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RESEARCH ARTICLE

Effect of engineered iron-oxide and copper oxide nanoparticle on the germination and growth on soybean (*Glycine max* L.)

■ **SANGHDEEP GAUTAM, PRAGATI MISRA, PRADEEP K. SHUKLA AND P. W. RAMTEKE**

SUMMARY

The field of nanotechnology is one of the most active areas of research in modern materials science. Nanoparticles are known as a stimulating agent for plant growth and the activation of metabolic processes in plant and animal organisms.This research was planned to evaluate the potential effect of iron-oxide (Fe_2O_3) and copper-oxide (CuO) nanoparticles on soybean. The seeds to exposed soybean to different concentration of Fe and Cu NPs were surface sterilised and inoculated in Murashige annd Skoog media. Soybean seeds responded differently toward the pre- inoculated treatment at various concentrations of the nanocrystallineiron-oxide and copper oxide. Soybean seeds showed significant change in germination and seedling vigour index (SVI) as the concentration of nanoparticle increased.

Key Words : Iron-oxide,Copper-oxise, Nanopartcle, Seedling vigour index

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Agricultural production needs to maximize on sustainable basis to meet the continuously increasing food demand of rapidly growing population. Global warming may lead to increased probability of drought and temperature stresses. Continuously increasing use of agro-chemicals is threatening human health and environment. Producing more and quality food from diminishing land and water while sustaining agricultural resource base in an environment friendly way is a formidable challenge of this century. To increase productivities in a resource efficient way agriculture needs to be reinforced and revitalized with innovating science-based technologies

(Hafeez *et al.*, 2015). In recent decades, nanotechnology products have been intensively applied in agriculture. Nanoparticles are known as a stimulating agent for plant growth and the activation of metabolic processes in plant and animal organisms. Nanotechnology as a novel technology has potential to solve many problems in different fields of science and industry and has found its position and functions in agriculture. Nanotechnology has various functions in all stages from production, processing, storage, packing and transportation of agricultural products (Scott and Chen, 2003). Effects of nano particles on plants can be beneficial (seedling growth and development) or non-beneficial (to prevent root growth) (Zhu *et al.*, 2008)

Soybean [*Glycine max* (L.) Merr.,Fabaceae] is one of the most important crops for human and animal consumption and the most important organic components of soybean seed are proteins (about 40%) and oil (about 20%). It is extensively cultivated in India particularly in its central part. Due to having useful compounds such as unsaturated fatty acids, protein, mineral salts and plant secondary metabolites such as isoflavin, soybean has many important roles in human and animal nutrition.

Iron and copper are one of the essential micro elements for plant growth and play an important role in the photosynthetic reactions. Iron activates several enzymes and contributes in RNA synthesis and improves the performance of photosystems (Malakouti and Tehrani, 2005). Application of iron in low–iron soils can increase grain yield in soybean (GhasemiFasaei *et al.*, 2006).

MATERIAL AND METHODS

Synthesis of iron-oxide nanoparticles :

Nanocrystalline iron oxide with sizes from 30 to 60 nm wassynthesised following the aqueoussolution reduction method with sodium hydroxide asa reducing agent (Mascolo *et al.*, 2013). $FeSO_4.7H_2O$, $FeCl_3$ and NaOH were purchased from Merk chemical Reagent Company. For preparing 0.3M of $FeSO_4.7H_2O$, 4.15 g of $FeSO_4.7H_2O$ was dissolved in 50ml of deionised water and stirred for 10 min in 100 ml flask. In another beaker 4.85 g of $FeCl_3$ was dissolved in 50 ml of deionised water to make 0.6M solution of $FeCl_3$. For preparing 2M NaOH, 8g of NaOH was dissolved in 100ml of deionised water in 500 ml flask and stirred continously.

For the prepration of iron oxide nanoparticle 50 ml each of $FeSO_4.7H_2O$ and $FeCl_3$ was mixed together in a 200 ml beaker on magnetic stirrer. NaOH was added drop by drop into the beaker (at an average rate of one drop per second) to reduce the solution till the pH of 10±0.5 was obtained. A dark black precipitate confirms the formation of iron-oxide nanoparticle. Precipitate was removed from supernanat by centrifugation at 12,000 rpm for 15 min and repeateadly washed with absolute ethanol (Fig. A).

Fig. A : Synthesized crystalline iron-oxide nanoparticle

Copper oxide nanoparticle :

Copper (II) oxide nanoparticle was procured from Sigma Aldrich. The size was < 50nm particles size (TEM), molecular weight 79.55, (TEM, surface area 29 m^2/g, form nano power).

Soybean seed treatment :

Soybean seeds of variety JS335ds was procured from Chander Shekhar Azad University of Agriculture and Technology, Kanpur. Soybean seeds were soaked overnight in water dispersed with varying amount of iron oxide and copper oxide nanoparticle, respectively (200ppm, 400ppm, 600ppm, 800ppm and 1000ppm) along with control (distilled water) on a rotatory shaker. After being removed from the soaking, the treated seeds were ready for inoculation.

In vitro seed germination :

The germination experiment was carried out in plant tissue culture laboratory, Department of Molecular and Cellular Engineering, SHIATS, Allahabad for determining the treatment effect on seed germination and seedling vigour. Soybean seeds were sterilizedin laminar air flow containing 20 per cent sodium hypochlorite solution for 5min to ensure surface sterility and rinsed thoroughly with sterile distilled water followed by 0.1 per cent mercuric chloride solution for 2 min and repeated washing with sterile distilled water for six times. To investigate the promotory or inhibitory effects of nanoparticles on soybean plant growth, treated and surface sterlized seed were inoculated on Murashige annd Skoog (MS) agar supplimented with same concentartion of iron oxide nanoparticle used for the treatment. For every treatment a triplicate of culture bottle were prepared with five seed per culture bottle.

Statistical analysis :

Data collected were subjected to statistical analysis using software WASP2.1 (Web Agri Stat Package) for interpretation of results and inferences.

RESULTS AND DISCUSSION

The results obtained from the present investigation as well as relevant discussion have been summarized under following heads :

Characterization of the SDMP :

Scanning electron microscopy provided further insight into the morphology and size details of the iron oxide nanoparticles. Comparison of experimental results showed that the diameter of prepared nanoparticles in the solution was about 30-60 nm (Fig. 1).

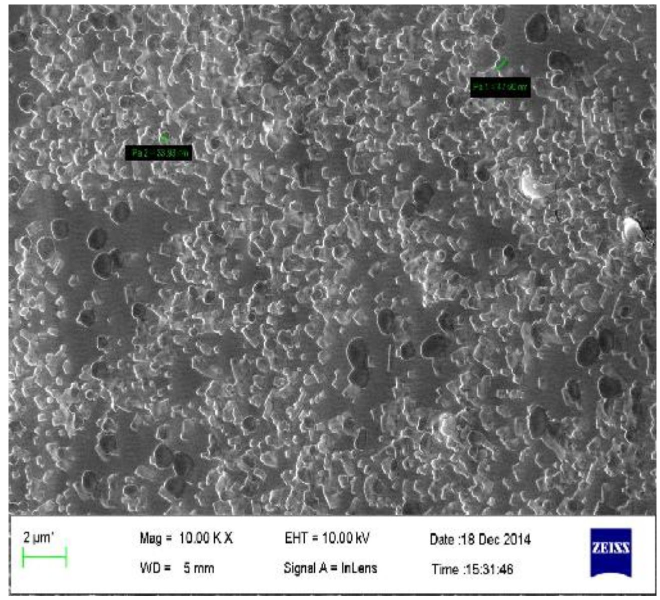

Fig. 1 : **SEM micrographs of iron-oxide nanopa rticle prepared by the aqueous chemical reduction method**

Seed germination and seedling vigour :

Soybean seeds responded differently toward the pre-sowing treatment at various concentrations of the nanocrystalline iron-oxide and copper oxide. The effect of nanocrystalline Fe, Cu on soybean germination and

Table 1 : The effect of iron-oxide and copper-oxide nanoparticles on germination and vigour (*in-vitro*)

Nanoparticle	Germination percentage	Root length	Shoot length	Seed vigour index (SVI)
Control				
	66.66	1.2	14.1	1019.89
200 ppm				
Iron oxide nanoparticle	73.33	3.43	20.6	1759.92
Copper oxide nanoparticle	76.66	2.3	17.66	1530.71
400 ppm				
Iron oxide nanoparticle	86.66	4.5	25.76	2617.13
Copper oxide nanoparticle	63.33	1.06	13.26	906.88
600 ppm				
Iron oxide nanoparticle	66.66	4.9	20.16	1666.5
Copper oxide nanoparticle	56.66	0.85	9.5	586.43
800ppm				
Iron oxide nanoparticle	56.66	7.9	10.3	1031.21
Copper oxide nanoparticle	43.33	0.71	6.7	321.07
1000 ppm				
Iron oxide nanoparticle	4.66	7.2	11.8	886.54
Copper oxide nanoparticle	23.33	0.53	4.4	115.01

aSeedlingvigor index (SVI)=germination (%)×(root length (cm) + shoot length (cm) $p > 0.05$

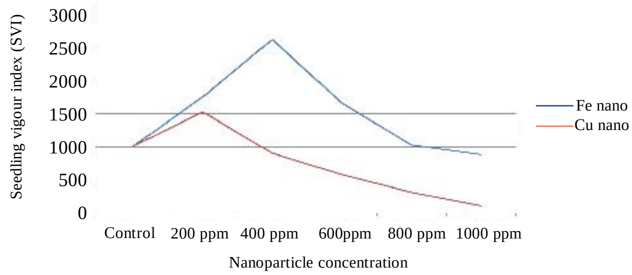

Fig. 2: Effect of iron-oxide and copper-oxide nanoparticle on growth

vigour depicted Table 1 and Fig. 2 showed that 400ppm and 200 ppm concentration, respectively demonstrated the best result in germination and seedling vigour compared to the higher doses.

The experimental data presented in Fig. 2 proved that increasing the doses exceeding 400ppm in case of Fe and 200ppm in Cu could inhibit the germination rate and seedling vigour. The nanocrystalline iron-oxide demonstrated the strongest promoting effect on germination and seedling vigour (Table 1, Fig. 2): at 400ppm concentration. Germination rates increased by 23.07 per cent and 15 per cent in Fe and Cu nanoparticles, respectively compared to the control sample, while seedling vigour index (SVI) increased 61.03 per cent and 50.08 per cent, respectively as compared to control. These results of nanoparticle doses are in good agreement with other researchers (Prasad *et al.*, 2012 and Pavlov and Folmanis, 1999) and implieda possibility to minimize the optimal doses toward smaller values.

Conclusion :

Nanocrystalline iron-oxide particle size ranging from 30 to 60 nm have been synthesized by the aqueous chemical reduction method using sodium hydroxide as there ducing agent. Nanocrystalline iron-oxide and copper oxide treated soybean seeds responded in various ways to different concentrations and exhibited the best biological effects on soybean growth and development, with the SVI surpassing 2.5 times and 0.5 times in Fe and Cu nanoparticles, respectively. Beyond the dose 400ppm and 200ppm of the nanocrystalline Fe and Cu, respectively studied expressed an inhibitive effect on germination and growth of the treated soybean plant. The effect of Fe nanoparticle was more evident in respect to Cu nanoparticle in terms of plant growth and development. It is evident that nanocrystalline iron-oxide and copper-oxide with extra low doses can be used for agricultural application.

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