



Effect of Biosynthesized Nanoparticles on the Germination Profile of *Zea mays* Under Salinity Stress

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Abstract	Article History
<p>Production of crops for optimum human sustainability has faced diverse challenges in the agricultural sector globally. Mitigating these challenges in food production has been prioritized in different agricultural-related fields of research globally. This study was carried out to evaluate the effect of biosynthesized nanoparticles on germination profile of <i>Zea mays</i> under salinity stress. Garden soil samples were collected at Oba, Idemili South LGA, Anambra State using a soil auger. Filamentous fungi were isolated and purified using a standard microbiological technique. The fungal isolates were characterized based on cultural and microscopic features. Fungal biomass and natural nanoparticles were prepared using standard mycological technique. The biosynthesized nanoparticles were characterized using ultraviolet-visible spectrophotometric technique while the effects of the biosynthesized nanoparticles on the germination indices of <i>Zea mays</i> under salinity stress were ascertained using standard germination test. The filamentous fungal isolates were identified as <i>Fusarium</i> species and <i>Aspergillus niger</i>. The biosynthesized nanoparticles as detected by UV-VIS spectrophotometer were calcium (ii) oxide nanoparticles (CaONPs) and potassium nanoparticles (KNPs). CaONPs showed higher absorbance (0.14) compared to KNPs (0.0275) at different wavelength, indicating more composition and potency. There was an increase in the % germination and germination speed in the presence of the biosynthesized nanoparticles under zero concentration (0 mM) of sodium chloride. Therefore, it is noteworthy that fungal species are capable of synthesizing nanoparticles, which are potent and environmental friendly, and can support the growth of <i>Zea mays</i>, even when there is a high salinity.</p> <p>Keywords: Calcium nanoparticle, Filamentous fungi, Potassium nanoparticle, Salinity, <i>Zea mays</i></p>	<p>Received: 10 Jul 2025 Accepted: 28 Jul 2025 Published: 06 Aug 2025</p>  <p>Scan QR code to view*</p> <p>License: CC BY 4.0*</p>  <p>Open Access article.</p>
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1. Introduction

Salinity has been described as an accumulation of salts in the soil (Zörb *et al.*, 2019). Excessive salt in the soil serves as a stress to soil fertility, due to the negative interaction with soil microorganisms and the integrity of the absorption property of the root (Tsegaye *et al.*, 2021). Basically, there two metallic ions that commonly found in soil-enriched salt namely: calcium ion, sodium ion, chloride ion, and sulphate ion (Chhipa, 2019). Human activities such as irrigation, are

capable of introducing salt into the soil. Salt as an abiotic factor contributes immensely in the shortage of food globally and tackling the effect has been a major threat in agricultural practices, in terms of food production and availability (Murthy *et al.*, 2021).

Research has shown that stress due to salt accumulation in the soil is highly intense to seed during germination process (Satish *et al.*, 2015). Germination process is an essential stage in food production, because, the planted seed must undergo

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decomposition stages, which result to seed germination under conducive environmental conditions in the soil and in the atmosphere (Satish *et al.*, 2015). The presence of salt in the soil decreases seed germination index and leads to poor absorption of water by the decomposing seed. The poor absorption of water is due to the loss of water from the seed to the soil, which has higher concentration of salt (osmotic pressure).

Certain crops such as *Zea mays* had been optimized by humans for consumption purposes globally (Satish *et al.*, 2015). When the crops are planted in high saline soil, there is always a mechanism that curtails the effect of salt such as an increase in the production proline, which ensures that structure of cells is stabilized and removal of reactive oxygen species, which emanated due to stress of salinity (Parihar *et al.*, 2014). Also, there is production of antioxidant enzymes such as glutathione reductase and catalase that limit the effect of high salinity. This clearly indicates that crops that are not capable of mitigating the effect of high salinity cannot thrive in high salinity soil, which is one of the major challenges in agricultural sector (Parihar *et al.*, 2014).

It is worthy to note that chemically synthesized nanomaterials had been optimized in the neutralization of high salinity in the soil (Tsegaye *et al.*, 2021). These artificial nanoparticles are capable of neutralizing the high salinity nature of soil, which invariably normalizes salt concentration in the soil. The nanoparticles are capable of scavenging reactive oxygen species generated due to response to high salt content in the soil. Also, nanomaterials enhance the activity of enzymes found in essential soil microbes, there increasing their activities, and the ratio of sodium ion and potassium ion is drastically minimized due to application of nanoparticles. However, these chemically synthesized nanoparticles are not properly applied by most farmers, which pose adverse effects on crops such as damage to plant cell and even death of crops has been documented (Satish *et al.*, 2015). Also, the presence of the chemicals in the environment poses threat to living organisms including humans such as bioaccumulation when they enter the food chain (Satish *et al.*, 2015). This effect of chemically synthesized nanoparticles has necessitated for an alternative method of synthesizing nanoparticles, which could be of high efficacy, cost effective, and friendly to the environment (Satish *et al.*, 2015).

Several studies had been documented on the effect of nanoparticles on germination of crops under salinity stress such as Chhipa (2019), Tsegaye *et al.* (2021), and Murthy *et al.* (2021) but few studies are available on the effect of biosynthesized nanoparticles on germination profile of *Zea mays* under salinity stress. Hence, the aim of this study is to evaluate the effect of biosynthesized nanoparticles on germination profile of *Zea mays* under salinity stress.

2. Materials and Methods

Selection of the Desired Plants

The plant chosen for this study were *Zea mays*. It is an annual plant with a short growing season and has been identified as a hyperaccumulator because of its fast growth rate, high accumulation and translocation from root to shoot. Seeds of

Zea mays were purchased from the market, bagged and kept in the dark at room temperature until required for use.

Isolation and Purification of the Isolates: By adopting the method of Uba *et al.* (2018), soil samples were collected using soil auger at the depth of 10 cm and transported to the laboratory for processing (Uba *et al.*, 2019a; 2019b; Nnaka *et al.*, 2024). A stock solution of the soil samples was prepared by weighing one gram in a weighing balance ((DC-300), which was transferred to 100 mL of distilled water in a conical flask (pyrex). Ten - fold serial dilution was done in which 9 mL of distilled water were dispensed into test tubes (pyrex) each and 1 mL of the stock solution was dispensed into the first test tube, and was vortexed after which 1 mL was taken from the tube using another pipette, and was transferred into the second test tube. The process continued until the last tube after which 1 mL was discarded. Inoculation was carried out by aseptically inoculating 1.0 mL of the sample on PDA using pour plate method, and the incubation was done inverted at $32 \pm 2^\circ\text{C}$ for 7 days. After incubation, the grown colonies were sub-cultured by streaking a single colony aseptically on sterile poured plate (60 mm OD \times 55 mm ID \times 13mm high). All the plates in triplicates were incubated inverted at $32 \pm 2^\circ\text{C}$ for 7 days (Yew *et al.*, 2014; Dokubo *et al.*, 2024).

Identification of the Isolates

The sub- cultured isolates were identified using their colonial and morphological descriptions. The colonial description was carried out to determine the appearances of the isolates on agar media plates which includes; colours, edges, texture, surface, and reverse pigmentation (Yew *et al.*, 2014; Uba *et al.* 2019c).

Preparation of the Fungal Supernatant

For fungal biomass preparation, the selected fungal cultures were grown separately in the liquid medium containing (g/L): yeast, 3; peptone, 10; glucose, 20. Flask containing medium was incubated for 7 days at $28 \pm 2^\circ\text{C}$. After the incubation, the biomass was harvested and washed with distilled water. Then, 6 g of the biomass was added to 100 mL of deionized water and further incubated for 72 hr. After the incubation, the filtrate was obtained by passing the suspension through Whatman No. 1 filter paper (Moustafa, 2017; Uba and Obiefuna, 2023). Both filtrates were combined together to obtain a filtrate mixture.

Preparation and Characterization of Myco - Synthesized Calcium Oxide (CaONPs) and Potassium Nanoparticles (KNPs)

Exactly 10 g and 14.5 g calcium nitrate and potassium chloride were transferred into a 100 mL volumetric flask and dissolved to the mark. Then, 50 mL of each salt solution was transferred into a beaker containing 50 mL of the prepared fungal biomass filtrate mixture. The mixture was heated to 80°C and was continuously stirred using a mechanical stirrer for 20 min. Thereafter, 2 mL of 5 % sodium hydroxide solution was added to the colloidal solution while stirring until the mixture became intense yellow and dark in colouration. It was then centrifuged at 3000 rpm for 30 min, placed on an aluminum foil and dried in an oven at 90°C for 18 hr, then later transferred into an amber bottle and refrigerated below 4°C for further analysis

(Aliyu *et al.*, 2023; Elee *et al.*, 2024, Uba *et al.* 2024; Ubani *et al.* 2024).

Characterization of the Biosynthesized Nanoparticle

UV-VIS spectroscopic analysis

UV-visible spectrophotometric analysis was conducted on the nanocomposite sample using a UV-visible spectrophotometer (Apel 3000UV) with a slit width of 2 nm, using a 10 nm cell at room temperature. The extract was examined under visible and UV light in the wavelength ranging from 200 - 1100 nm as described by Dokubo and Uba (2023), Okafor *et al.* (2023) and Uba and Obiefuna (2023).

Application Experiments of CaONP and KNP on *Zea mays* seeds

In this research, the seeds of *Zea mays* were used as plant material. Firstly, seeds divided into six equal groups. Then, each group were separately soaked for 4 h in distilled water (control), 6.25, 12.5, 25, 50, and 100 % CaONPs and KNPs, respectively. Finally, they were allowed to air-dry overnight on a filter paper at 22 ± 2 °C. Seeds pre-soaked both with different concentrations of CaONPs, KNPs and distilled water were divided further into 5 equal sub-groups. Then, standard germination test was applied to all groups with different doses of NaCl concentrations (0, 40, 80, 160, and 250 mM NaCl). Germination tests were carried out using 4 replicates of pre-soaked with different concentrations of CaONPs and KNPs under different NaCl doses in the Petri dishes (12 cm) in the dark at 25 ± 2 °C. All Petri dishes were placed in a germination cabinet in randomly design for a period of 9 days (Koca *et al.*, 2020).

Statistical Analysis

Data was statistically analyzed using one-way ANOVA on Graph pad Prism 8.0.1 software. The results were presented as mean \pm S.D. (standard deviation) and data from different treatments and control was compared by Duncan's multiple-range test at $p < 0.05$ (Uba *et al.*, 2024).

3. Results

Cultural and Morphological Characteristic Features of the Fungal Isolates

The cultural and morphological characteristic features of the fungal isolates are presented in Table 1. The result revealed that the fungi had various colours ranging from white, cream, and brown. The isolates had macrocodia with septate hyphae and reverse pigmentation was colourless.

Table 1: Morphological features of the selected plant growth promoting bacterial strains

Isolate code	Colonial morphology	Microscopic morphology
PSB1	Colonies growing rapidly, 4.5 cm in four days, aerial mycelium white to cream, becoming bluish-brown when sporodochia are present.	Macroconidia are formed after 4-7 days from short multiple branched conidiophores which may form sporodochia. They are three to five-septate (usually three-septate), fusiform, cylindrical, often moderately curved, with an indistinct pedicellate foot cell and a short blunt apical cell, 28-42 x 4-6 μ m. Possible fungus is <i>Fusarium</i> species
PSB8	It has a darkly pigmented colony with white aerial mycelium, light brown conidial masses and dark brown reverse side.	The conidia are cylindrical with obtuse ends. The conidia length and width is 14 x 5.14 μ m. Possible fungus is <i>Aspergillus niger</i>

Characteristics of the Biosynthesized Nanoparticles

The characteristics of the biosynthesized nanoparticles are shown in Figures 1a and b. The UV - VIS spectral profile revealed that Calcium oxide nanoparticle (CaONPs) recorded the highest absorbance of 0.14 at the wavelength of 550 nm followed by the wavelength of 350 nm, which recorded an absorbance of 0.05. Meanwhile, potassium nanoparticle recorded the highest absorbance of 0.0275 at the wavelength of 360 nm.

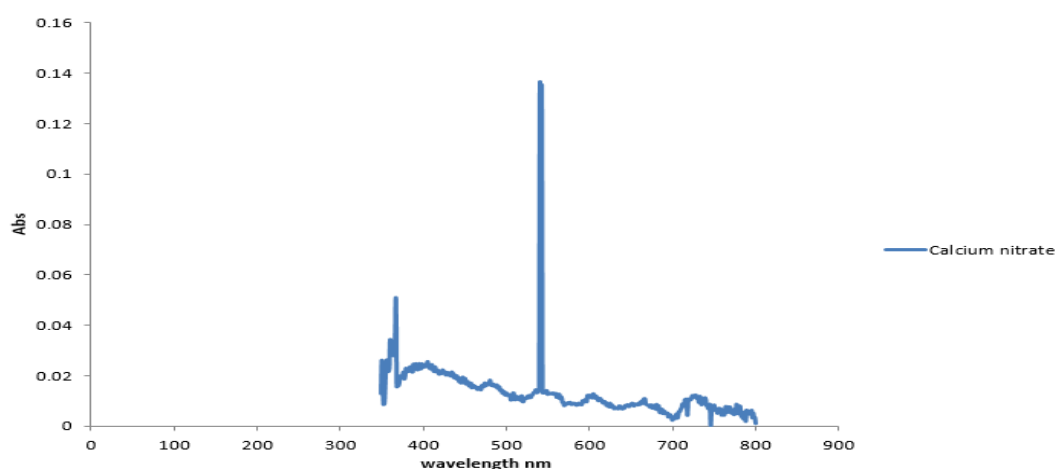


Figure 1 (a): UV - VIS spectral profile of biosynthesized calcium nanoparticle

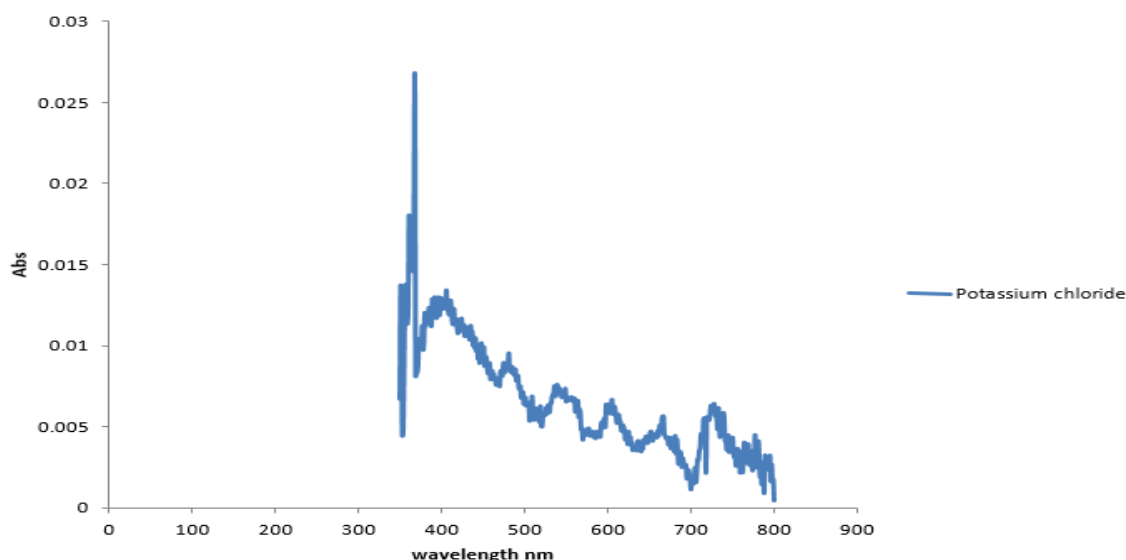


Figure 1 (b): UV - VIS spectral profile of biosynthesized potassium nanoparticle

Effects of Biosynthesized Nanoparticles on Germination Indices of *Zea mays* Under Salinity Stress

The effects of biosynthesized NPs on germination indices of *Zea mays* are shown in Figures 2, 3, 4, and 5. In Figure 2, the highest % (80%) of germination was recorded at zero concentration of sodium chloride in the biosynthesized CaONPs while 85% germination was observed at zero

concentration of the same solute in the biosynthesized KNPs (Figure 3). Similarly, in Figure 4, the maximum speed (3.5%) was recorded at the highest concentration of CaONPs that had zero concentration of sodium chloride while the maximum speed (6.2%) was recorded at the highest concentration of KNPs that had zero concentration of sodium chloride (Figure 5).

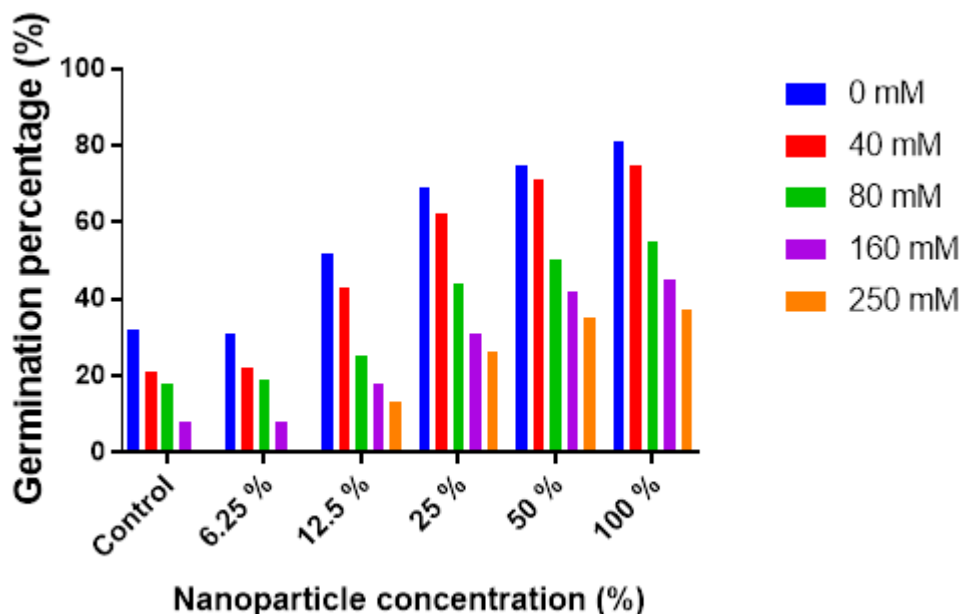


Figure 2: Germination percentage of *Zea mays* seeds exposed to different doses of sodium chloride under the effect of CaONPs

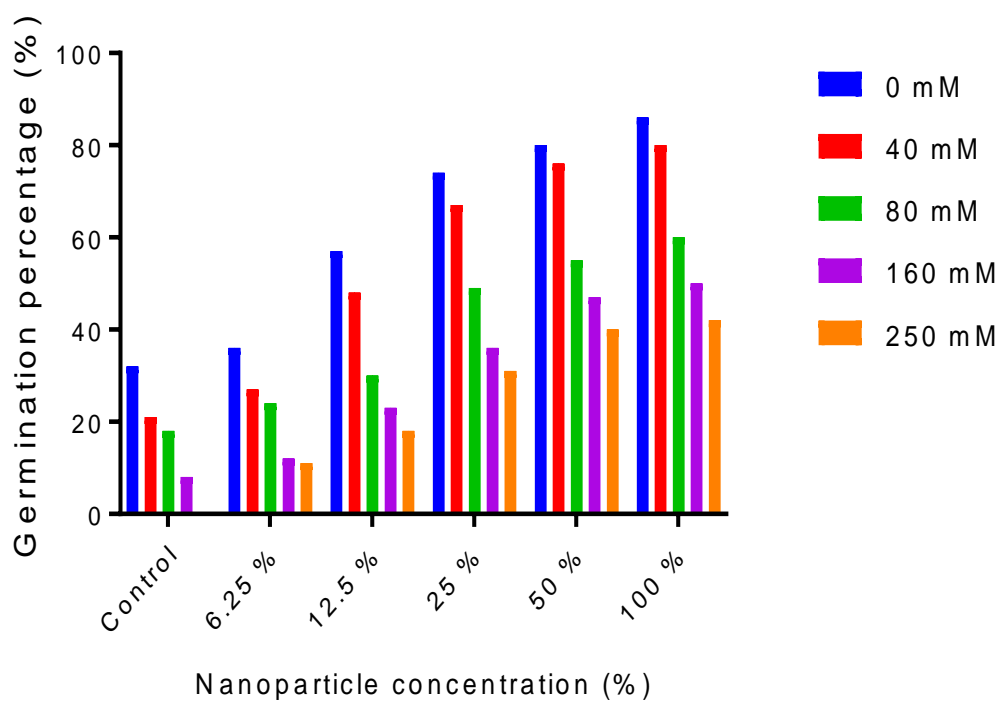


Figure 3: Germination percentage of *Zea mays* seeds exposed to different doses of sodium chloride under the effect of KNPs

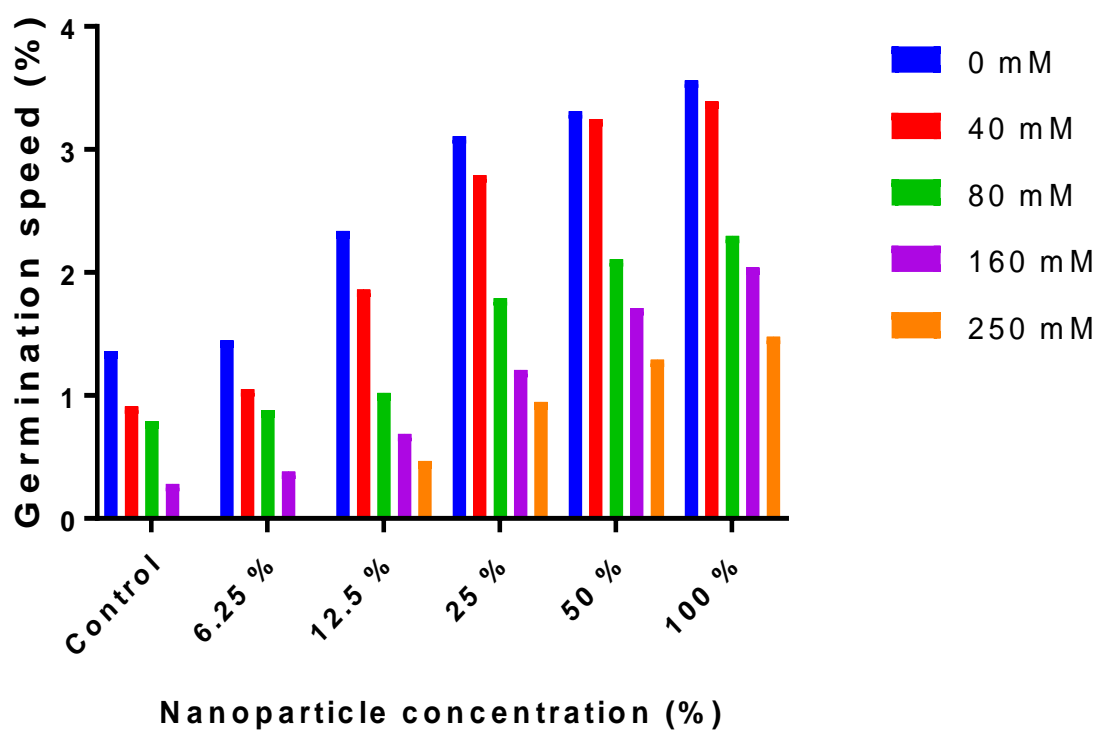


Figure 4: Germination speed of *Zea mays* seeds exposed to different doses of sodium chloride under the effect of CaONPs

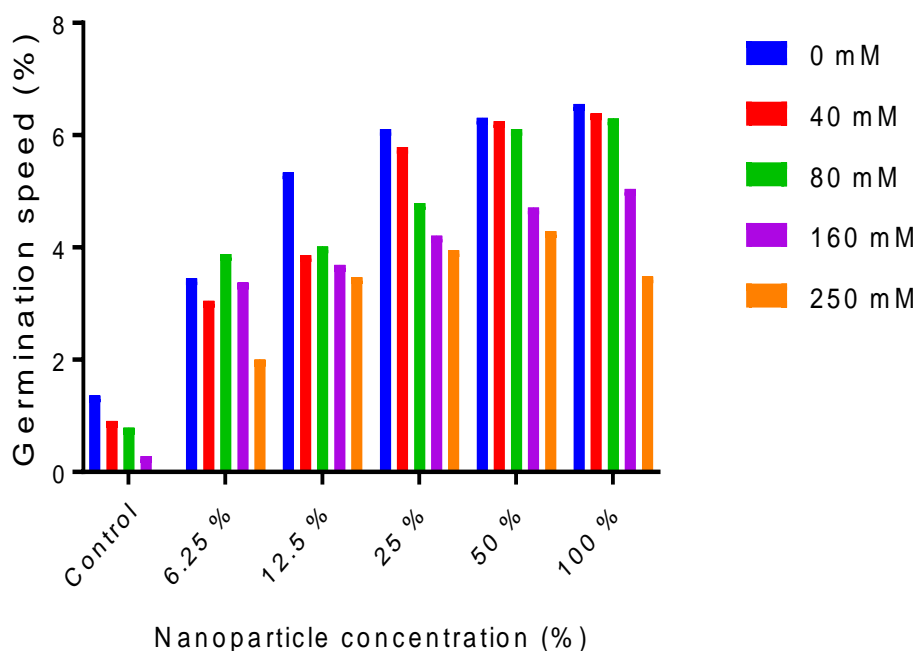


Figure 5: Germination speed of *Zea mays* seeds exposed to different doses of sodium chloride under the effect of KNP5

4. Discussion

Crop cultivation is highly essential practice that ensures that food is available for human consumption (Sharma *et al.*, 2017). Growing of crops can be hampered by different environmental factors, which could be physical, chemical, and biological (Sharon *et al.*, 2010). Tackling these factors that affect germination is essential to enhancing productivity in agricultural sectors globally. This study has revealed that the UV-Vis spectral profile of biosynthesized nanoparticles were specifically calcium oxide nanoparticles (CaONPs) and potassium nanoparticles (KNPs). This corresponds to the findings of other researchers (Sharon Sharon *et al.*, 2010; Ashraf *et al.*, 2013; Sharma *et al.*, 2017; Koca *et al.*, 2020) who evaluated the ability of microorganisms to synthesize nanoparticles. The variation that was observed in the absorbance of the two nanoparticles could be attributed to composition, size, and shape of particles. For instance, in this study, the absorbance of CaONPs was higher than the absorbance of KNP5 which also be attributed to variation wavelength and the metallic oxide nature of the nanoparticles (CaONPs). This conforms to the observation of Sharma *et al.* (2017). The characteristic features of CaONPs and KNP5 suggest their potentials in enhancing plant growth under stress conditions. The porous and layered structure of CaONPs could improve soil calcium levels, essential for cell wall stability and signaling in plants. Similarly, the spherical KNP5 can enhance potassium availability, crucial for various physiological processes including osmoregulation and enzyme activation (Ashraf *et al.*, 2013). The nanoparticles' ability to improve nutrient solubility and availability can play a critical role in mitigating the adverse effects of salinity stress on plants. These findings correspond to the observation of Khan *et al.* (2019) that reported that enhanced nutrient profile provided by these nanoparticles can support better germination and growth of

crops such as *Zea mays* which are often challenged by salinity stress. The result indicated that continuous increase in salinity stress adversely affects seed germination speed and plant growth by disrupting water and nutrient uptake. The finding of the study showed that *Zea mays* plant when exposed to different doses of sodium chloride under the effect of CaONPs and KNP5 applied to them at different concentrations, the *Zea mays* seed plant germination percentage, and germination speed increased as the concentration of the nanoparticles increased while the control sample showed no growth impact. The findings of the study corroborate to the work of Sharon *et al.* (2010) who reported that there was an increase in yield by the application of nanoparticles, stating that nanoparticles are excellent growth simulator (Sharon *et al.*, 2010).

5. Conclusion

In this study, the use of NPs has been postulated to play an essential role in alleviating ionic toxicity and osmotic stress. It is clear that the nanoparticles synthesized by the green synthesis method can have a high positive effect on the germination and yield of the *Zea mays* plant seeds and will be very crucial for countries trying to curtail soil salinity emerging from continuous loss of nutrient, which could also improve the soil quality by application of biosynthesized nanoparticles. Moreover, the response of *Zea mays* seeds grown under saline stress showed improved growth in the presence of CaONPs and KNP5, though CaONPs seemed to be more active.

Conflict of Interest

Authors declare no conflicts of interest.

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