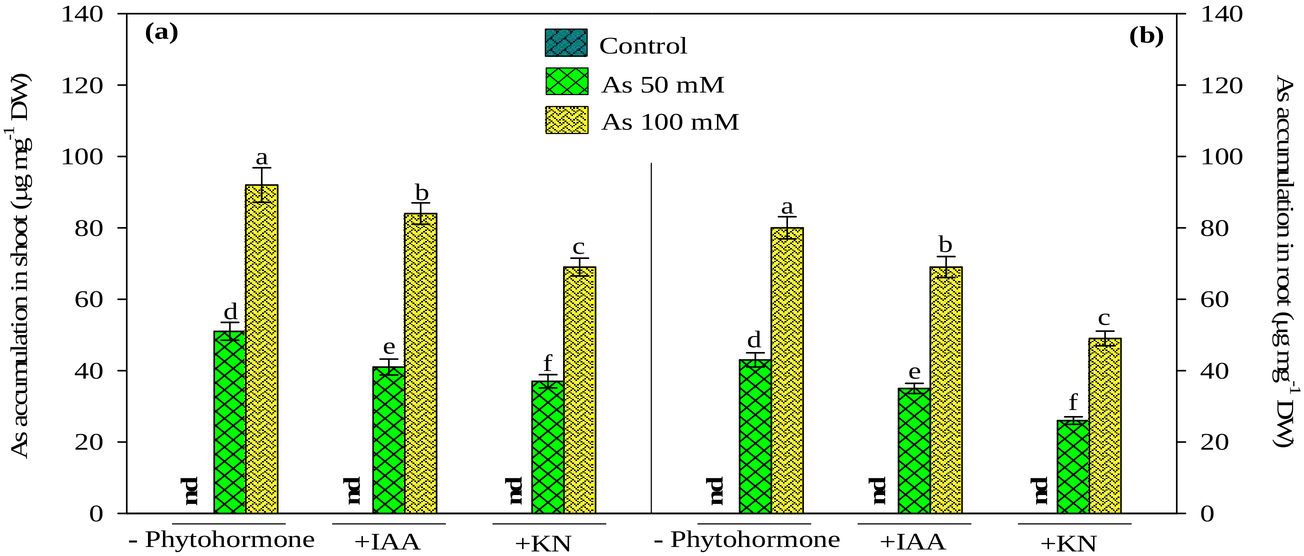

Fig. 2: Impact of IAA and KN on as accumulation in shoot (a) and root (b) of rice seedlings under as toxicity. Data are mean ± standard error of three independent experiments. The bars followed by different letter show significance level of difference at P<0.05 between treatments according to Duncan's multiple range test.

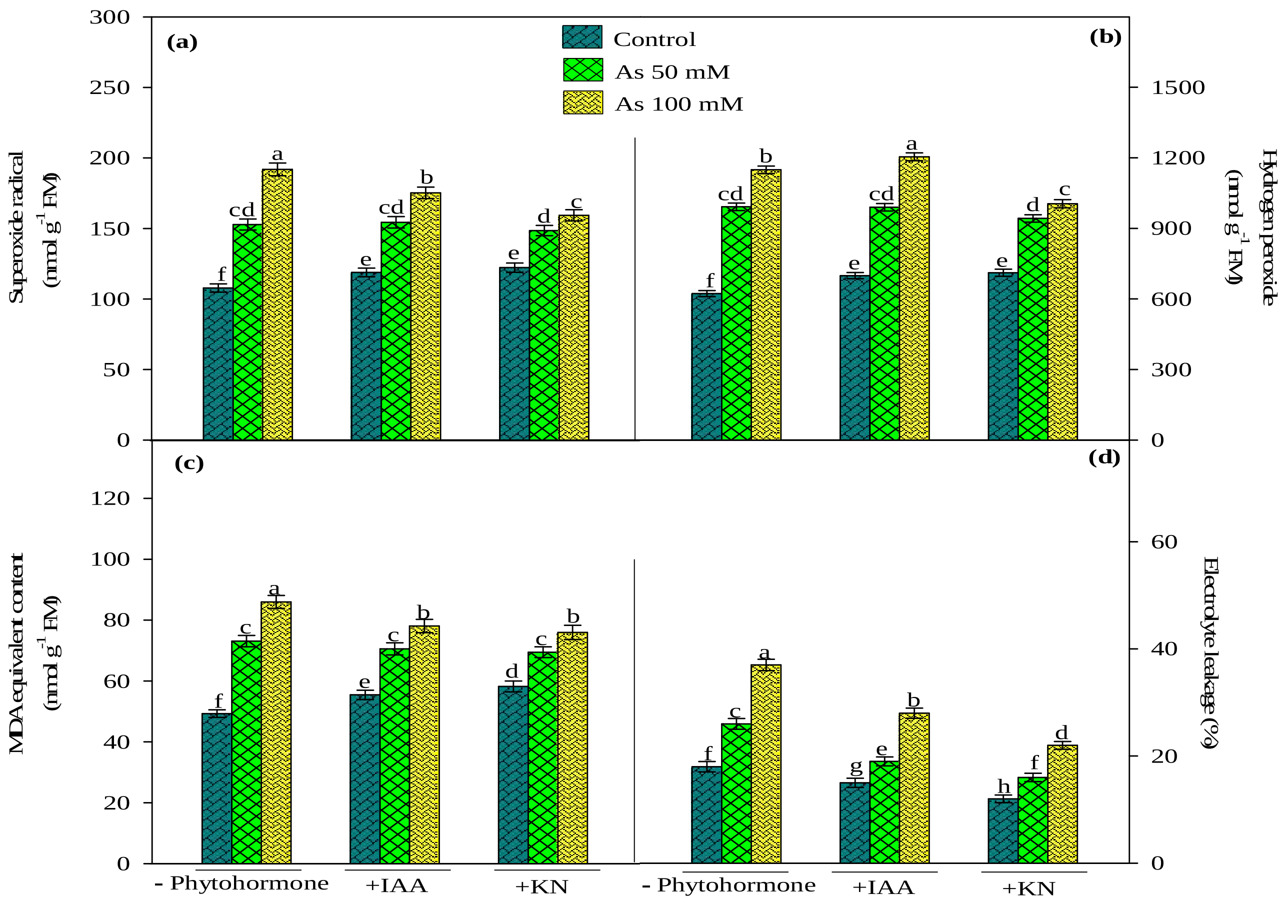

Fig. 3: Impact of IAA and KN on superoxide radical (a), hydrogen peroxide (b), MDA equivalent content (c) and electrolyte leakage (d) of rice seedlings under as toxicity. Data are mean ± standard error of three independent experiments. The bars followed by different letter show significance level of difference at P<0.05 between treatments according to Duncan's multiple range test.

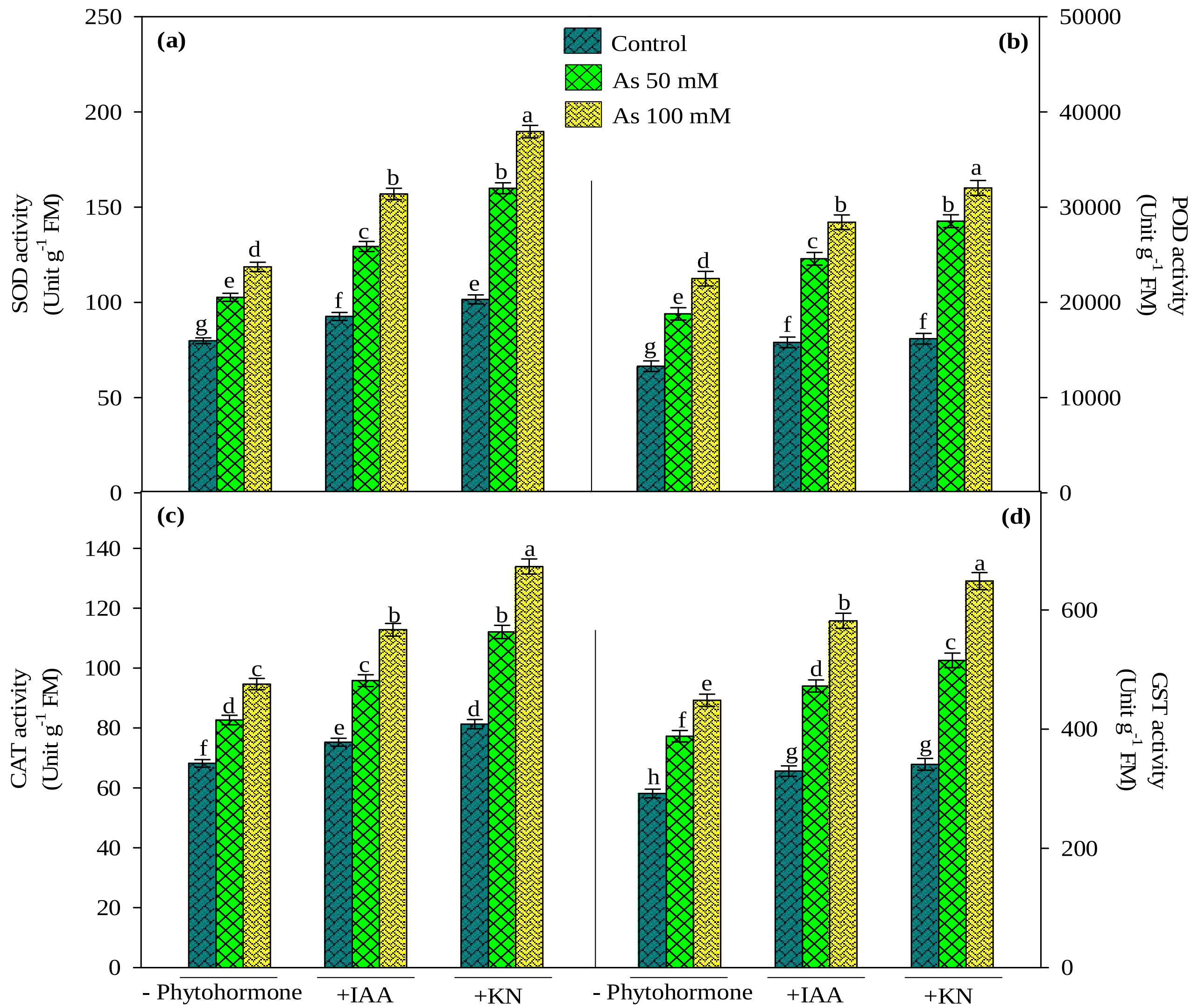

Fig. 4: Impact of IAA and KN on enzymatic antioxidants SOD (a), POD (b), CAT (c) and GST (d) activities of rice seedlings under as toxicity. Data are mean ± standard error of three independent experiments. The bars followed by different letter show significance level of difference at P<0.05 between treatments according to Duncan's multiple range test.

Table 1: Impact of IAA and KN on photosynthetic pigments content i.e., chlorophyll *a*, chlorophyll *b* and carotenoids of rice seedlings under as toxicity.

Treatments	Photosynthetic pigments content (mg g^{-1} FM)		
	Chlorophyll *a*	Chlorophyll *b*	Carotenoids
Control	1.912 ± 0.071^{d}	0.461 ± 0.007^{c}	0.510 ± 0.008^{e}
As 50 mM	1.561 ± 0.058^{f}	0.352 ± 0.006^{g}	0.428 ± 0.007^{f}
As 100 mM	1.321 ± 0.041^{g}	0.298 ± 0.004^{h}	0.399 ± 0.005^{g}
+ IAA 10 µM	2.22 ± 0.092^{b}	0.546 ± 0.009^{b}	0.644 ± 0.009^{b}
As 50 mM + IAA 10 µM	1.89 ± 0.087^{d}	0.452 ± 0.007^{d}	0.579 ± 0.008^{d}
As 100 mM + IAA 10 µM	1.74 ± 0.062^{e}	0.401 ± 0.006^{f}	0.535 ± 0.007^{e}
+ KN 10 µM	2.46 ± 0.102^{a}	0.577 ± 0.009^{a}	0.707 ± 0.010^{a}
As 50 mM + KN 10 µM	2.14 ± 0.092^{c}	0.492 ± 0.008^{c}	0.658 ± 0.009^{b}
As 100 mM + KN 10 µM	1.99 ± 0.091^{d}	0.441 ± 0.007^{e}	0.632 ± 0.008^{c}

Data present here are mean ± standard error of three independent experiments. Values within same column followed by different letters show difference at P<0.05 level between treatments according to Duncan's multiple range test.

Table 2: Impact of IAA and KN on non-enzymatic antioxidants NP-SH, cysteine and proline contents of rice seedlings under as toxicity.

Treatments	Non-enzymatic antioxidants (nmol g^{-1} FM)		
	NP-SH	Cysteine	Proline
Control	1890 ± 59^{i}	36 ± 0.88^{h}	270 ± 5.88^{g}
As 50 mM	2612 ± 104^{f}	45 ± 1.02^{f}	358 ± 8.90^{d}
As 100 mM	3067 ± 107^{e}	51 ± 1.10^{e}	408 ± 9.15^{cd}
+ IAA 10 µM	2059 ± 84^{h}	41 ± 0.90^{g}	299 ± 6.21^{f}
As 50 mM + IAA 10 µM	3454 ± 118^{d}	55 ± 1.19^{d}	415 ± 9.58^{c}
As 100 mM + IAA 10 µM	4006 ± 119^{c}	63 ± 1.37^{b}	484 ± 10.68^{b}
+ KN 10 µM	2261 ± 89^{g}	44 ± 0.91^{f}	322 ± 6.99^{e}
As 50 mM + KN 10 µM	4195 ± 126^{b}	61 ± 1.27^{c}	485 ± 10.68^{b}
As 100 mM + KN 10 µM	4912 ± 154^{a}	73 ± 1.49^{a}	594 ± 12.99^{a}

Data present here are mean ± standard error of three independent experiments. Values within same column followed by different letters show difference at P<0.05 level between treatments according to Duncan's multiple range test

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