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Assessment of Physiological and Biochemical Changes in Rice Seedlings Exposed to Bulk and Nano Iron Particles

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ABSTRACT

Metallic nanoparticles have huge applications in all the fields including agriculture and its allied sciences. This study aims at evaluating possible implications of iron oxide nanoparticles on the growth and development of rice (Oryza sativa L.). Rice is feeding hunger of half of the World's population and yet found to be highly sensitive towards iron deficiency especially at seedling stage. The results obtained through hydroponic study reveals growth stimulatory effects of rice seedlings exposed to various metallic form of iron bulk and nano at concentrations at 100, 200 and 400 ppm. Nanoparticle treated seedlings experienced less stress compared to their bulk counterparts. Together, physiological and biochemical results show that iron nanoparticles could be utilized for overcoming Fe deficiency especially during seedling development.

Key words: *iron oxide nanoparticles, rice, nanofertilizer, DLS, NTA*

INTRODUCTION

With the advent of nanotechnological interventions in different scientific fields results into rapid growth and utilization of a number of engineered nanoparticles of different sizes and physicochemical properties[1]. Metallic nanoparticles have tremendous applications in agriculture which can immensely improved the nutrient use efficiency by crops, reducation in dosage coupled with enhancement of growth. Nanoparticles of zinc, iron, hydroxyapatite phosphorous, cerium oxide etc. are applied on various model plant systems for assessing their growth stimulatory effects.

Iron is one of the most important element needed in less amount but the higher or critically low amount in the soil may leads to toxicity or deficiency, respectively and can severely damaged the crop[2]. Indian soils are naturally found to be deficient in iron[3] and a crop like rice (*Oryza sativa* L.) is highly sensitive to iron deficiency[4]. Iron deficiency in rice starts at the seedling stage exhibiting iron chlorosis in younger leaves which in severe deficiency leads to death of seedlings.

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These results in loss of valuable seedlings which could be overcome by application of iron fertilizers. Based upon the chemical nature, different forms of Fe fertilizers are available which includes (i) chelated – Fe fertilizer (ii) in-organic Fe and (iii) organic Fe fertilizers[5]. The chelated fertilizer are expensive and applied to high-value crops, soluble in-organic Fe fertilizer improve the Fe content to a considerable limit depending upon the nature of soil and organic fertilizer can get easily enter into the soil and leads to leaching leading to reduction in the fertilizer effects[6]. Therefore, to improve the Fe content in plants, new mode of applications can certainly enhance the nutrient use efficiency of crops.

Direct estimation of elemental response using soil as a substrate leads to erroneous results due to the complexity and nature as well as physical and chemical structure problems associated with soil. In comparison, hydroponic systems is the proven method for estimation for plant biology research especially for abiotic stresses like higher or critically low concentration of elements[7]. Rice is one of the most important model crop as well as best suited for comparing the bulk and nano iron forms under controlled conditions.

Nanotechnology-based fertilizer and pesticides formulation can significantly increase the uptake efficiency and efficacy, respectively by the plants as well as a reduction in application dosage may reduce the cost of cultivation coupled with environment-friendly can revolutionize the agriculture sector[8]. Growth stimulatory response of various nanomaterials has been reported which includes zinc oxide nanoparticles (9), silver nanoparticles (10), nanoanatase TiO_2 (11), alumina NPs (12), cerium oxide NPs (13), iron oxide NPs (14) and hydroxyapatite nanophosphorous (15).

In view of this, the present study was aimed at characterizing the effects of FeO NPs on most widely grown cereal crop rice variety Jaya to shed light on the effects of NPs on morpho-physiological and biochemical response over a three different concentrations (100, 200 and 400 ppm) under hydroponically grown *in vitro* conditions. A possible positive impact of nanoparticles over rice seedlings is highlighted under the study.

MATERIALS AND METHODS

Preparation of iron oxide nanoparticles (FeO NPs) suspension

Iron oxide nanoparticle (FeO NPs) of <50 nm were procured from Sigma, Alrich, USA and used as supplied. These particles were characterized for their size, zeta potential using Malvern Zetasizer (Model: ZS90) and Nanosight (NS 500), respectively following standard operating procedures. Different suspension of FeO NPs were prepared using Milli Q (18 M Ω) water and the final concentration in the hydroponic solution was adjusted to 100, 200 and 400 ppm.

Plant materials

Rice seeds (variety: Jaya) were supplied by Main Rice Research Station, Anand Agricultural University, Nawagam, Gujarat and only good quality seeds showing uniform size and shape without any morphological damage were screened and surface sterilized using hypochlorite solution before treating with different bulk and nano iron forms.

Seeds of rice variety Jaya supplied by Main Rice Research Station, Anand Agricultural University, Nawagam was used in the experiment. The variety was selected as it shows susceptibility towards iron deficiency in early stages as well as it is still prefer variety for sowing by the rice farmers of Gujarat state, India.

Hydroponic study

The seeds were immersed in the hydroponic solution over a paper towel support. The test tubes were kept under illumination chamber with 14-h photoperiod, 25/20°C day/night temperature and 65% relative humidity. The germination rate was measured when 65% of control roots were 5 mm long while ungerminated seeds were removed to avoid bacterial or fungal growth in the media and the seedlings were allowed to grow for 10 days under light (340 μmol m^{-2} s^{-1}). A single treatment is represented by five seeds per test tube with five replications in a completely randomized design. Morpho-physiological observations like total chlorophyll content (mg g^{-1} of fresh weight), shoot and root length (cm), fresh and dry weight (g) and biochemical observations like total protein content (mg g^{-1} of fresh weight), anti-oxidant enzyme activities like peroxidase (µmole mg $protein^{-1}$ min^{-1}), super-oxide dismutase (unit mg^{-1} protein) and catalase (µmole H_2O_2 mg $protein^{-1}$ min^{-1}) has been carried out on completion of incubation period i.e. on 10th day.

Enzyme extraction and activity assay

Enzyme extraction was carried out at the end of incubation period i.e. on 10th day and the fresh seedlings were grinded with pre chilled four ml of 0.1 M phosphate buffer (pH 6.0), 0.1mM EDTA, 0.3% (w/v) Triton X-100 and 4% (w/v) PVP for SOD assay or containing 4mM DTT, 2mM EDTA and 2%(w/v) PVP for catalase and peroxidise enzyme assay. The homogenised mixture was centrifuged at 4°C and 10,000*g* for 15 min and the supernatant was collected in a fresh tube. All the enzyme activity was carried out as described by Chou *et al.,* (2011) and total protein was estimated following Lowry method using BSA as the standard[17].

Statistical analysis

The morpho-physiological and biochemical results are represented as mean ± SE (n = 5). Nanoparticle suspension analysis was carried out using in built analysis software (A) For Malvern ZS 90 – version 7.04 and (B) For Nanosight NS 500 –version 2.3.

RESULTS

Characterization of FeO NPs

Physical characterization of commercially available FeONPs was found to be in agreement with the supply of manufacturer. The size of nanoparticles was found to be 39.81 ± 0.31 nm having polydiserpsity index of 0.36 (Fig. 1). Stability of nanoparticles is one of the most important criteria as far as their behaviour in the solution is concerned. Nanoparticle tracking analysis reveals the zeta potential of FeO NPs to be -0.49 mV (Fig. 2A). Zeta potential results coupled with polydispersity index suggest the monodispersive nature of the nanoparticle in solution. Higher concentration of nanoparticle in solution leads to aggregation of particle and hence the determination of particle concentration was carried out. The particles concentration per mL was found to be 39.01 x 10^8 for FeO NPs (Fig. 2B).

Effect of FeO NPs on morpho-physiological parameters

Nanoparticle effect was found to be growth stimulatory at the end of incubation period in hydroponic solution for rice seedlings. Overall enhancement in morpho-physiological response was observed for all the parameters studied. Maximum shoot and root length was observed for 100 ppm FeONPs treated seedlings compared to other treatments. Among bulk particle treatments, the increment was found to be maximum for 400 ppm $FeSO_4$ treated seedlings. Similar growth response was observed for fresh and dry weight i.e. 100 ppm exposed FeONPs accumulated maximum fresh and dry weight compared to all other treatments. The morpho-physiological observation for nano and bulk particles of iron showed completely reverse trend for concentration i.e. with increasing concentration of iron oxide nanoparticles all the studied parameters showed inhibition of growth while all the ferrous sulphate treated seedlings exhibits growth enhancement as the concentration increases from 100 to 400 ppm (Table 1).

Total chlorophyll content was recorded higher in leaves exposed with 400 ppm of both FeO NP and $FeSO_4$ whereas, least chlorophyll content was recorded in control seedlings. (Fig. 3). Total chlorophyll content increase significantly in all the seedlings exposed to nanoparticles showing higher accumulation of chlorophyll content per gram of fresh samples compared to bulk particles.

Effect of FeO NPs on total protein and anti-oxidants status of rice seedlings

Accumulation of total protein was recorded maximum in seedlings exposed to 400 ppm of FeO NPs while minimum was observed in 100 ppm $FeSO_4$. Overall, the protein content was higher in nanoparticle treated seedlings followed by bulk and least in control. (Fig. 4).

Increase in anti-oxidant activity of defence enzymes boost over all response of plants towards various abiotic and biotic stresses. In the present investigation, increment in the various anti-oxidant enzymes like SOD, CAT and POX has been observed. The SOD activity was found to be higher in seedlings receiving 400 ppm of nanoparticle treatment followed by 400 ppm of $FeSO_4$ and control (Fig. 5). Similar response for both the anti-oxidnats enzyme POX (Fig. 6) and CAT (Fig. 7) has been observed wherein increment of enzyme activity increases in 400 ppm FeO NPs compared to all other treatments.

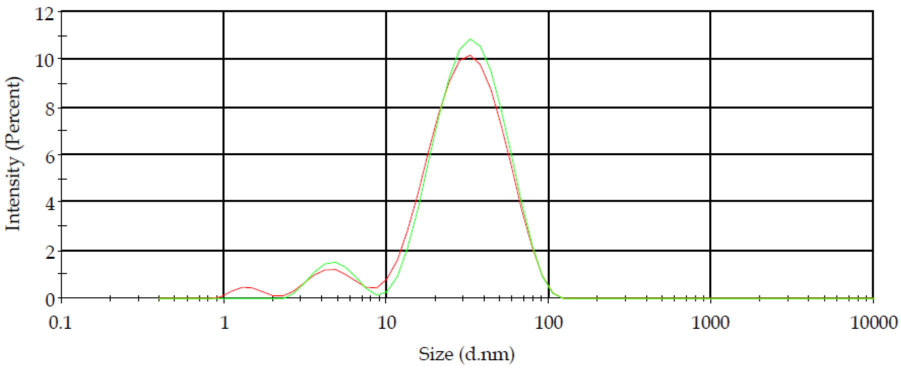
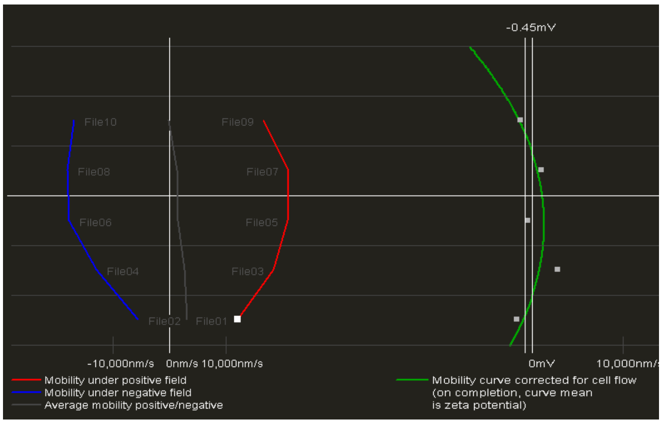
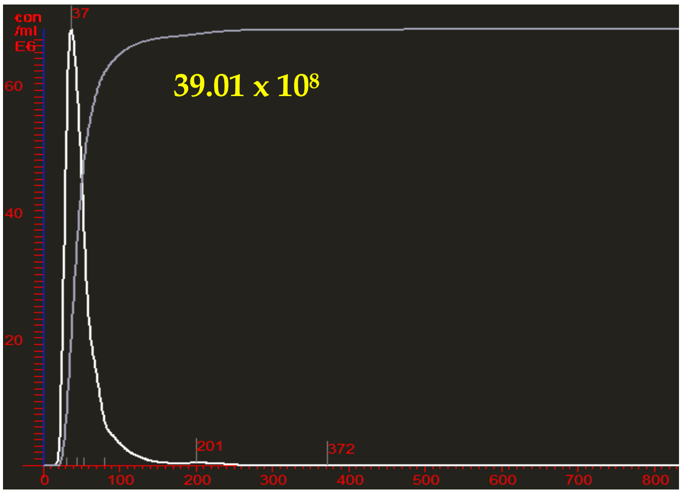


Fig. 1: Size distribution by intensity of commercially available iron oxide nanoparticles using dynamic light scattering



(A)



(B)

Fig. 2: (A) Zeta potential (mV) measurement and (B) particles per ml of commercially

available iron oxide nanoparticles

Table 1: Effects of bulk and nano iron particles on rice seedlings variety Jaya after ten days for shoot and root length (cm), fresh and dry weight (gm)

Treatment	Shoot length (cm)	Root length (cm)	Fresh weight (gm)	Dry weight (gm)
Control	3.59 ± 0.38	0.9 ± 0.12	0.06 ± 0.01	0.03 ± 0.00
100 ppm FeONP	**8.56 ± 0.30**	**2.2 ± 0.10**	**0.15 ± 0.01**	**0.10 ± 0.01**
100 ppm $FeSO_4$	6.19 ± 0.67	0.9 ± 0.09	0.11 ± 0.01	0.06 ± 0.01
200 ppm FeONP	8.07 ± 0.36	1.9 ± 0.05	0.13 ± 0.01	0.08 ± 0.01
200 ppm $FeSO_4$	7.20 ± 0.41	1.2 ± 0.09	0.12 ± 0.01	0.07 ± 0.01
400 ppm FeONP	6.54 ± 0.48	1.7 ± 0.09	0.13 ± 0.01	0.07 ± 0.01
400 ppm $FeSO_4$	**7.93 ± 0.69**	**1.6 ± 0.10**	**0.13 ± 0.01**	**0.09 ± 0.01**
S. Em.	0.439	0.094	0.01	0.005
C.D. (0.05)	1.29	0.27	0.03	0.013
C.V.%	12.84	12.67	16.48	11.52

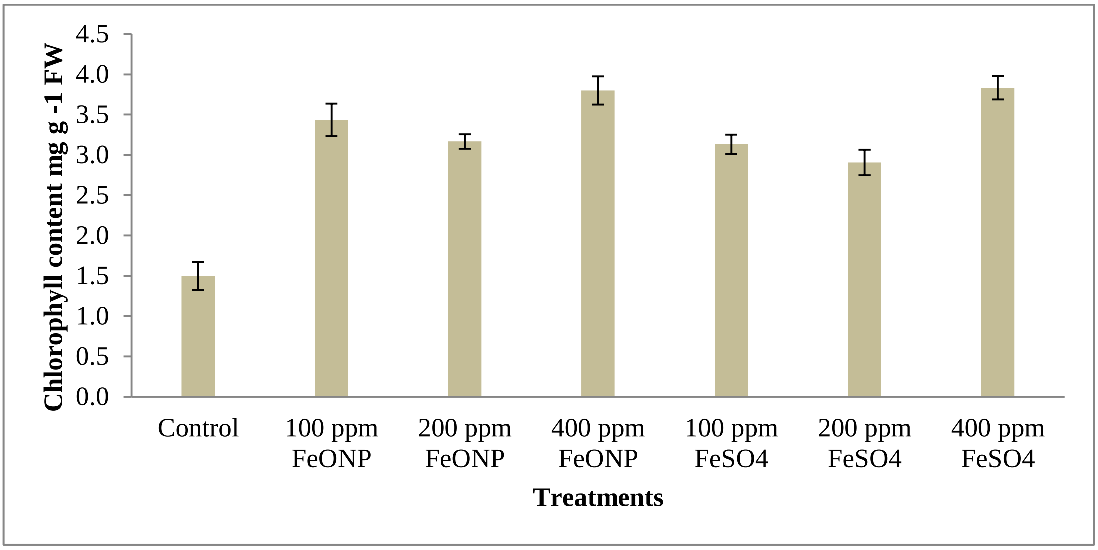


Fig. 3: Chlorophyll content (mg g^{-1}) in leaves of rice harvested at 10^{th} day

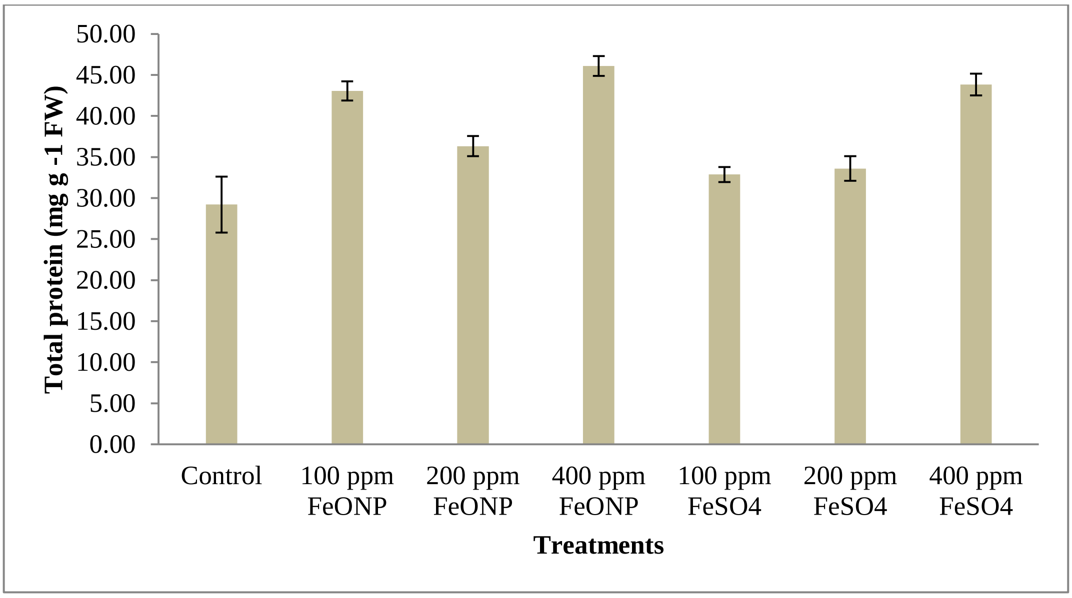


Fig. 4: Total protein content (mg g^{-1}) leaves of rice harvested at 10^{th} day

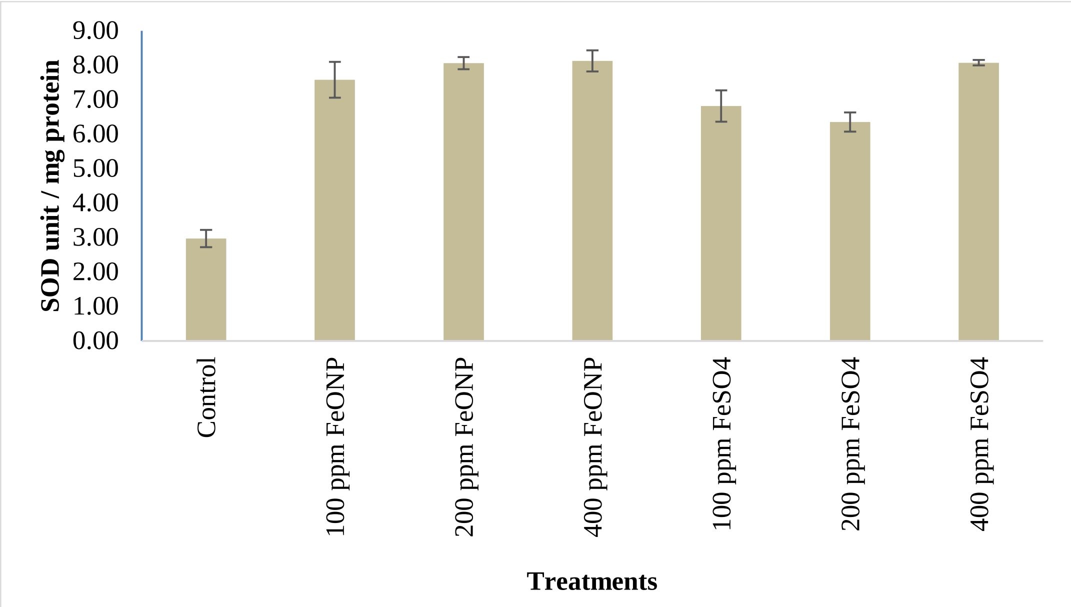


Fig. 5: Superoxide dismutase activity in leaves of rice harvested at 10th day

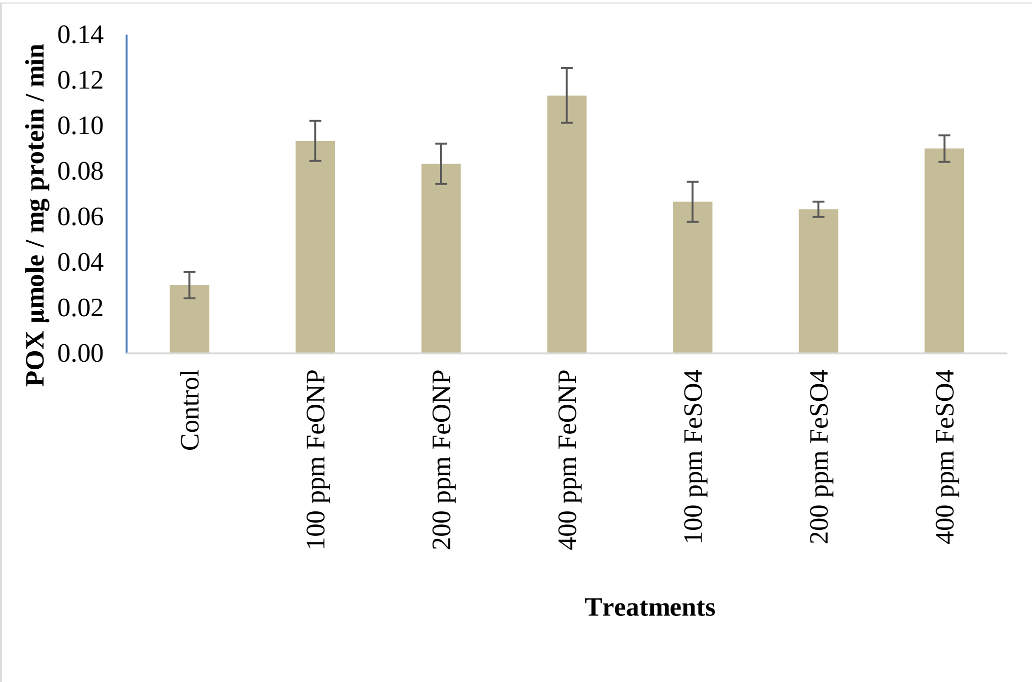


Fig. 6: Peroxidase activity in leaves of rice harvested at 10th day

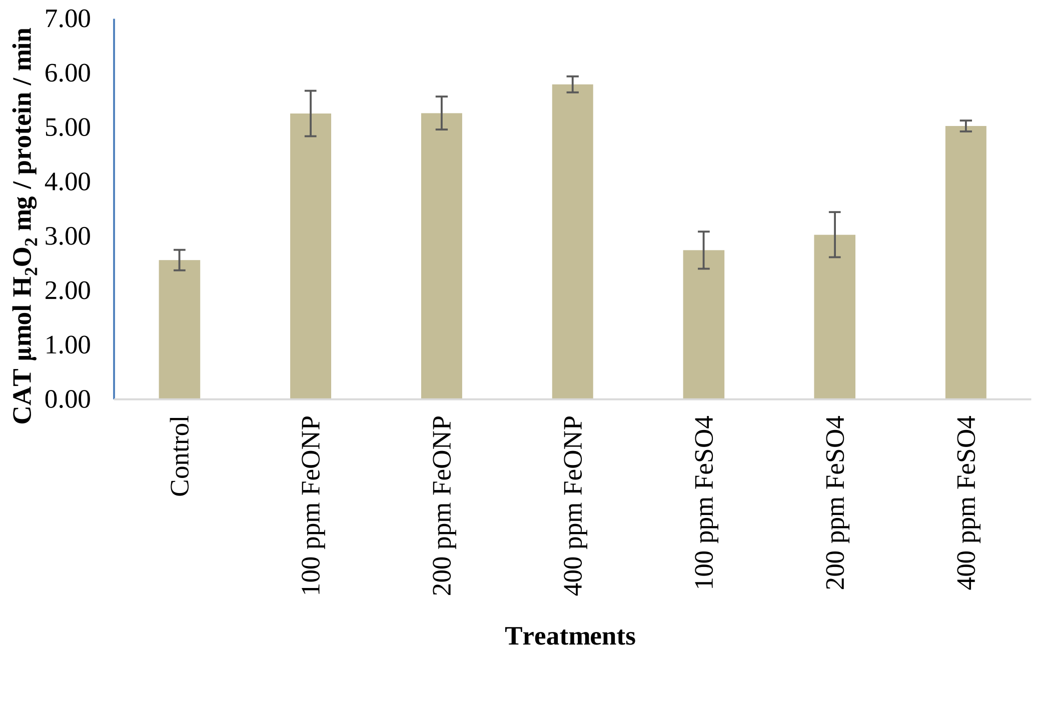


Fig. 7: Catalase activity in leaves of rice harvested at 10th day

DISCUSSION

Among various problems, death of seedlings at nursery stage due to deficiency of one or more nutrient element has drastically increased the overall production cost of farmers. Good quality seedlings can substantially decrease the mortality rate in the field as well as can enhance the production per hectare. Rice is grown on a variety of soils worldwide which often lacks important nutrient like zinc, iron, sulphur etc. Among various nutrients, rice seedlings are highly susceptible to iron deficiency which leads to death of seedlings in severe cases.

Traditionally, ferrous sulphate is applied to overcome iron deficiency, however, the amount of applications and the nutrient absorption efficiency of seedlings is very low[18]. These inability of seedlings due to large particle size of ferrous sulphate leads to overall loss to farmers. Reduction of size of these larger particles through nanotechnological intervention can enhance the nutrient uptake efficiency of seedlings as well as reduction of application dosage owing to enhance reactivity of nanoparticles. Till date there are very few reports on positive impact of nanoparticles due to utilization of higher concentration of nanoparticles. The present investigation therefore aims at assessing the impact of iron oxide nanoparticles on hydroponically grown rice seedlings.

Commercially available iron oxide nanoparticles dispersion was characterized using DLS and NTA. The behaviour of nanoparticle in dispersion tends to change and may leads to aggregation and formation of larger size particles. The results obtained in present investigation reveals uniform size with lower polydispersity index and good stability as observed through zeta potential measurement. Similar results were reported by Kouhi and co-workers, (2014) wherein size and zeta potential measurement of bulk and nano forms of zinc was assessed and found to be optimum which further inhibits aggregation of particles.

Maize and cabbage seedlings exhibited increased in fresh weight upon treated with silver and zinc nanoparticles[20]. The enhancement could be due to increased in plant growth regulators like cytokininins and gibberlins which are directly responsible for cell division and elongation, respectively. Zheng *et al.*, (2008) have also reported that nano-TiO_2 could promote photosynthesis and improve spinach growth. The results indicates that more number of reaction centers are in an 'open state' to carry out light reaction. Presence of higher number of open or oxidized electron acceptors in PS-II decreases the probability of generation of reactive radicals[22].

Accumulation of protein was found to be more in the present investigation in the nanoparticle treated seedlings compared to bulk and control seedlings. Similar increase in protein content was observed in leaves of maize treated with silica nanoparticles[23]. Iron is involved as co-factor for optimum activity of a number of enzymes which may be attributed due to metabolic balance between induction of proteins.

Superoxide dismutase isoenzymes catalyzes dis-mutation of super oxide anion leading to production of hydrogen peroxide which gets reduced to water by either catalases or peroxidases[24]. Antioxidant enzymes activity was found to be enhanced for all the three studied enzymes. Similar enhancement was observed by Vannini *et al.*[25], for silver nanoparticle and silver nitrate treated *Erucia sativa* leaves. The increase in CAT and POX activity in all the nanoparaticle treated seedlings suggest that the FeO NPs could effectively modify these enzyme activity under hydroponically grown rice seedlings. Similar increased in CAT activity was reported by Zhao *et al.*[26], in ten day old maize seedlings exposed to zinc oxide nanoparticles. The increase in activity clearly suggest that nanoparticle treatment could stimulate the peroxidase activity and helps the plant to overcome the abiotic stresses. Similar increment in growth parameters at lower concentration of ZnO NPs has been reported by Liu and co-workers, (2015) in maize. The growth enhancement by ZnO NPs may be attributed to release of Zn^{2+} ions more for plant growth.

CONCLUSION

The commercially available iron oxide nanoparticles could be effectively utilized for assessing its effects on plants as the supplied nanoparticle dispersion was found to be optimum in size, poly dispersity index with good stability as revealed by zeta potential. Further, the physiological and biochemical studies conducted in the present investigation could also be utilized for evaluating the growth inhibitory or promontory effect of metallic nanoparticles. Finally, from the study it can be concluded that nanoparticles if applied at optimum concentration can enhance the growth of plants both morpho-physiologically as well as biochemically.

Acknowledgement

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Conflict of Interest

The authors declare that they have no conflict of interest.

Authors contribution:

M. Mankad designed and conducted the experiment and prepared the manuscript with the assistance of A. Patel and P. Mankad; R. S. Fougat and Subhash N., mentor the whole experiment and G. Patil checked and corrected the manuscript.

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