



"EFFECT OF VARIOUS FACTORS ON SEED GERMINATION STUDIES OF ZINNIA ELEGANS"

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ABSTRACT;

The present studies were conducted to develop in vitro cost-effective micropropagation, ex vitro seed germination, and the effectiveness of green ZnO nanoparticles on *Zinnia elegans*. Cost-effective micropropagation was performed using three types of media. MS1 medium supplemented with (NAA 0.5mg/l and BA 1mg/l), MS2 medium supplemented with (NAA 0.5mg/l and BA 1.5 mg/l), and Isabgol cost-effective media supplemented with (0.5mg/l NAA and BA 1 mg/l) were used for in vitro establishment of the culture. Tap water was used instead of distilled water and table sugar instead of sucrose powder for production. It was observed that the production rate is slower than with standard MS media. Ex- vitro seed germination was done on the best six plants of *Zinnia elegans*. Data was noted for seed germination percentage, seed vigour, Max average shoot length, Max average root length, and Max average secondary root length. P-value, dry weight, and wet weight of an ex-vitro seed germination were noted down. Effects of green ZnO nanoparticles on Ex- vitro seed germination were obtained by using three plant extracts *Rhododendron arboretum*, *Cannabis sativa*, and *Santalum album*.

Keyword- Cannabis, Cost-Benefit Analysis, Dietary Sucrose, Germination, *Rhododendron santalum* Seeds, Zinc Oxide, Plant Extracts Water

1. INTRODUCTION

Zinnia (Zinnia elegans), is a member of the Asteraceae family and originates from Mexico. The magnificent annual summer flower, zinnia is quickly gaining popularity due to its wide array of vibrant blossoms. Its flowers have extended vase lives, strong stems, bright, consistent hues, and disease resistance (Dole, 1999). The petals of the blooms may be arranged in a single row or take on a dome-like shape and be colored purple, lilac, or crimson. They may

grow from seeds and can be used as an intercrop. The zinnia is an indigenous flower to North America, coming from Mexico and Central America. Zinnia is grown straight from seeds in India, they are grown as a container plant, cut flower, and bedding. Like lengthy shelf life, extensive marketing as a cut flower, and availability in different forms and colours are the reasons for its commercial importance. It is suitable for growing in all environments, except for poorly aerated soils, which may result in root illnesses because they cannot resist arid and hot climate conditions. It has been using various approaches to address the obstacles involved in zinnia propagation including limited viability of seeds, low germination rates, and high risk of disease infection which further restricts seed production. Overcoming this issue, we discovered that the nanoparticle extraction method is highly beneficial for the productivity of flowers. Nanotechnology deals with nanoscopic dimensions ranging from (1nm to 100nm), these nano compounds are rapidly and completely absorbed by plants fixing their nutrient shortages and growing. Nanoparticles of Gold (Au), Silver (Ag), Copper (Cu), Zinc (Zn), Aluminium (Al), Silica (Si), Zinc oxide (ZnO), Caesium oxide (Ce₂O₃), Titanium dioxide (TiO₂), and Magnetised iron (Fe) have various applications in agriculture. Zinc Oxide a micronutrient, is essential for plant growth and development under its optimum range. In several plant species, diverse effects have been reported after nanoparticle application, such as the stimulation or inhibition of seed germination/seedling growth activation of genes involved in metabolism, induction of photosynthesis or reactive oxygen species generation, and chromosomal aberrations. NPs containing herbicides, fertilizers, or nanoencapsulated nutrients, which are more effective on plants, are applied in agriculture and horticulture. This study intended to investigate and compare the effects of different green ZnO NPs based on plant extracts at distinct concentrations. Additionally, various attempts have been made in the present investigation to explore the possibility of cost reduction in mass multiplication of Zinnia by the benefit of cheaper medium components to improve the cost effectiveness of plant tissue culture technology.

MATERIAL AND METHODS

Synthesis of Green Zinc Nanoparticles: We acquired the synthesis of green zinc nanoparticles (Zn-NPs) using the methods of Kathad and Gajera. The presence of brownish-blue particles indicates the formation of zinc nanoparticles. We prepare the nanoparticles by washing them with ethanol, drying them in a hot air oven at 60 °C, and dissolving them in 100 ml of distilled water. We characterized these nanoparticles with a UV-visible spectrophotometer.

Source of Explant: Zinnia seeds sourced from Sector 26, Grain Market in Chandigarh served as the basis for the explants. To remove contamination, we rinsed the seeds under running water for a few minutes. After that, we soaked the seeds for 10 minutes in a solution of Teepol. Before rinsing the seeds three or four times with distilled water, we treated them with a fungicide for 10 minutes. To remove residual sterilant, we cleaned the surface with autoclaved distilled water two or three times after sterilizing it with 0.1% mercuric chloride for three minutes. For the surface sterilization, we used a laminar airflow.

Preparation of MS Media: Zinnia elegans shoots that had already been planted were subjected to mass multiplication in a Murashige and Skoog (MS) medium that included 3 grams of sugar and varying amounts of growth hormone regulators like NAA and BA. MS1 media supplemented with 0.5 mg/l of NAA and 1 mg/l of BA. In MS2 media 0.5 mg/l of NAA and 1.5 mg/l of BA were added. We utilized a cost-effective method to establish explants and multiply *Z. elegans* cultures in vitro. One approach was to use standard MS medium supplemented with BA 1mg/l + NAA 0.5 mg/l. Another option was to use BA 1.5mg/l + NAA 0.5mg/l, which involved substituting sugar for sucrose, tap water for distilled water, and Isabgol for agar. All the media had their pH levels adjusted between 5.6 and 5.8 using either 1 M NaOH or HCl.

Ex-vitro Seed Germination:

We placed the seeds on petri dishes containing soaked tissue paper with distilled water under a laminar air flow. We then moved the petri dishes to the tissue culture room. After 2-4 days, we saw that the seeds had begun to sprout. We deposited five surface-sterilized *Zinnia* seeds on each Petri dish. We nanosuspensions each of the three types of green Zn nanoparticle samples in distilled water at concentrations of 100%, 50%, and 25%. Before observing seed germination after a few days, we sterilized Zn-NPs for 1.5 hours. Next, we placed the seeds onto filter paper- soaked petri plates. Assessing morphological data After the seeds germinated, we measured their root and shoot lengths. Incubate the petri plates in a light/dark cycle of 16:8, at a temperature of 25 ± 2 °C and a relative humidity of $70 \pm 10\%$. *Zinnia* treated with Zn-NPs had their germination data supplemented after seven days, using the following formula: % Seed germination = Number of seeds germinated / total number of seeds x 100 Preparation of extract and Biochemical assays

Data Collection and Statistical Analysis:

We observed the in-vitro germination and growth of the plants on control media, media containing different concentrations of growth hormone regulators, and media using isbagol instead of agar for *Zinnia* germination. We calculated the germination percentage after the radicals emerged. We calculated the percentage seed germination by dividing the number of germinated seeds by the total number of seeds, multiplied by 100. We recorded the data for shoot length and root length in centimetre (cm) after 15 days of culture. We also calculated the seed vigour index and tolerance indices for *Zinnia elegans*.

We experimented with a completely randomised design with three replicates, repeating each treatment three times. Data is statistically analyzed by analysis of variance (ANOVA) and tested for significance using Fisher's protected least significant difference (LSD) at $p \leq 0.05$.

Results and discussion

Effectiveness of Green Zinc Oxide Nanoparticles on Seed Germination Assay of *Zinnia*

NPs have extremely high levels of chemical reactivity and biological activity because of their smaller size and higher surface-to-volume ratio. These nanoparticles are exposed to different tissues and fluids, quickly cling to surface macromolecules with which they clash and interfere with other proteins' and enzymes' regulatory mechanisms. Because of their small particle size, metal oxide nanoparticles (NPs) produce more free radicals and reactive oxygen species (ROS) than other nanoparticles. Although the NPs have some detrimental effects on the environment, they can also be helpful to crops. Small amounts of zinc and silver are valuable to healthy development and seed germination. The ZnS treatment significantly affects the length of the root shoot, the pigment content, and the germination of seeds. It also increases antioxidant activity.

Ag₂S, on the other hand, is an antibacterial agent that affects germination. The ZnS, Ag₂S NPs, and their NCs control the uptake of nutrients, enhance light absorption, and boost metabolism to influence the germination. It was also observed that all the other concentrations of nanoparticles were found to be inhibitory except 25% percent and 50% concentrations of Santalum album and 100% concentration of Rhododendron arboretum of green ZnO nanoparticles. Only a 30 per cent germination rate was observed in these concentrations. At 100% concentration, it was the best among all the nanoparticles used in this study. The average root length was (3.06 ± 0.12) cm and the shoot length was (2.5 ± 0.081) cm. Then the p-value of 100% concentration was 0.0026 which was significant. No seed germination was reported at any other Rhododendron arboretum concentration (50%, 25%) based on green ZnO nanoparticles. Among different concentrations of Santalum album based on green ZnO nanoparticles tried for ex-vitro seed germination of *Zinnia elegans* only 25% and 50%

concentration was found to be effective average root length obtained at these concentration(25%,50%) was (0.53 ± 0.32) cm, (2.2 ± 1) .and the average shoot length was (2.06 ± 0.309) cm, (2 ± 0.08) cm. In the case of *Cannabis sativa*, there was no seed germination at any concentration(100%, 50%, 25%). The impact of Zn-NPs at low concentration on the maximum mean shoot length and leaf length, further increase in Zn-NPs concentration results in accumulation of Zn-NPs in the shoot as well as in the leaf too resulting in browning of the shoot which may be due to the absorption of Zn-NPs via roots. And that some plants can uptake NPs and accumulate them in their tissues. In the present study, data analysis of shoot length and root length of *Zinnia* treated with Zn-NPs was observed and showed a positive response for growth, being a micronutrient, acts as a metal ion for metalloenzymes involved in electron transport, protein and carbohydrate biosynthesis which plays an important role in plant regeneration thus improving the growth parameters of plants treated with Zn-NPs. Growth parameters including shoot length and shoot biomass were reduced when the concentration of Zn-NPs went above 1000 mgL⁻¹ compared to the control. The number of roots increases with an increase in Zn-NP concentration in the current study. Our findings compare with other studies In 2021 N thakur et al. reported that Seedlings of wheat treated with Cu-NPs and seed germination rate were increased as compared with the control plate which had only distilled water and the germination rate was 100%. Reduction was also observed due to fungal contamination of the treated seedlings compared to the control. However, in contrast, many available studies observed a negative impact of Cu -NPs on percentage seed germination with an increased concentration due to the size, shape, and toxic nature of these particles. The toxicity of Cu-NPs may arise due to the formation of metal oxide. The lack of a negative impact of Cu-NPs on % seed germination may be because of the presence of the protective seed coat. According to A. H. Alsaeedi *et al.* (2019), all nano-silica treatments markedly increased the percentage of germination when compared to seeds that were left untreated with nano-silica (control). On the other hand, after 10 days, treatments with 200 and 300 mg L⁻¹ nano-silica resulted in 100% final germination percentage, whereas 77.7% of control seeds germinated. In 2020 R. Pazak *et al.* reported that Ag-NPs were more effective at cold temperatures, at cool temperatures, notable variations were seen, except for the seed sample subjected to Ag-NP treatment at 2.5 mg dm⁻³. In the case of the "Bali" cultivar and the "Delfina" cultivar, the germination capacity of seeds treated with Ag-NPs at 0.25 and 1.25 mg dm⁻³ was higher than that of the control seeds by 9.3% and 9.8%, respectively. According to H. Guo et al. (2022), when wheat seed samples were treated with green ZnO-NPs at a moderate dosage of 62 mg/L, their root length increased significantly ($p < 0.005$) compared to other concentration levels by 50%.Furthermore, ZnO-NP treatment at 50 ppm has been shown to promote seedling growth and decrease excessive ROS formation, but 500 and 1000 ppm concentrations have been shown to have detrimental effects on rice seedling growth.

Ex - vitro Seed Germination:

Ex- vitro seed germination was done on the best six plants of *Zinnia elegans*. Data was observed Max average shoot length was $(6.48 \pm 1, 27)$ cm, Max average root length was (2.31 ± 0.954) cm and Max average secondary root length was (1.83 ± 0.37) cm. According to PJ Zale (2022), M551 and Knudson C had the fastest initial germination rates; however, after two weeks, the germination rate had dropped. The germination process on P723 started slowly, but by week 20, seedlings were growing and the overall germination was higher than on the other two media.

Some of the seedlings even made it to stage 5, while others only reached stage 4. The Tulasnella isolates were used in an experiment by Y Ozdener Kompally *et al.* (2022). The seeds inoculated with this isolate developed up to S3 (the first photosynthetic leaf) and S4 (seedling with advanced leaves). The germination rates of the seeds with these isolates were

93.2%, 94%, and 90%, they germinate better in the presence of fungus. S. Oli et al. (2023). They tested the effects of urea and graphene-based metal composite (GMC) on the germination and performance of *B. Chitria*. GMC showed maximum germination at 30 ppm (75%) and urea at 15 ppm (79%).

Findings reveal non-significant effects of GMC which can be used as an alternative fertilizer.

Seed Vigour Index (S. V.I.):

A seed can germinate quickly and uniformly to develop later under different environmental conditions largely dependent on its vigour. The network of molecular processes that regulate seed germination and seedling emergence is called seed vigour. Seed vigour index is calculated by multiplying germination (%) and seedling length (mm). Maximum average seed vigour in Ex- vitro conditions was obtained (729. According to A. H. Alsaedi *et al.* (2019), all nanosilica treatments had greater VI than control seeds. 200 mgL⁻¹ nano-silica was found to give seeds the greatest measured VI. Elevated CO₂ at 720 ppm decreased seed nitrogen content, substrate availability, and subsequent translocation, according to a paper by Lamichaney *et al.*, (2019). As a result, atmospheric [CO₂] exceeding 610 ppm decreased rice seed vigour U Bose et al., 2020 inside it Numerous parameters, including the quality of messenger RNAs retained throughout the seed maturation stage, proteostasis, DNA integrity, cell metabolism, and connections with hormone pathways, have been revealed to play a vital role in seed vigour through experiments utilizing "omics-based" techniques. Reduced ability of seeds to perform all physiological processes during germination is associated with loss of seed vigour. Reduced seed vigour results from physiological ageing, which also affects protein synthesis, enzyme activity, and the integrity of cell membranes. The validated markers and novel markers reported by S. Sahoo et al. (2020) that were shown to have a better R² value will help enhance rice seed vigour P Magena *et al.* (2021) reported that germination frequency in all cultivars was influenced by seed vigour and the storage time of seeds. The performance of the seeds for germination in vitro, and the growth of the seedlings were all influenced by the decline in seed vigour. Pearson's correlation value for the germination frequency analysis showed a favourable link between germination set against storage length and seed vigour. Deterioration of seed vigour was mirrored in the decline in the frequency of shoot development from explants produced from seedlings. It also showed that seed germination was poor.

N Thakur *et al.* 2021 reported that the seed vigour index of PBW 677 decreased significantly with increasing copper nanoparticle concentrations. The seed vigour index of PBW 725 was independent of the copper nanoparticle concentrations, as the Seed vigour index was variable at lower concentrations. It was found to be maximum at a 5ppm concentration of Cu-NPs. However, the Seed vigour index was reduced to a much lower value at high copper nanoparticle treatment (100 ppm), which was observed for both varieties significantly higher with increasing copper nanoparticle concentrations.

Dry weight and Wet weight:

Dry weight in a plant mainly refers to the weight of a plant that has been measured after drying the plant or part of a plant at higher temperatures. Here plants are dried completely to remove all the moisture or water content in the plant cells and tissue. Dry weight of the best six plants was observed in Ex- vitro seed germination (0.018kg). Wet weight in the plant, containing the chemical of interest is not dried to remove water. The amount of the chemical found in subsequent analysis is expressed as the weight of the chemical divided by the total weight, including any water present, of the material that once contained it. The wet weight of the best six plants was observed (0.315g).

A. H. Alsaedi, *et al.* in 2019 presented data, application of nano-silica by 200 mg L⁻¹ achieved the highest value Wet weight, dry weight, and RWC recording 0.317 and 0.022 g germinant

and 93.0 %, respectively. However, the difference between other nano-silica treatments (e.g. 100, 300, and 400 mg L⁻¹) was insignificant. L Rossi *et al.* (2019) zinc oxide nanoparticle (ZnO NPs) positively affected the wet weight and dry weight of roots and leaves, with wet weight being increased by 37% (root) and 95% (leaves) when compared to the control. The dry weight increase was 28%, 85%, and 20% in roots, stems, and leaves, respectively. The net photosynthetic rate increased 55% in response to ZnO-NPs treatment at the end of the experiment.

H.A.Begum *et al.* (2021) The wet and dry weight determines the effects of UV radiation on germination and growth. On the 16th day of germination, dry and wet weights decreased compared to the control. In both species (*Brassica rapa* L. and *Eruca sativa* L.). According to results published by A. H. Alsaeedi *et al.* in 2019, using 200 mg L⁻¹ of nano-silica produced the highest values of wet weight, dry weight, and RWC, recording 0.317 and 0.022 g germinant and 94.0 %, respectively. According to Rossi *et al.* (2019), the favourable effects of zinc oxide nanoparticles (ZnO-NPs) on both wet and dry weight were reported. For roots, stems, and leaves, the increases in dry weight were 28%, 85%, and 20%, respectively and the wet weight of roots and leaves increased by 37% in roots and 95% in leaves compared with the control. At the conclusion of the trial, the application of ZnO-NPs raised the net photosynthetic rate by 55%. Begum H.A. *et al.* (2021) reported that wet and dry weights were used to determine the effect of UV radiation on seed germination and growth. In comparison to the Control, both the Wet and Dry weights dropped on the sixteenth day of germination in the presence of UV in (*Eruca sativa* L. and *Brassica rapa* L.) both species. In the *Scirpus grossus* plant, the Wet weight and dry weight of the plant and the water content inside the plant can be determined which starts at 90% and decreases to 70% on the 30th day of germination.

CONCLUSION

In conclusion, the results presented here show that it is possible to improve the production of *Zinnia elegans* plants in vitro. Moreover, *Zinnia elegans* seeds and seedlings seem to be rewarding objects for further fundamental studies concerning the relationship between cytokinin and the systems that regulate their activities. Dry seeds containing quiescent embryonic cells represent novel objects on which to study the development of these functions. Ex vitro seed germination shows the seed germination percentage of *Zinnia elegans*. Value of Seed vigour, Moisture content, Maximum average shoot length, root length, and secondary root length. In ex- vitro seed germination, P-value was also observed which is significant and the seed germination of six plants was 83% Systematic studies to highlight the effect of ZnO-NPs on *Zinnia elegans* seedlings. The results revealed that ZnO NPs at concentrations of 100% 50%, and 25% had different results in three different green nanoparticles of plant extract (*Cannabis sativa*, *Rhododendron arboretum*, *Santalum album*) in seed germination, shoot/root elongation, there was no seed germination in case of *Cannabis sativa*, in case of *Rhododendron arboretum* seed germination observed only in 100% concentration and in *Santalum album* seed germination was observed at (50%,25%). The p-value was observed in the case of 100% concentration of *Rhododendron arboretum* but the P-value was not observed in the case of *Cannabis sativa*. In the *Santalum album* only at 50%and 25% concentration was a P-value observed. The information above suggests that ZnO-NPs had a beneficial effect on *Zinnia elegans*' root and shoot lengths at varying lower concentrations. It also shows notable alterations in the metabolic profiling of the plant. These results provided insights into the application of reduced ZnO nanoparticle concentrations to increase *Zinnia* production compared to synthetic fertilisers, this will produce higher-yielding plants and provide a safer alternative to direct soil applications, making it less harmful to soil bacteria.ss

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