

# Effects of Iron Oxide Nanoparticles on Chick Pea (*Cicer Arietinum*): Physiological Profiling, Chlorophylls Assay and Antioxidant Potential

Dr. Animesh Naskar<sup>1</sup>, Mitrabrata Goswami<sup>2</sup>, Aritra Ganguly<sup>3</sup> Rounak Ghosh<sup>4</sup>

<sup>1</sup>Assistant Professor & HOD, Department of Food Technology, Hemnalini Memorial College of Engineering, Maulana Abul Kalam Azad University of Technology, West Bengal -741246, India

<sup>2</sup>Assistant Professor, Department of Food Technology, Hemnalini Memorial College of Engineering, Maulana Abul Kalam Azad University of Technology, West Bengal -741246, India

<sup>3,4</sup>B.Tech in Food Technology, Hemnalini Memorial College of Engineering, Maulana Abul Kalam Azad University of Technology, West Bengal -741246, India

\*\*\*

**Abstract** - Seed priming potential of chick pea was investigated on exposure to different concentrations of Iron oxide nanoparticles (IONP). No obvious phytotoxicity was found to be shown on IONP priming whereas FeCl<sub>3</sub> and Fe<sub>2</sub>O<sub>3</sub> promoted adverse effects on seed priming. The germination was considerably improved on IONP priming and no significant impact on chlorophylls content observed in comparison with hydroprimed and unprimed control. Interestingly, the amount of lipophilic nonenzymatic antioxidants was increased in chick pea leaves on IONP priming at higher concentrations (150 and 200 mg L<sup>-1</sup>).

**Key Words:** Chick pea, Seed priming, Iron oxide nanoparticle, Antioxidant potential, Chlorophylls content.

## 1. INTRODUCTION

Crop yield is one of the major concerns for increasing population in the recent decade. Advancement of crop yield with effective method is an imperative scientific task. Chick pea (*Cicer arietinum*) is wealthy source of protein, fats, carbohydrates, as well as minerals cultivating in India, Middle Eastern cuisine, and Mediterranean region [1]. Chick pea is also famous in Philippines as they preserved it in syrup to eat as sweets. However, seed deterioration is one of the major problems due to exposure of adverse environmental conditions. Simple procedure to improve seedling vigour is known as seed priming which may reverse the negative impacts on seed deterioration. In this regards, priming has gained enormous importance to improve the physiological state, germination, and seedlings development of the seed [2]. Recently, nanotechnology offers novel opportunities in the field of agricultural biotechnology [3]. Exposure of nanoparticles can modulate the biochemical processes resulting to have positive and negative impacts on plant growth. Although the development of plants depends on the size, concentration, chemical composition and reactivity of nanoparticles [4]. Notably, the overall practice and information is relatively low in this perspective till date.

This research mainly focused on seed priming of chick pea using different concentrations of IONPs with profound

investigation of the seedling development, photosynthetic pigments, and antioxidant potential.

## 2. MATERIALS AND METHODS

### 2.1 Seed and chemicals

Chick pea (*Cicer arietinum*) was procured from local market. Iron oxide nano particle (IONP) with purity of 99.9%, 2,2'-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), and 2,2-diphenyl-1-picrylhydrazyl (DPPH) was supplied from Sigma-Aldrich. The particle size and specific surface area of IONP were <50 nm and 180 m<sup>2</sup>g<sup>-1</sup>, respectively. Ferric chloride (FeCl<sub>3</sub>.6H<sub>2</sub>O), iron(III) oxide (Fe<sub>2</sub>O<sub>3</sub>) powder (<6 μm), and potassium permanganate (KMnO<sub>4</sub>) were obtained from Merck, India.

### 2.2 Effect of IONP, FeCl<sub>3</sub>, and Fe<sub>2</sub>O<sub>3</sub> on seed priming

Chick pea seeds with uniform-sized were taken for priming under the effect on IONP, FeCl<sub>3</sub>, and Fe<sub>2</sub>O<sub>3</sub> solution. Before priming the seeds were sterilized using 10% sodium hypochlorite for 5 min followed by three-time washing with double distilled water and then air-dried at ambient temperature. Priming of seeds was carried out in different concentrations of FeCl<sub>3</sub>, Fe<sub>2</sub>O<sub>3</sub> and IONP solutions (20 mL) ranging from 10 to 200 mg L<sup>-1</sup>. Afterward, the seeds were taken out and air-dried. The primed and hydroprimed (control) seeds were sown in the plastic pots (8 cm width and 20 cm length) containing potting soil and placed in a glasshouse which was maintained at 12/25 °C (minimum night -time/maximum daytime temperature) with a photoperiod of natural dusk and dawn transition. Leaf samples were harvested from each experiment on the 9<sup>th</sup> day for chlorophyll quantification and antioxidant measurement was accomplished by leaf disc method.

### 2.3. Seed germination and seedling growth

After sterilization, IONP primed with different concentrations of IONP solution ranging from 10 to 200 mg L<sup>-1</sup>, hydroprimed and unprimed seeds were placed on in Petri plates using filter paper. The filter papers were moistened with double distilled water (5 ml) for

hydroprimed seed, and with 5 ml of IONP solution of different concentrations for respective IONP primed seeds. All the plates were incubated at 25°C for three days allowing germinating. The seed germination was recorded in each day, and the days (mean number) on the basis of 50% germination was determined after completion of three days. Afterward, seedlings were placed gently on the sterile test tube containing a 1% agar medium wherein the radicle was positioned just inside the agar. After 8 days of maintenance with germinating conditions seedlings were separated from medium to measure shoot and root length.

## 2.4. Chlorophylls determination

In order to determine the quantification of chlorophyll a and b, the frozen leaves of each treated plant were taken and ground manually. 20 mg of each sample was placed to separate microcentrifuge tubes (2 ml). Methanol (1.5 ml) was poured into these tubes and mixed through vortex for 10 s. Mixtures were kept in an incubator for 30 min under the dark condition. Afterward, the samples were centrifuged at 10500 rpm for 10 min. The sup of each sample (200 µL) was poured into a 96-well plate. A microplate reader was used to measuring the absorbance at 652 and 665 nm. The amount of chlorophyll a and b were determined by Wellburn's equation [5].

## 2.5. Antioxidant assay using Leaf disc-based method

The leaf disc assays in terms of DPPH, ABTS, and potassium permanganate reduction (PPR) were employed to analyze the relative antioxidant activities of experimented seedlings like different IONP primed, hydroprimed, and unprimed seedlings [6].

## 3. RESULTS AND DISCUSSION

### 3.1 Phytotoxicity measurement

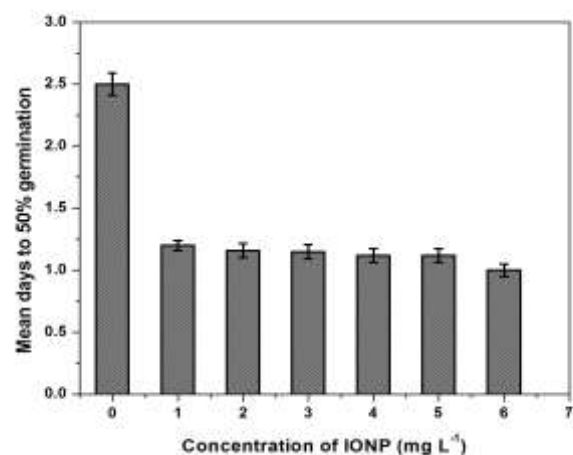
Analysis of phytotoxicity was carried out on the basis of chlorophylls and nonenzymatic antioxidants levels of hydroprimed, different IONP primed, FeCl<sub>3</sub> primed and Fe<sub>2</sub>O<sub>3</sub> primed seeds. Results show that chlorophylls content decreased in treatment with Fe<sub>2</sub>O<sub>3</sub> and FeCl<sub>3</sub> (150 and 200 mg L<sup>-1</sup>) hydroprimed seed. In addition, there was no change in chlorophylls content on IONP primed seeds under different concentrations (Data not shown). On the other hand, nonenzymatic antioxidant potentials of all types of primed seeds revealed an almost similar effect (Data not shown). Hence, IONP was found to be nonphytotoxic in comparison with studied Fe<sub>2</sub>O<sub>3</sub> and FeCl<sub>3</sub>.

### 3.2 Effect of IONP on Seedling Development, chlorophylls content, and antioxidant potential

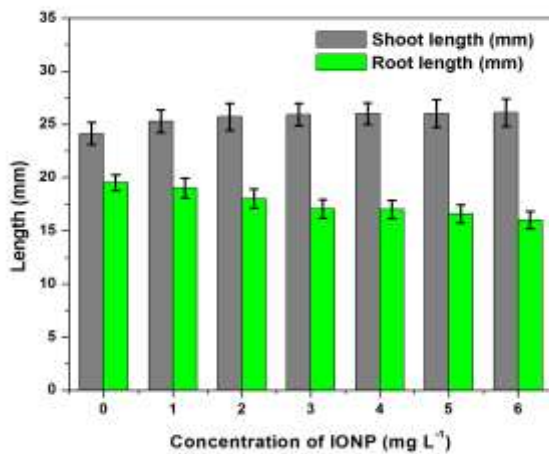
Fig. 1 and 2 show the results of 50% germination on mean days, seedling development, stem length and root length of IONP primed seeds under different concentrations. The

germination was significantly improved for IONP priming and hydropriming treatment compared to unprimed control. Besides, chick pea seedlings were found to be almost similar to both IONP priming and hydropriming treatment resulting in root and stem length. According to results, there were no significant changes in chlorophylls content of studied IONPs priming treatment compared to hydroprimed and unprimed control (Fig. 3). This is reported that IONP influences the synthesis of chlorophylls. Although, it is dependent on concentrations, seed types and plant species [7].

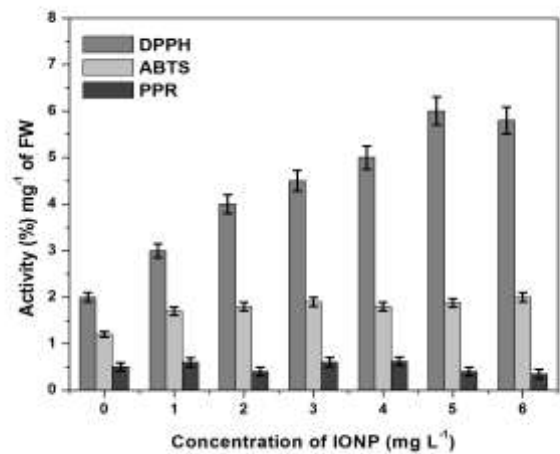
This is pertinent to mention that metallic nanoparticles can modulate the stress of reactive oxygen species (ROS) in plants resulting in the alteration of nonenzymatic and enzymatic antioxidant defenses [2]. This fact was well reflected in these experiments showing the increment of nonenzymatic antioxidant levels (DPPH assay) with IONP priming at higher concentrations (150 and 200 mg L<sup>-1</sup>). Whereas no obvious changes in antioxidant levels were found to be observed under PPR and ABTS assays in comparison with hydroprimed and unprimed control (Fig. 4). Thus, the outcomes of experiments suggest that the increment of lipophilic nonenzymatic antioxidants is noteworthy in chick pea leaves under IONP priming at higher concentrations (150 and 200 mg L<sup>-1</sup>).



**Fig -1:** Effect of different priming treatments on mean days to get 50% germination; 0)Unprimed, 1) hydroprimed, 2) 10 mg L<sup>-1</sup> IONP, 3) 50 mg L<sup>-1</sup> IONP, 4) 100 mg L<sup>-1</sup> IONP, 5) 150 mg L<sup>-1</sup> IONP, and 6) 200 mg L<sup>-1</sup> IONP



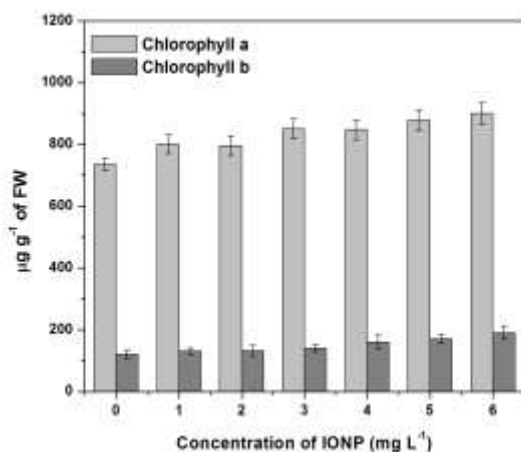
**Fig -2:** Length of root and stem of a chick pea in response to different IONPs and hydropriming treatments; 0)Unprimed, 1) hydroprimed, 2) 10 mg L<sup>-1</sup> IONP, 3) 50 mg L<sup>-1</sup> IONP, 4) 100 mg L<sup>-1</sup> IONP, 5) 150 mg L<sup>-1</sup> IONP, and 6) 200 mg L<sup>-1</sup> IONP



**Fig -4:** Seed priming with different concentrations of IONPs on antioxidants; 0)Unprimed, 1) hydroprimed, 2) 10 mg L<sup>-1</sup> IONP, 3) 50 mg L<sup>-1</sup> IONP, 4) 100 mg L<sup>-1</sup> IONP, 5) 150 mg L<sup>-1</sup> IONP, and 6) 200 mg L<sup>-1</sup> IONP

#### 4. CONCLUSIONS

In summary, IONP was found to be nontoxic for chick pea seed priming, seedlings development compared to the exposure of Fe<sub>2</sub>O<sub>3</sub> and FeCl<sub>3</sub>. IONP priming can be useful sustainably to enhance the nonenzymatic antioxidant potential in chick pea plant tissue. Nevertheless, a thorough study is required to apply this distinctive priming methodology through IONP in a later phase.



**Fig -3:** Seed priming with different concentrations of IONPs on chlorophylls; 0)Unprimed, 1) hydroprimed, 2) 10 mg L<sup>-1</sup> IONP, 3) 50 mg L<sup>-1</sup> IONP, 4) 100 mg L<sup>-1</sup> IONP, 5) 150 mg L<sup>-1</sup> IONP, and 6) 200 mg L<sup>-1</sup> IONP

#### REFERENCES

- [1] B.P.N. Chaurasia, B.M. Bara, Influence of priming on seed yield parameters of chickpea (*Cicer arietinum* L.), *Journal of Pharmacognosy and Phytochemistry*, 7 (2018) 753-755.
- [2] D.M. Kasote, J.H. Lee, G. Jayaprakasha, B.S. Patil, Seed priming with iron oxide nanoparticles modulate antioxidant potential and defense-linked hormones in watermelon seedlings, *ACS Sustainable Chemistry & Engineering*, 7 (2019) 5142-5151.
- [3] I. Plaksenkova, M. Jermaļonoka, L. Bankovska, I. Gavarāne, V. Gerbreders, E. Sledevskis, J. Sņikeris, I. Kokina, Effects of Fe<sub>3</sub>O<sub>4</sub> nanoparticle stress on the growth and development of rocket *Eruca sativa*, *Journal of Nanomaterials*, 2019 (2019).
- [4] H. Maswada, M. Djanaguiraman, P. Prasad, Seed treatment with nano-iron (III) oxide enhances germination, seeding growth and salinity tolerance of sorghum, *Journal of agronomy and crop science*, 204 (2018) 577-587.
- [5] R. Wellburn, The spectral determination of chlorophylls a and b, as well as total carotenoids, using various solvents with spectrophotometers of different resolution, *Journal of plant physiology*, 144 (1994) 307-313.
- [6] D.M. Kasote, G.K. Jayaprakasha, B.S. Patil, Leaf disc assays for rapid measurement of antioxidant activity, *Scientific reports*, 9 (2019) 1-10.
- [7] J. Hu, H. Guo, J. Li, Q. Gan, Y. Wang, B. Xing, Comparative impacts of iron oxide nanoparticles and ferric ions on the growth of *Citrus maxima*, *Environmental pollution*, 221 (2017) 199-208.