

BIO-INSPIRED ZINC OXIDE NANOPARTICLES FROM ONION PEELS: THEIR ROLE AS SOYBEAN NANO-FERTILIZERS

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ABSTRACT

Onion peel waste poses significant environmental pollution challenges. However, nanotechnology offers a sustainable solution by transforming this waste into nano-fertilizers with targeted delivery, specificity, and controlled release. This study synthesized zinc oxide nanoparticles (ZnONP) using onion peel extract and characterized them through advanced techniques, including field emission scanning electron microscopy (FE-SEM), Fourier transform infrared spectroscopy (FTIR), and particle size distribution (PSD). FE-SEM analysis revealed spherical particles ranging from 95.35 to 124.5 nm, while PSD showed a size distribution between 50 to 400 nm with a zeta potential of -17.9 mV. FTIR analysis identified organic molecule capping from onion peel extract, indicated by bands within 1000 - 1800 cm^{-1} . The synthesized ZnONP were tested as a nano-fertilizer on soybean plants. At a concentration of 500 mg kg^{-1} , ZnONP significantly improved soybean seed germination by 75 % compared to control seeds. Additionally, they promoted root and shoot growth and increased chlorophyll content in soybean leaves. Chlorophyll a, chlorophyll b, and total chlorophyll levels improved by 55.7 %, 61.1 % and 57.5 % respectively, as a relative value compared to the control condition. These findings demonstrate that ZnONP derived from onion peel waste can serve as an effective nano-based nutrient source, offering an environmentally friendly approach to improving agricultural productivity.

Keywords: nano-fertilizer, ZnO nanoparticles, soybean, chlorophyll, seed germination

INTRODUCTION

Globally, fertilizer application is considered one of the most effective factors for increasing crop production and ensuring food security [1]. Typically, spraying and broadcasting are the two most common methods of applying conventional fertilizers to crops. As a result of

leaching, drift, runoff, evaporation, soil moisture hydrolysis, photolytic and microbial degradation, a very low concentration reaches the targeted site, which is much lower than the minimum desired concentration. A significant portion of the fertilizer content is lost to the environment and cannot be utilized by plants, resulting in economic and environmental

losses [2, 3]. The excessive use of fertilizers and pesticides leads to increased resistance of pathogens and pests, reduced soil microflora, decreased nitrogen fixation, and the destruction of bird habitats due to pesticide bioaccumulation [4]. To maximize crop nutrition while minimizing environmental pollution, it is essential to optimize the use of chemical fertilization. Therefore, other fertilization methods can also be valuable in providing the necessary nutrients for plants grow and production, while keeping soils healthy and the environment pollution-free [5].

Modern agriculture relies on inorganic fertilizers after the green revolution. Synthetic fertilizers are inorganic fertilizers used to improve plant growth and increase crop yields. They can be used through soil application, foliar feeding, or fertigation (irrigation water). The use of nano-fertilizers has been proposed as a way to improve fertilizer use efficiency through fertigation, precision fertilization, and limited application [1].

Using nanotechnology, it is possible to build smart fertilizers as new facilities to increase nutrient use efficiency, ensuring environmental sustainability, economic stability, and reducing environmental pollution cost by exploring nanoscale or nanostructured materials as fertilizer carriers or controlled-release vectors [6 - 8]. The word "nano" is a prefix derived from the Greek word for "dwarf" and refers to a particle smaller than 100 nanometers [9].

In the field of agriculture, nanoparticles are increasingly used as fertilizers. These particles are classified as conventional fertilizers using primary (N, P, K) and secondary (Ca, Mg, Zn) mayor components, which provide essential microelements for plant growth. Various types of nanomaterials are available, such as single-walled and multi-walled nanotubes, magnetized iron nanoparticles, copper (Cu) [10 - 12], aluminium (Al) [13], silver (Ag) [14 - 16], gold (Au) [17, 18], zinc (Zn) and zinc oxide (ZnO) [19 - 22], silica [23 - 25], cerium oxide (Ce₂O₃) [26 - 28], and titanium dioxide (TiO₂) [29, 30]. Nano-fertilizers have the potential to boost crop production through

improved seed germination, seedling growth, and photosynthesis rate [31].

Plants require zinc as essential micronutrient. Zinc is a major component of enzymes, a cofactor in protein synthesis, and an energy component in plants. ZnO nanoparticles (ZnONP) are important for seed germination, chlorophyll production, fertilization, and pollen function [32]. Minimal concentration of ZnONP has great potential for improving crop growth and development [33]. Because these nanoparticles are site-specific and have controlled delivery, they offer significant advantages over traditional fertilizer which can leach and runoff due to rain [34, 35]. The higher concentration of ZnONP did not disturb soil microbes, such as nitrogen-fixing microbes and methane-oxidizing microbes [36]. The absence of zinc has negative effects on the plant. In many plant species, photosynthesis is inhibited by chlorosis, slowed growth, and white necrotic spots on the leaves [37, 38].

Copper (Cu) is another micronutrient that plays an important role in agriculture by aiding in several metabolic processes, such as photosynthesis, and also due to its antifungal action in preventing the development of crop diseases [39]. An increase in seed germination of *Hordeum vulgare* was reported in the concentration range of 0.005 to 0.25 mg mL⁻¹ [40]. Various plants, such as *Eruca sativa* [41], *Lactuca sativa*, and *Raphanus sativus* [42], show a positive effect or no effect at low concentrations of Cu nanoparticles, but an inhibitory effect at high concentrations of Cu nanoparticles.

Further studies show that iron nanoparticles, such as magnetite (Fe₃O₄) and maghemite (γ-Fe₂O₃), improve fertilization efficiency in cases of deficiencies. These nanoparticles promote plant growth by regulating hormones and antioxidant enzyme activity [43 - 46]. Considering the importance of metal nano-fertilizers in agriculture, zinc oxide nanoparticles can be synthesized by various physical, chemical, and biological methods. Biological methods are safe and eco-friendly. The green synthesis method synthesizes zinc

oxide using plant extract, fungi, bacteria, and microbes. Green synthesis reduces pollution and saves the environment.

If zinc oxide nanoparticles are synthesized using waste materials such as vegetable waste, this represents not only a green synthesis but also an eco-friendly approach. In 2019, the UN reported that 931 million tons of kitchen food waste were generated globally, with onion peels being a common type of waste, particularly in India [47].

Onion peels contain minerals such as K, Ca, Mg, Fe, Zn, Mn, Se, total phenolic, flavonoids (Quercetin), and lauric acid. Seed germination plays an important role in the plant life cycle, and quercetin has been proven to play a vital role in the process of seed germination. Quercetin is one of several flavonoids that act as a signalling molecule, regulating gene expression in genes that are involved in seed dormancy and germination in plants [48]. In photosynthesis, quercetin alleviates salt-induced toxicity by maintaining chlorophyll content and promoting better plant growth [49]. As a powerful antioxidant, quercetin strengthens the plant antioxidant mechanism, providing tolerance to biotic and abiotic stresses such as salinity and heavy metals [49, 50]. Overall, quercetin facilitates essential plant growth processes such as seed germination, pollen growth, antioxidant defence, and photosynthesis, contributing to improved plant growth and stress tolerance.

In this study, a green synthesis method was used to synthesize ZnONP from onion peel. Their use as nano-fertilizer on soybean growth was studied to check their impact on seed germination of selected crop, root and shoot length, fresh and dry weight and chlorophyll content.

MATERIALS AND METHODS

Zinc acetate (FINE CHEM industries, Mumbai, India), sodium hydroxide (FINE CHEM Industries, Mumbai, India), onion peels from the vegetable market, Pimpri

market, Pune, and soybean seeds from the local market were used for the purpose of this study.

Preparation of onion peel extract

The onion peels obtained from the vegetable market are cleaned several times with double-distilled water to remove all dust. The onion peels were completely dried in a hot air oven at 40 °C for three days. Once completely dried, the peels were ground into powder using a home grinder. The powder was then sieved to obtain a fine powder. Furthermore, 10 g of dried onion peel powder was weighed and mixed with 100 mL of double-distilled water, heated at 60 °C for 20 min, with continuous stirring for 24 h. It was later cooled to room temperature and then passed through Whatman filter paper (0.2 µm). The filtrate was stored in a refrigerator for further use. Quantitative and qualitative examination of onion peel extract was carried out using standard techniques.

Green synthesis of ZnONP using onion peel extract

About 50 mL of 0.02 mol/L zinc acetate solution was prepared and kept in a stirrer (Figure 1). Parameters such as pH and amount of onion peel extract were optimized, and pH 10 was used throughout the experiment. With vigorous swirling, 0.5 mL of aqueous onion peel extract was added dropwise with 50 mL of zinc acetate solution and stirred with a magnetic stirrer for two hours. A 2M sodium hydroxide solution was used to maintain the pH of the solution at 10 throughout the experiment. The preparation of ZnONP was indicated by a colour change from pale yellow to white. To separate the solution from the residue, the white mixture was centrifuged for five minutes at 4000 rpm. The solution was decanted and the precipitate was retained. To remove contaminants from the pale white precipitate, it was washed with ethanol and double-distilled water. The resulting pale white precipitate was dried in an oven to obtain a completely dried powder.

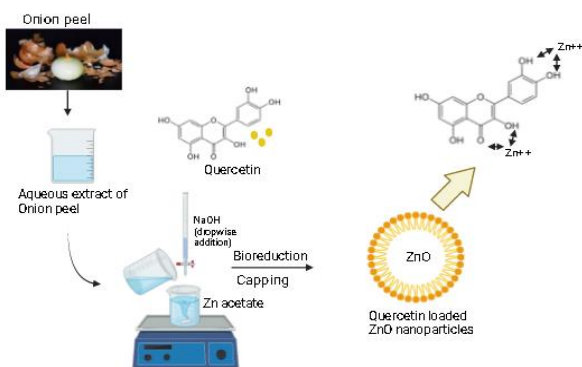


Figure 1. Schematic representation of the bio-fabrication of ZnONP

High Performance Liquid Chromatography (HPLC) analysis of ZnONP

HPLC analysis of onion peel extract and green synthesized ZnONP was performed using quercetin as a standard. The mobile phase consisted of methanol and 0.5 % ortho-phosphoric acid in distilled water in a ratio of 70:30. The analysis was performed on a C18 column (4.6 mm × 160 mm × 5 μm) with a flow rate of 1 mL/min, a PDA detector set at 375 nm, an injection volume of 20 μL, with a total run time of 8 min. A stock solution of quercetin was prepared by dissolving 1 mg of quercetin (purchased from Sigma Aldrich) in 100 mL of HPLC-grade methanol, and serial dilutions were made to achieve concentrations ranging from 1 to 6 μg/mL to determine the linearity range. A hydro-alcoholic onion peel extract at 100 μg/mL was also prepared for analysis. For ZnONP, 1 mg of nanoparticles was dissolved in 10 mL of methanol, sonicated for 45 min, and then centrifuged at 3000 rpm for 25 min. The resulting supernatant was injected into the HPLC system for analysis. Quantification was based on external 5-point calibration curves with R^2 using reference standards. All the samples were analysed three times, and the results were expressed in μg/mg of sample solvent.

Characterization of ZnONP

The synthesized ZnONP were morphologically characterized by FESEM. Furthermore, these particles were analysed for PSD by dynamic

light scattering method and FTIR. These studies helped to confirm and identify the formation of ZnONP. For FE-SEM, NovaNano SEMNPEP303 was used. For analysis, a green synthesized ZnONP powder sample was made into a thin film using carbon-coated tape. Excess powder was removed with blotting paper. The film on the SEM grid was then dried under a mercury lamp and then used to determine the surface structure of ZnONP. For Fourier-transform infrared spectroscopy (FTIR) analysis (Shimadzu), the sample and KBr was mixed in the ratio of [1:100] and the measurement was performed in the range of 400 to 4000 cm^{-1} wavenumber scale. For zeta potential analysis and particle size distribution, a nanoparticle size analyser was used. The dried powder was diluted with double-distilled water in a ratio of 10:1, and then double sonicated using a bath sonicator (Ikon industries ultrasonic bath and probe sonicator) for 5 min each, and then the analysis was performed.

Seed treatment and growth conditions

An evaluation of seeds vitality was performed by suspending seeds in water that had undergone two distillations. Those seeds that sank to the bottom were selected for further study. The seeds were washed twice in double-distilled water. The seeds were further divided into four groups, and each group was soaked for 10 - 12 h in control solution (untreated aqueous solution), onion peel extract, and in two different concentrations of ZnONP suspensions, as shown in Table 1. The soaked seeds were sown in different pots to assess plant growth and development. No other fertilizer or irrigation was applied during the entire period.

Agricultural land in the Pune region served as the soil for the experiment. The coarse-grained soil with a loamy-sandy texture was sieved and a sample was taken for further physico-chemical analysis.

Table 1. Groups of seeds soaked in different solutions

Groups	Solution
Group 1	Control
Group 2	Aqueous onion peel extract
Group 3	ZnONP (concentration - 200 mg kg ⁻¹)
Group 4	ZnONP (concentration - 500 mg kg ⁻¹)

Plant growth measurements

Root length and shoot length

Plants in each group were carefully uprooted from the soil two weeks after sowing the seeds (14-days-old plants). Four to five plants in each group with intact roots and shoots were carefully uprooted from the soil. The roots of the plants were carefully shaken to remove excess soil, and the shoots were cut just above the soil surface. Clumps of soil trapped between the roots were also removed. After transferring the roots to a beaker and gently shaking the roots in water, the roots were cleaned with a brush. Roots were extracted from the soil extract and further processed to determine root length, while the shoot length was measured on a meter scale. Root length was measured from the point below the hypocotyls to the tip of the root. Shoot length was measured from the base of the cotyledons. A thread and scale were used to measure root and shoot lengths.

Percentage of seed germination

Germination percentage is an estimate of the viability of a seed population. The following equation was used to calculate the germination percentage:

$$GP = \frac{\text{seeds germinated}}{\text{total seeds that were planted}} \times 100$$

Relative seed germination rate was also calculated by considering the ratio of Ns

(number of seeds that germinated in the sample) and Nc (number of seeds that germinated in the control).

Fresh weight and dried weight

The fresh weight of the roots and shoots of the plants was measured using an electric balance. Furthermore, both roots and shoots were sun-dried for 8 days, after which the weights of the plants were measured again in order to construct a growth graph for analysis.

Chlorophyll estimation method

Fresh leaf extracts were homogenized in 80 % acetone solution. A calcium carbonate solution was added to stabilize the chlorophyll content. The residue was filtered. Using a UV/visible spectrophotometer, measurements were made at 663 nm and 645 nm on a 10 ml filtered solution. The experiment was carried out with three replicates; each replicate contained about five seedlings per sample or treatment. The content of chlorophyll pigments was calculated using the following equations [51]:

$$C_a (\text{mg g}^{-1} \text{ fresh leaf}) = 0.0127 \times D_{663} - 0.00269 \times D_{645}$$

$$C_b (\text{mg g}^{-1} \text{ fresh leaf}) = 0.0299 \times D_{645} - 0.00468 \times D_{663}$$

C_a - chlorophyll a, C_b - chlorophyll b

Statistical analysis

Data were statistically analysed using GraphPad Prism 8. The standard error was calculated, and one-way analysis of variance (ANOVA) was performed on the data with four replicates to determine the level of significant difference between treatment and control. The significance level for the data was ****P < 0.0001, ***P < 0.001, **P < 0.01, and ns P > 0.05.

RESULTS AND DISCUSSION

Green synthesis of ZnONP

The addition of zinc acetate dihydrate to *Allium cepa* peel extract triggered a series of physicochemical changes, including a rapid colour change within minutes. Due to its natural pigments and phytochemicals, the extract is originally yellow in colour. During this process, the colour may change from pale yellow to light brown or off-white, indicating the formation of zinc oxide nanoparticles. As a result of the interaction between the bioactive compounds in the extract and zinc acetate dihydrate, where phytochemicals act as natural reducing and stabilizing agents, the colour of the extract changed from pale yellow to off-white. When dissolved in water or plant extract, zinc acetate dihydrate dissociates into Zn^{2+} (zinc ions) and acetate ions (CH_3COO^-). In addition, the interaction between bioactive compounds in the extract and zinc acetate dihydrate reduces Zn^{2+} ions and forms zinc hydroxide, which is then dehydrated to form ZnONP [52, 53]. As a result of this process, it has been shown that plant-based extracts can be used as natural reducing and stabilizing agents in the green synthesis of nanoparticles. The colour changes of the reaction mixture from pale yellow to pale white indicates and confirms the formation of ZnO NP (Figure 2).

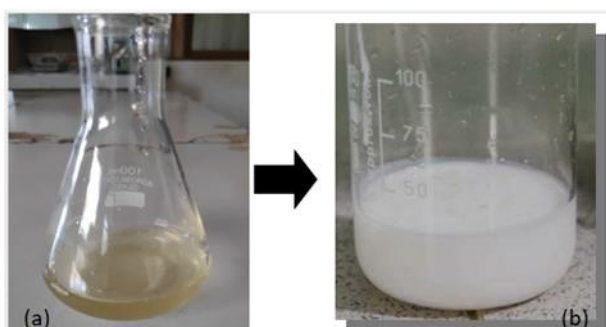


Figure 2. Synthesis of ZnONP: a) before, b) after

HPLC analysis of onion peel extract and synthesized ZnONP

For the determination and quantification of specific phytochemicals that are responsible for reducing and capping process in the preparation of ZnONP, the HPLC study was performed. In this study, the HPLC procedure was optimized for efficient quantification of quercetin in synthesized ZnONP [54]. Under the optimized conditions, a sharp and well-defined peak for quercetin was observed at a retention time of 5.808 min (Figure 3a) in standard solution with linearity in the range of 1 - 6 $\mu\text{g/mL}$ and a regression coefficient (R^2) of 0.9971 (Figure 3b). The hydro-alcoholic onion peel extract showed a peak at a retention time of 5.738 min (Figure 3c) and the synthesized ZnONP also showed the presence of plant growth-promoting substance, i.e. quercetin with a peak at a retention time of 5.858 min (Figure 3d). The identification of quercetin in the onion peel extract was confirmed by comparing the retention time of the sample chromatogram with that of the reference standard. The quercetin peak in the sample solution matched the retention time of the standard solution, confirming the presence of quercetin in the hydro-alcoholic onion peel extract and synthesized ZnONP. The results of HPLC quantification (Table 2) indicate the presence of quercetin in ZnONP with a concentration of 2.4 $\mu\text{g/mg DW}$. The presence of flavonoids derivatives on the surface of nanoparticles is consistent with the results of previously published articles that quantify the flavonoids in various green synthesized nanoparticles using HPLC [54 - 56].

Table 2. HPLC analysis of the biosynthesized ZnONP

Peak	Ret. time	Area	Concentration
ZnONP	5.858	26522	2.4 $\mu\text{g/mg DW}$

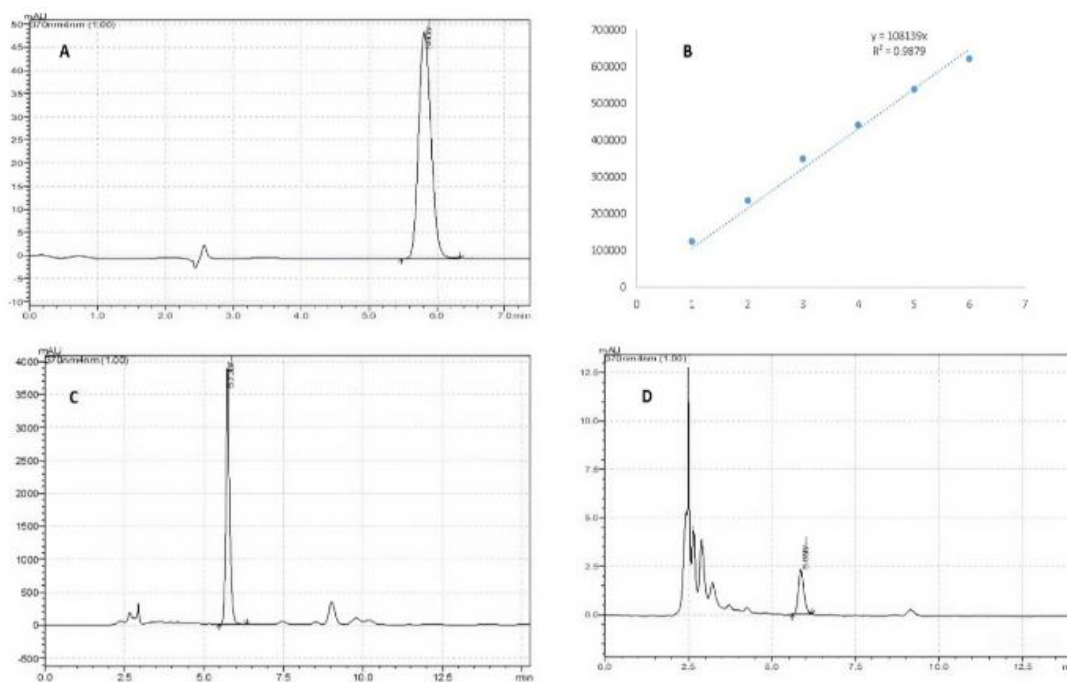


Figure 3. Quantification of quercetin a) chromatogram of standard quercetin, b) linearity graph for standard quercetin, c) onion peel extract d) synthesized ZnONP

Results of characterization of ZnONP

Morphological analysis of ZnONP by FE-SEM

A SEM analysis was performed to analyse the structure of synthesized zinc oxide nanoparticles using onion extract. SEM images show the size and the aggregation of nanoparticles. The average size of the nanoparticles ranged from 95.35 to 124.5 nm and had a spherical shape, as shown in Figure 4.

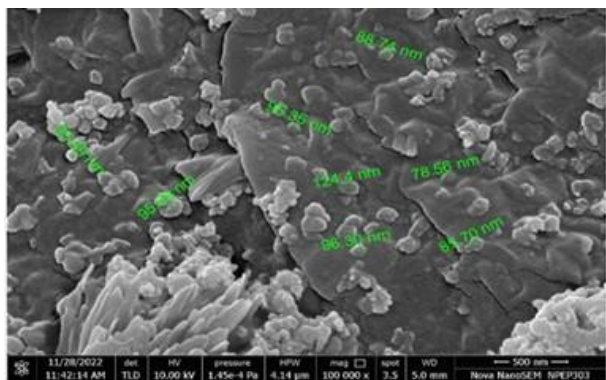


Figure 4. FE-SEM image of ZnONP synthesized from onion peel extract. Green text represents the size of the nanoparticles

Since the synthesized nanoparticles are smaller in size, they are uniform in nature and less particles aggregation is observed. Thus, the use of plant extract for formation of metal nanoparticles provides a dual benefit in cost and also acts as reducing/capping agent [57].

Particle size distribution of ZnONP

Figure 5 shows a graph of the particle size distribution of ZnONP. The particle size distribution of the synthesized ZnONP varied from 100 to 400 nm. The findings were consistent with previously produced nanoparticles [58, 59]. ZnONP synthesized by a green method with an average particle size ranging from 58 to 94.36 nm in diameter were also reported [60]. The overall particle size appears to be larger than the actual size of the nanoparticle due to agglomeration of particles, resulting in the formation of larger particles. The average particle size was 214.7 nm, and the zeta potential was -17.9 mv. The polarity was found to be negative, and therefore it was found to be stable.

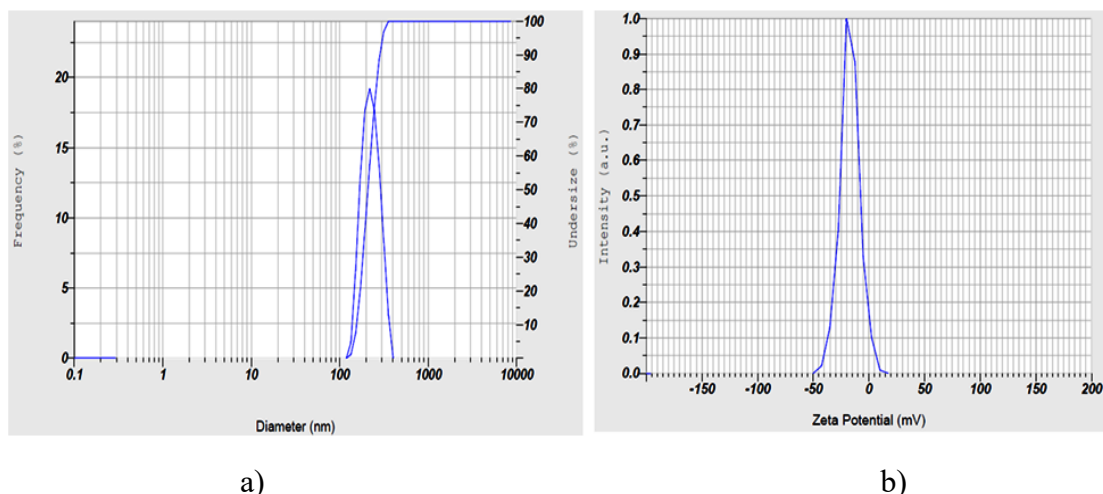


Figure 5. a) ZnONP particle size distribution graph, b) zeta potential of ZnONP

FTIR study of synthesized ZnONP

The peak at 639.75 cm^{-1} corresponds to the Zn-O bond and indicates the formation of ZnONP. Additional peaks at 2778.05 cm^{-1} and 1576.31 cm^{-1} were observed, corresponding to C-H stretching vibrations and stretching vibration peaks for C=O. The stretching vibrations of C-O at 1008.77 cm^{-1} were observed (Figure 6). The appearance of a band in the spectrum ranging from 639.75 cm^{-1} to 1008.77 cm^{-1} is related to the formation of ZnONP, and the findings are in good agreement with the literature [61, 62].

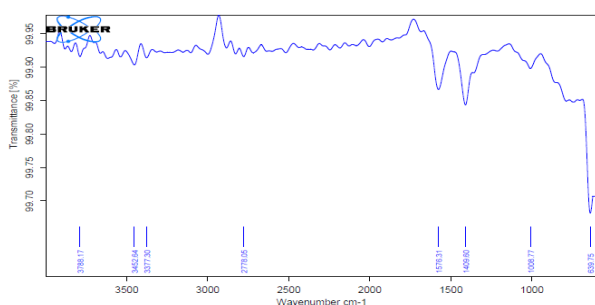


Figure 6. FTIR spectra of ZnONP

Results of plant growth measurements

Soil analysis

The properties of the soil used in the study are shown in Table 3.

Table 3. Physicochemical properties of the soil

Parameter	Content
Total nitrogen	0.75 %
Phosphorous (kg m^{-2})	0.001186
Potassium (kg m^{-2})	0.08894
pH	6.98
Sulphur (mg kg^{-1})	15.85
Zn (mg kg^{-1})	3.8
Fe (mg kg^{-1})	7.98
Mn (mg kg^{-1})	28.75
Cu (mg kg^{-1})	2

Percentage of seed germination

Seed germination is a crucial process that affects crop yield and quality. To improve these, it is of great significance to understand the molecular aspects of seed dormancy and germination. The result of the seed germination rate is shown in Figure 7. 50 % of the seedlings germinated when the control solution was used, while 33.33 % germinated when the aqueous onion peel extract was used, which increased to 66.6 % when the nanoparticles were added at a dose of 200 mg kg^{-1} . The highest percentage of seed germination (75 %) was observed after treatment with nanoparticles at a concentration of 500 mg kg^{-1} . In conclusion, the obtained results indicate that an optimal concentration of Zn, along with the onion peel extract, is required for seeds to germinate at the best possible rate. Moreover, ZnONP may alleviate both abiotic and biotic stresses in plants, and

also serve as bio stimulants, increasing germination rates and overall plant growth [63].

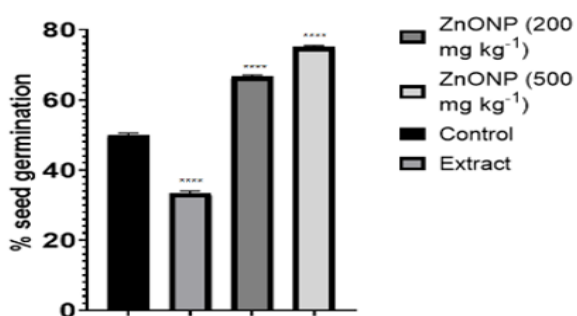


Figure 7. Effect of ZnONP on soybean seed germination. All the data are the mean of five replicates (n = 3), and vertical bars show standard errors (\pm SE)

Furthermore, this study showed that in the absence of Zn, seed germination was reduced when seeds were treated with onion skin extract alone. The low germination percentage observed in seeds treated with onion peel extract may be due to the presence of allelochemicals in the onion peel extract, which could affect seed germination [64, 65]. Previous studies have found that onion peel extract inhibits the growth of mustard and garden cress seeds [66]. At higher

concentrations of onion peel extract, the increase in inhibitory substances is more pronounced and can drastically change critical physiological processes necessary for germination. At lower concentrations, the presence of fewer bioactive compounds makes it less inhibitory compared to higher concentrations [67]. Therefore, the pattern of effects of onion peel extract is concentration-dependent, indicating possible allelopathic effects of the onion peel extract [68].

Root and shoot length

The results of the study showed a significant increase in plant growth after the application of zinc nano-fertilizer. The maximum sapling root length of 6.3 cm was observed at a ZnONP concentration of 500 mg kg⁻¹ on the 14th day after planting (Figure 8a). The maximum sapling shoot length of 4.5 cm was also observed at a ZnONP concentration of 500 mg kg⁻¹ (Figure 8b). Compared to saplings treated with control solution and onion peel extract, saplings treated with ZnONP had longer roots and shoots.

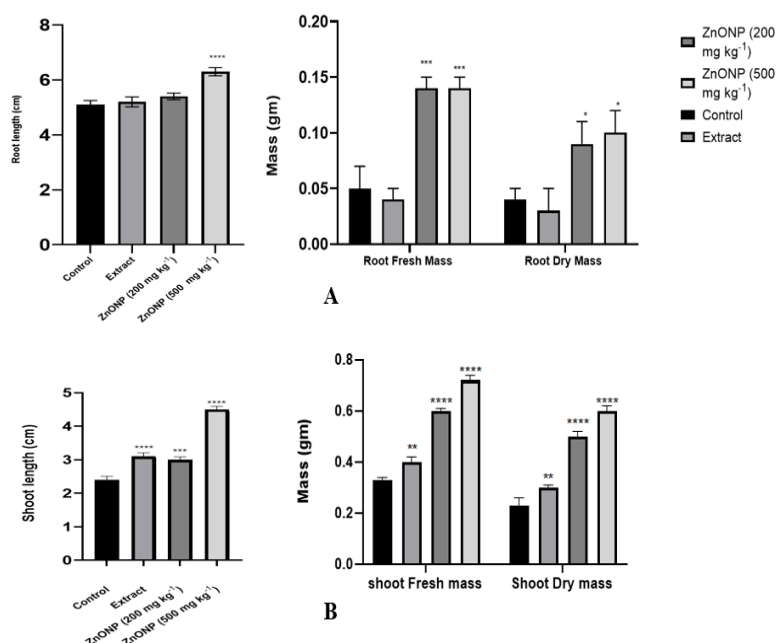


Figure 8. Effect of ZnONP on: a) root length, fresh and dry weight of roots, b) shoot length, fresh and dry weight of shoots of soybean plants. All data are the mean of five replicates (n = 3), and vertical bars show standard errors (\pm SE)

Furthermore, the maximum fresh weight of roots and shoots was obtained at 500 mg kg⁻¹, i.e. 0.14 g and 0.72 g, respectively. The fresh weight of roots and shoots of control saplings was lower, i.e., 0.05 g and 0.33 g, respectively. In the case of soybean roots, the maximum dry weight was 0.100 g at a ZnONP concentration of 500 mg kg⁻¹, and the lowest dry weight was 0.03 g when onion peel extract was applied (Figure 8a). Regarding shoot dry weight, the highest value of 0.60 g was obtained with a ZnONP concentration of 500 mg kg⁻¹ (Figure 8b), while the lowest value was obtained in the case of the control.

Therefore, it can be concluded that Zn is a micronutrient needed by the plant and that it promotes plant growth, but at higher concentrations these nanoparticles can lead to phytotoxicity. It can induce enzyme and auxin production, promoting protein synthesis and carbohydrate metabolism. It also facilitates starch synthesis and chlorophyll synthesis and promotes normal growth and maturation of seeds and stalks [69, 70]. In addition, quercetin helps maintain plant health and processes such as photosynthesis, pollen production, and seed germination, and protects plants from biotic and abiotic stresses, such as salinity, heavy metals, and UV radiation, due to its strong antioxidant capabilities [71, 50]. Furthermore, toxicity can result in short roots and shoots, leading to reduced weight, which can ultimately affect the plant growth [72, 73].

Chlorophyll content estimation

Chlorophylls are light-harvesting pigments that are an integral part of the photosynthetic process. Chlorophyll concentration data will provide information on the photosynthetic potential of the plant. The effects of ZnONP on the difference in the chlorophyll pigments content of the soybean plant were investigated and shown in Figure 9. The results show that ZnONP at a concentration of 500 mg kg⁻¹ increased chlorophyll content in soybean leaves compared to the control solution. Compared to the control, chlorophyll *a* increased by approximately 55.7 % (from 0.0106 mg g⁻¹ to 0.0165 mg g⁻¹), chlorophyll *b*

increased by 61.1 % (from 0.0054 mg g⁻¹ to 0.0087 mg g⁻¹), and the total chlorophyll content increased by 57.5 % (from 0.0160 mg g⁻¹ to 0.0252 mg g⁻¹). These findings suggest that ZnONP treatment significantly increases chlorophyll accumulation in soybean leaves. Consequently, it is likely that these results are due to the improvement of the nutrient uptake of the nano-fertilizer and are consistent with some of the previous results [74]. The process of photosynthesis is one of the most important physiological processes in plants and directly affects plant growth, biomass, yield, and quality.

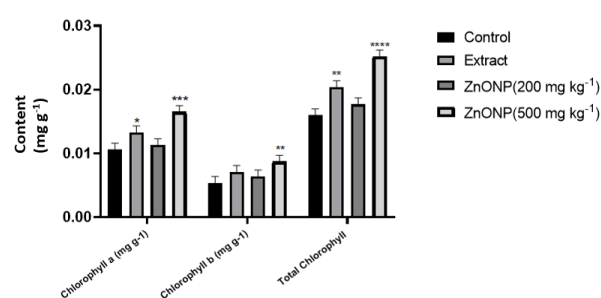


Figure 9. Effect of ZnONP on the content of chlorophyll *a*, chlorophyll *b*, and the total chlorophyll in soybean leaves. All data are the mean of five replicates ($n = 3$), and vertical bars show standard errors (\pm SE)

CONCLUSION

Green synthesis of ZnONP using onion peel extract is a simple, cost-effective, and environmentally friendly way to form ZnONP, and has proven to be suitable for application in agricultural fields due to the simplicity of the process [75, 76].

Based on FE-SEM analysis, the particles were found to be nano-sized (95.05 nm) and spherical in shape. The results of this study show that ZnONP has a greater impact on soybean seed germination and chlorophyll content than treatment with control solution and onion peel extract. The effects of nanoparticles on plants are synergistic, as plants treated with ZnONP showed an increase in growth and development.

In addition, this study may help other researchers in further research and development of ZnONP as an environmentally friendly and sustainable approach with a high potential for commercialization in the coming years.

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