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Comparative analysis of plant growth promoting activity of iron, silver and zinc oxide metal nanoparticles

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Abstract— Nanotechnology is a developing field in science and technology with diverse applications. Among the different types of nanomaterials synthesised, metal nanoparticles are widely used due to their enhanced catalytic, chemical and other physical properties. The field of agriculture is undergoing major quest for innovative methods to enhance the productivity of the crops. Metal ions are vital in plant growth as macro and micro elements for carrying out a number of physiological processes. These metal ions in the form of nanoparticles with multi-faceted properties are most of the time promoting the plant growth. Growth and development of plants is known to be influenced by a number of metal oxide nanoparticles and there are many established studies indicating the improvement of plant systems in the presence of the metals as nanoparticles. A comparative study was carried out with biologically synthesized iron, silver and zinc oxide nanoparticles on the seed germination and plant growth in potted plants of potato *(Solanum tuberosum)*, carrot *(Daucus carota)*, and beetroot *(Beta vulgaris).* From the study it is evident that silver and zinc oxide nanoparticles have promoted early germination as well as increased the shoot length in potato plant when compared to iron nanoparticles. A significant increase in shoot length when compared to the control has been observed with zinc oxide nanoparticles. Simultaneously the effect on micro flora of soil has been analysed which showed an increased in the presence of iron and zinc oxide nanoparticles and decrease with silver nanoparticles. The statistical analysis by ANOVA test revealed that the significance value $p < 0.05$ which states that there is a significant impact of metal nanoparticles on plant growth.

Keywords*: Metal nanoparticles, Solanum tuberosum, Daucus carota, Beta vulgaris, Germination and Growth.*

I. INTRODUCTION

Nanotechnology is a blend of science, engineering and technology which deals with manipulating matter at nanoscale to obtain materials with unique properties which can be put to novel uses [1]. In the field of agriculture the nanomaterials are used as nanobiosensors in crop protection, carriers of agrochemicals and genetic manipulation of plants for development of insect resistant plants [2]. Among the different types of nanomaterials synthesized metal nanoparticles are used in detection and measurement of crop nutrient status, insects, pathogens, weeds, moisture level, soil fertility and temperature.

Accumulation of metal nanoparticles in plants occurs in cellular compartments like nuclei, cell wall and vacuoles and affect physiological and biochemical processes [3]. Silver nanoparticles are known to exhibit antibacterial, antiviral and antifungal properties [4]. They have significant applications in agriculture since they are known to selectively inhibit fungi, bacteria on seeds and provide an alternate source of fertilizer that will help in improving and enhancing the germination and growth of plants [5]. Zinc is an essential nutrient required by all living organisms and is considered as an essential micronutirent in the metabolism of plants. It has a vital role in the synthesis of indole acetic acid from tryptophan, and regulates the functions of stomata by retaining potassium content of protective cells [6]. Studies have shown zinc oxide nanoparticles having a profound effect on growth of plants like maize, zucchini, wheat, rice, cowpea, cucumber, soybean and green pea and influenced root elongation in ryegrass, radish and rape. [7]. Among metal ions required by plants for development, iron is an important element in crops as it is essential in maintaining the structure of chloroplast and chlorophyll synthesis. Though it is most essential and fourth abundant element, it is not accessible for the uptake of plants. Hence iron nanoparticles are used, which have large surface area and high reactivity, increasing the rate of absorbtion [8].

Metal ions play a vital role in plant growth as macro and micro elements hence agriculture utilizes nanotechnology to enhance the yield of plants for various uses. The response of plants to various metal nanoparticles depends on the type of metal, plant species and growth stage but excess concentration of metal nanoparticles may lead to toxicity.

Some plants are capable of up taking nanoparticles and the plant cells interact with these nanoparticles which modify their gene expression and leads to enhancement of plant germination and growth [9].

The present study focuses on comparative effect of iron, silver and zinc oxide metal nanoparticles on *Solanum tuberosum* (potato), *Beta vulgaris* (Beetroot) and *Daucus carota* (carrot) for enhancing early germination of seeds and growth of plants. The study also aims to configure variations in the effect with respect to plant species and the extent of promotion or inhibition in the patterns of plant growth and micro flora of the soil. Significance of the results obtained was also statistically tested by ANOVA which showed the impact of nanoparticles on the growth of plants.

Related Work

Areeba Farooqui *et al*., in 2016 showed that iron nanoparticles increased the frequency of germination, enhanced the root growth and shoot growth and simultaneously reduced seedling vigour of wheat on soaking in nanoparticle suspension [10]. Similarly silver nanoparticles have significant applications in agriculture as they can protect the plants by selectively inhibiting fungal and bacterial pathogens on seeds. Earlier studies also showed that when wheat was exposed to silver nanoparticles there was no significant effect observed on growth parameters [11]. The effect of zinc oxide nanoparticles when studied on chilli plant, showed inhibition of root elongation, increased germination rate and early flowering. Zinc oxide nanoparticles have also potential to boost the yield and growth of food crops and release fertilizers which save fertilizer consumption and minimize environmental pollution. Syed Mohamad Afrayeem *et al*., in 2017 showed that zinc oxide at higher concentrations increase the seed germination, root length, shoot length and seedling growth that indicates the higher concentrations is not harmful to the chilli plant [12].

II. METHODOLOGY

Synthesis of iron nanoparticles.

6.5g of neem flowers washed thoroughly and boiled in 30ml of double distilled water for 25 minutes. The solution was cooled and filtered by using Whatmann filter paper no.1 to obtain the neem flower extract.10ml of the extract was added to 100ml of ferric chloride solution for formation of iron nanoparticles that was identified visually by colour change.

Synthesis of silver nanoparticles.

Culture supernatant of overnight grown *Bacillus* (SAJSBVC51) was added to 100ml of 10mM silver nitrate solution and centrifuged for 10 minutes at 6000 rpm. This solution was incubated at room temperature for 20 minutes to observe the colour change.

Synthesis of zinc oxide nanoparticles.

Culture supernatant of overnight grown *Bacillus* isolate was added to 100ml of 10mM zinc nitrate solution, centrifuged for 15 minutes at 10,000 rpm and heated for 3-4 minutes for formation of zinc oxide nanoparticles as precipitate.

Characterization of metal nanoparticles.

Metal nano particles synthesized by biological methods were characterized by visual colour change, FTIR and SEM analysis.

Potted plant experiment on germination.

Selection of potato tubers having eyes was done while beetroot and carrot seeds were purchased from Sunrise Kitchen Garden and used for planting. Three pots (A, B and C) were taken and soil containing a mixture of neem, coir peat and vermicompost was added. The sowing was done in a layer wise pattern. Soil was added at the bottom of the pot and then horizontally cut potato tubers with eyes were placed and another layer of soil was added on top of the potato tubers. This was repeated for 2 more times. The same method was carried out for the planting of beetroot and carrot plants. After sowing, 1ml of iron nanoparticles/50g of soil (3.4g/50g of soil), 1ml of sliver nanoparticles/50g of soil (21.3g/50g of soil) and 1ml of zinc oxide nanoparticles/50g of soil (22.3g/50g of soil) were added to pots A and B drop wise with help of a micropipette while pot C was taken as control. The pots were sprinkled with 10ml of water once a day and the height was measured at an interval of 7 days for 32 days.

Determination of variations in shoot length.

Experimental pots were periodically observed at an interval period of 7 days up to 32 days. The variation in plant shoot lengths was measured with a measuring scale with respect to presence and absence of nanoparticles. The correlation between the presence of nanoparticles and growth has been evaluated statistically.

Enumeration of Bacteria rhizospheric soil.

After the sprouting of plants (potato, beetroot and carrot) 1g of rhizospheric soil treated with metal nanoparticles was enumerated for the bacterial population present in it. The enumeration was done by dilution plate technique. 0.1ml from dilutions 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6} and 10^{-7}were plated with appropriate controls. The plates were incubated at 37 ^{o}C for 24 hours. Colony forming unit (CFU) was calculated for each plate to determine the effect of nanoparticles on rhizosphere bacteria.

III. RESULTS AND DISCUSSION

Generally plants are capable of taking up nanoparticles which may modify their gene expression and leads to enhancement of seed germination and plant growth. The present study makes a comparative analysis of biologically synthesized iron, silver and zinc oxide metal nanoparticle for enhancing early germination of seeds and plant growth in potted plants. Initial stages of the study started with the biological synthesis of iron, silver and zinc oxide metal nanoparticles.

Synthesis of iron nanoparticles.
Iron nanoparticles were synthesized by adding filter sterilized neem flower extract to 10mM $FeCl_3$ solution resulted in the transformation from yellow to green colouration as depicted in Figure 1 indicated the presence of iron nanoparticles.

Synthesis of silver nanoparticles.
Silver nanoparticles were synthesized using *Bacillu*s culture supernatant which was already established in the laboratory [13]. Addition of 1 ml of overnight grown culture supernatant of *Bacillus* to 100 ml of 10mM silver nitrate solution resulted in the formation of dark brown precipitation which indicated the formation of silver nanoparticles (Figure 2).

Synthesis of zinc oxide nanoparticles.
Microbial synthesis of zinc oxide nanoparticles was done using overnight grown *Bacillus* culture supernatant to 10mM Zinc nitrate solution which resulted in the observation of white precipitate indicating the formation of Zinc oxide nanoparticles.

Characterization of metal nanoparticles.
Biologically synthesized metal nanoparticles were characterized by FTIR analysis. This analysis is usually done to identify interaction of nanoparticles with the proteins. The peaks are specific for the functional groups of amino acids that make up the proteins. The peaks found in the Figure 4 revealed the formation of nanoparticles during the interaction with biomolecules. Characterization by SEM analysis is normally carried out to determine the size and shape of nanoparticles. SEM analysis pictures are represented in Figure 5, in which the size was found to be ranging from 4.7 nm to 127 nm and mostly spherical in shape.

Comparative analysis of metal nanoparticles effect on germination.
Three plants were taken for the comparative analysis of metal nanoparticles in potted plant experiments. Germination of plants was monitored at an interval of 7 days for 32 days which is depicted in Figure 6. Nanoparticles enriched pots showed faster germination of plants than their respective control pots. 50 % impact on the germination of potato plants was observed in the presence of silver and zinc oxide metal nanoparticles than compared to iron nanoparticles.

In the present study presence of iron nanoparticles has shown germination by fifth day, while silver nanoparticles and zinc oxide nanoparticles showed germination by third day of planting when compared with their respective controls which germinated by eight day. With respect to the controls, the test pots showed two days early germination patterns indicating impact of metal nanoparticles. Similar observations were also reported by N. Savithramma, *et al*., 2012 where preponement in the germination of rice seeds was found. The increased nutrient uptake by the seeds treated with nanoparticles was reported. [14].

Effect of Metal nanoparticles on plant growth.
During the experiments potato plant growth was monitored at regular weekly intervals and the obtained data is graphically represented in Figure 7. The ANOVA test was applied to check whether there is any significant difference in growth of potato plant with respect to Nano particles and also tested weekly growth of potato plant. Since $p < 0.05$, there is some significant difference in growth with respect to different Nano particles. Also, $p^* < 0.05$, there is a significant difference in weekly growth of potato plant. Also, the experimental studies observed that the growth (in cm) of potato plant is high in the pot with zinc nano particles.

Figure 8 represents comparison of different Nano particles (Iron, Silver and Zinc) used in growth of Beetroot Plant. Since $p < 0.05$, there is some significant difference in growth with respect to different Nano particles. Also, $p^* > 0.05$, there is no significant difference in weekly growth of beetroot plant. Also, the experimental studies observed that the growth (in cm) of beetroot plant is high in iron Nano particles pot where as there is no growth in 3rd and 4th weeks in control groups.

Figure 9 represents comparison of different Nano particles (Iron, Silver and Zinc) used in growth of Carrot Plant. The ANOVA test was applied to check there is any significant difference in growth of Carrot plant with respect to Nano particles and also tested weekly growth of carrot plant. Since $p < 0.05$, there is some significant difference in growth with respect to different Nano particles. Also, $p^* < 0.05$, there is a significant difference in weekly growth of carrot plant. Also, the experimental studies observed that the growth (in cm) of carrot plant is high in Silver Nano particles pot.

Enumeration of rhizospheric bacteria of soil.
No significant increase in bacterial colonies were observed when the rhizospheric soils of potato plant from the experimented pots were tested as CFU/ml**.** Bacterial colonies count was found to be similar with iron and zinc oxide nanoparticles while there was reduction in bacterial colonies with silver nanoparticles. There was a significant increase in the bacterial colonies in the rhizospheric soils of beetroot and carrot plant enriched with iron, silver and zinc oxide nanoparticles. Interestingly a decrease in the bacterial

colonies was noticed in the experimented pots containing silver nanoparticles (Figure 10).

Figure 1. A.10mM $FeCl_3$ and B. Iron nanoparticles

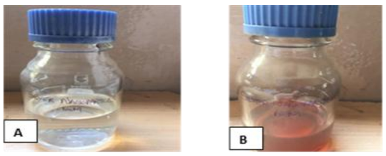

Figure 2. A.1mM $AgNO_3$ and B. Silver nanoparticles

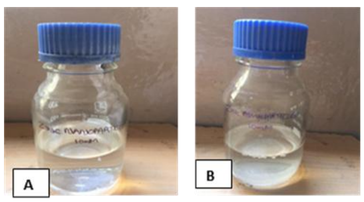

Figure 3. A.10mM $Zn(NO_3)_2$ and B. Zinc oxide nanoparticles

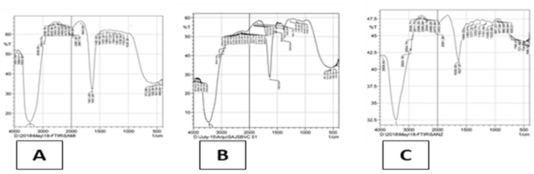

Figure 4. FTIR Analysis: A. Iron nanoparticles B .Siver nanoparticles C. Zinc oxide Nanoparticles

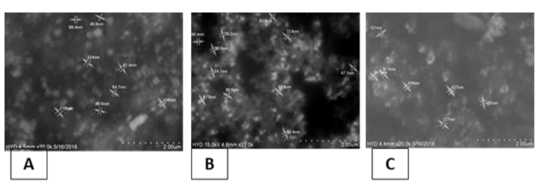

Figure 5. SEM Analysis: A. Iron nanoparticles B .Siver nanoparticles C. Zinc Nanoparticles

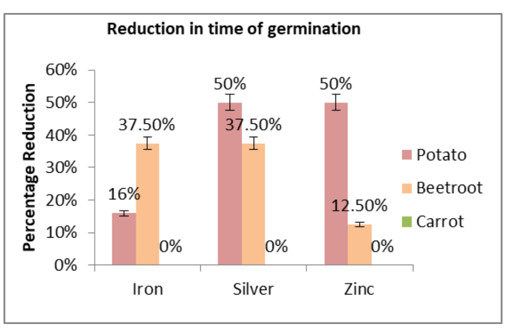

Figure 6. Comparative analysis of metal nanoparticles on germination.

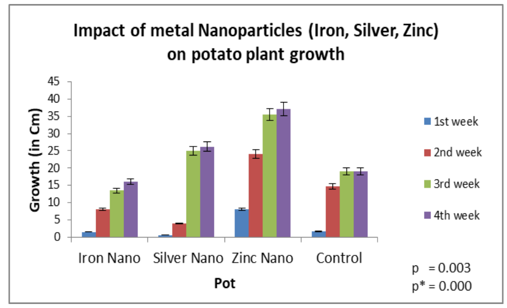

Figure 7. Impact of metal nanoparticles on height of potato. plant.

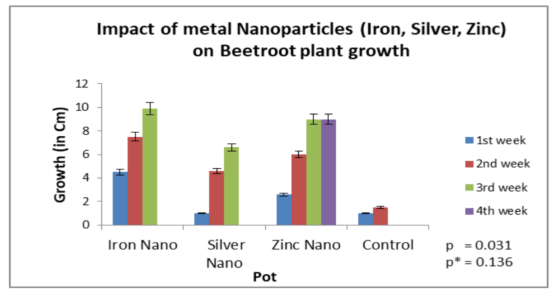

Figure 8. Impact of metal nanoparticles on height of beetroot plant.

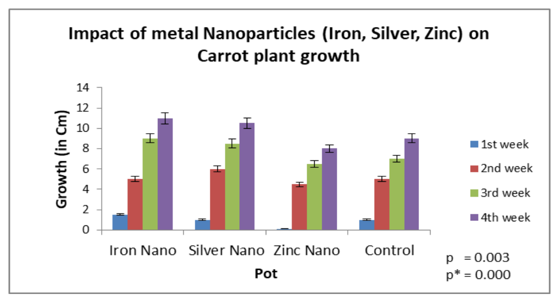

Figure 9. Impact of metal nanoparticles on height of carrot plant.

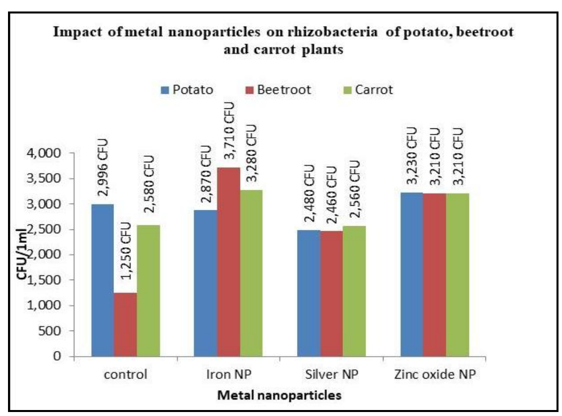

Figure 10. Impact of metal nanoparticles on rhizobacteria of potato, beetroot and carrot plants.

IV. Conclusion and Future Scope

Potted plant experiments with three metal nanoparticles were observed to influence the germination and growth of potato, beetroot and carrot plants. During the study it was observed that silver and zinc oxide nanoparticles were more effective on potato plant. Interestingly, soil enrichment with metal nanoparticles showed a variation in microbial population. Further optimization studies are required to effectively utilize nanoparticles for plant growth promotion.

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