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# Determination of Effect of Zno Nanoparticles of Invitro Cultrue of Cowpea Beans

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***Abstract: The cowpea is a annual herbaceous legume. The root nodules have capacity fo fix the atmosphere N2 that is beneficial for farming.This fixed N2 benefit other crops (intercropping) in various ways. The Zno NPs (Zinx Oxice Nanoparticles) has wide applications in agriculture as a fertilizer to increase the product yield and to limit the onset of fungal and bacterial diseases. The invitro germination of seed followed by the seedling growth needs the intervention of ZnO Np in cowpea beans. This research was developed to study the effect of various doses of ZnO Nps (Zinc Oxide Nanoparticles).***

***Index Terms: Cow pea, Dose, Germination, Nanoparticles, Zno, Invitro Germination***

## I. INTRODUCTION

The Vigna unguiculata (cowpea), the most important legume in the world is a very common yearly plant, particularly in Africa, Asia, South America and the United States. It is a strong pre-plant that can thrive in subpar conditions and poor soils along with the aid of nitrogen fixation, is able to raise the yield of the next product. The legume family member cowpea, which is used as a fodder in animal nutrition as well as a dry grain and green vegetable in human nutrition, has a protein content of 4.5–5.0% in fresh grains and fresh beans with 2.0–4.3%. Dry cowpea grains can attain protein content range from 20.42% to 34.60% after attaining maturity, depending on the variability and environmental factors. Additionally, cowpea grains have a carbohydrate content of 50–67%, 3.9% cellulose, 1.3% oil and 3.6% of ash. When compared to grain seeds, the protein in its seeds is deficient in methionine and cysteine and abundant in the amino acids tryptophan and lysine. The field of nanotechnology a multidisciplinary science (combines both science and technology) is the study of nanoscale materials [6]. Nanotechnology is described as the development of materials with a size smaller than 100 Micronutrients use in agriculture, nutrition, agricultural nanometers and the ability to manipulate them at the atomic level. applications, plant protection, pesticide distribution, nano sensors, and pesticide degradation are all areas of interdisciplinary research that are now under development. For reducing agricultural waste and environmental contamination, nanotechnology provides efficient ways to safeguard the health and conditions of the soil. Precision agriculture can be considerably enhanced by nanotechnological method [4]. In Nanotechnology, the aggregates of atom or molecules called nanoparticles have diverse physicochemical properties. These properties include higher surface to volume ratio, <100 nm size, lower melting point, high photo catalytic and antimicrobial activity [9]. The absorption capability of the Nano particles in the form of chemical compounds of plants is better when compared to bulk. This is because of their small size [9].

An important inorganic substance with a broad range is ZnO. This substance is utilised in a variety of products, including cigarette filters, light emitting diodes, laser diodes, skin irritant treatment, semi-conductors, and as concrete in industrial products like cosmetics, paint, and rubber. Due to the property of size, they are utilised more readily by plant cells and promote plant growth, and recognised as a crucial micronutrient for plants and grains. In order to study the structure and morphology of zinc oxide NPs, a critical micronutrient for plant metabolism, X-ray diffraction and transmission electron microscopy (TEM) are used. The production of nanoparticles has been rapidly rising worldwide. They are employed across a extensive range of fields, including optics, electronics, energy production, materials research, medicine, and the biological sciences [11]. Additionally, during the processes of manufacture, transport, use, and disposal, they are discharged into the environment. Despite the fact that plants expose to natural nanoparticles, the rise of artificial nanoparticles calls for thorough monitoring [9]**.** The ZnO nanoparticle would also stand as ecofriendly after monitoring. There are many factors that play a major role in association of Nanoparticles with plant cells. They include the basic size, surface covering and shape; their chemical composition, concentration [10], reactivity and mode of application. They would also include the plant characteristics like age and genotype of plant, stage of plant development [5]. These above mentioned factors have shown diverse effects on morphology and physiology of many plant species both in a positive and negative manner. They can promote or inhibiting seed germination; activate metabolism related genes; ROS (Reactive oxygen species) generation; chromosomal aberration; inducing or inhibiting of metabolism related genes and seedling growth.

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In horticulture and agriculture, nanoparticles that are more effective on plants, containing herbicides, fertilisers, or nano encapsulated nutrients are mostly used. Plant Genetic engineers have also shown interest in nano particles. Targeting genes can be released from nanoparticles into particular cellular organelles with ease when they penetrate through the cell wall. Additionally, the applications are not genotype-specific. Nanoparticles use is effective than technologies such as biolistic approaches or Agrobacterium Transformation.

Metallic zinc (Zn) is a crucial micronutrient for the overall growth and development of plants and is engaged in numerous enzymatic and physiological activities. In many plant species, zinc deficiency causes white necrotic patches on the leaves, chlorosis, stunted growth and photosynthesis [11] inhibition. Zn acts as a important metal component in several enzymes, It also acts as a cofactors participate in chlorophyll biosynthesis and energy production. It acts as a catalyst in the phosphate, lipid, carbohydrate[11] and protein metabolism. Zn influences capability for water intake and transport, impacts seed viability and radical growth, and lessens negative effects of heat, drought, or salt stressors. Additionally, zinc actively participates in the production of gibberellins and auxins. In a plant organism, high zinc content can harm cell function and interfere with various crucial processes because it displaces other minerals with a similar charge and dimension.

CNT (carbon nanotubes), Au, Ag, $TiO_2$ and ZnO nanoparticles are among the most repeatedly produced nanoparticles worldwide. ZnO Nano Particles are produced on an annual basis in between 10–100 times more than that of any other nanomaterial, at 550 and 5550 tonnes. ZnO nanoparticles (whitish powder) are inorganic compounds can be produced by chemical methods (precipitation, vapour transport, and hydrothermal processes), as well as biological methods through various plant extracts.

Zinc oxide nanoparticles were utilised by many different goods, including ceramic sunscreen cosmetics. They were used in optical devices, solar cells, gas sensors, biosensors, catalysts, paints, rubber products, semiconductors, and drug delivery systems [13]. Zn fertiliser is anticipated to be the best option in agriculture for foliar and soil applications. Moreover, ZnO nanoparticles are used to prevent and treat infections caused by diseases in plants. As the plant is equipped with micronutrients, the people with micronutrient deficits could be benefitted from this uptake. To date, very limited studies have been conducted to look into how ZnO nanoparticles affect plants [13].

The growth and development of plant are stimulated by ZnO nanoparticles, although the results change depending on the genotype. ZnO nanoparticles have a harmful effect on plants, and this effect is size-dependent, meaning that smaller particles are more hazardous than bigger ones, and the toxic effects are worse as concentration rises. Additionally, the shape of ZnO nanoparticles influences both how well they interact with cell membranes and how well they can enter cells.

## II. MATERIALS & METHODS

### A. Production of Zno Nano Particles

A 0.9M aqueous solution of Sodium hydroxide is prepared in a 100 mL beaker. This solution is thoroughly mixed and heated at $55^0$C. Later, the 0.45 M $Zn(NO_3)_2$ solution was added slowly. This entire mixture is allowed to settle and incubated for about 2 hrs for precipitation reaction. The Zinc Oxide nanoparticles get precipitated and get settled. The precipitated ZnO Nano Particles were then vacuum dried at $60^0$C after being washed with millipore water, ethanol, and then water. Such produced nanoparticles were put in an airtight screw cap flask (10 mL) and kept for later research.

Zinc oxide NPs was diluted with deionized water to a capacity of 50 ml, sprayed over the soil, and manually stirred to provide a uniform distribution of Zn. To compare this, the deionized water is considered as control and this is sprayed over the soil. Each treatment was repeated three times. The watering was continued to 60% of its water holding capacity. Later, it is allowed to acclimatise for one day before planting [14].

### B. Explant

The cowpea was collected from the mada forest, Korangi, Kakinada. The cowpea seeds were soaked overnight. The plumule parts of the embryo were then taken out and used as explants

#### *a. Shoot Length*

In many literature studies, the shoot length varied constantly. The application of ZnO nanoparticles was correlated with the length of the shoot. The increase of shoot length was directly proportional to the dosage of the ZnO nanoparticles. The different dosages like 20mg/L, 40mg/L, 60mg/L, 100mg/L, 150mg/L. Besides these treatments, an explant is grown separately without the addition of any ZnO NP, which is labelled as control. Shoot length was calculated by taking from the base of the root –hypocotyl transitions zone upto the base of cotyledons. The shoot length was measured by using a scale along with thread

#### *b. Root Length*

According to some research findings, the root length also varied constantly. The application of ZnO nanoparticles was correlated with the length of the root. The increase of root length was directly proportional to the dosage of the ZnO nanoparticles. The different dosages like 20mg/L, 40mg/L, 60mg/L, 100mg/L, 150mg/L. Besides these treatments, explants are grown separately without the addition of any ZnO NP, which is labeled as control. Root length was calculated by taking from the point below the hypocotyls to the end of the tip of the root. The root length was measured by using a scale along with thread

#### *c. Germination Index:*

The germination index is a measure of the percentage and speed of germination. This indicates a slight difference between each variety in response to temperature and light regime.

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This depends on the relative seed shoot length varied constantly. This is determined by taking Seed germination rate (RSG) and relative root growth (RRG) Germination index (GI) = (RSG\RRG)×100 Relative Seed germination rate (RSG) = $(S_c/S_s)$ *100 Relative root growth (RRG)= $(R_s/R_c)$*100 Where $S_s$ is no of seed germinated in sample $S_c$ is the no of seed germinated in control $R_s$ is the avg. root length in sample $R_c$ is the avg. root length in control

*d. Fresh Weight*

The fresh weight of root and shoot of seedlings was determined by weigh of the root and shoot seperately on electric balance. The explants of the which are prior treated with ZnO Nanoparticles of various dosages like 20mg/L, 40mg/L, 60mg/L, 100mg/L, 150mg/L were allowed to grow invitro and the obtained root and shoot seedling are weighed for fresh weight.

*e. Dry Weight*

The dry weight of root and shoot of seedlings was determined by placing the seedlings in a hot air oven at $60^0$C for 48hrs, cooled to room temperature and weighed. The explants of the which are prior treated with ZnO Nanoparticles of various dosages 20mg/L, 40mg/L, 60mg/L, 100mg/L, 150mg/L were allowed to grow invitro and the obtained root and shoot seedling are now weighed for dry weight.

*f. Seed Germination Percentage*

Seed germination (%) = $(S_c/S_s)$ *100

Where $S_s$ is no of seed germinated in sample $S_c$ is the no of seed germinated in control

## III. RESULTS AND DISCUSSION

Our study deals with the effect of ZnO nanoparticles on the growth of the plants. This was tested on cowpea seeds where the ZnO nanoparticles were used in different concentrations like 20mg/L, 40mg/L, 60mg/L, 100mg/L, and 150mg/L (Figure 2). The seed or explants that were not treated by any, is considered as control (Figure 1)

When we compare the results from shoot length to the ZnO nanoparticles concentration, the highest growth of shoot i.e 15.9 cm was observed with lowest concentration of ZnO nanoparticles i.e., 20mg/L. The highest concentration of the ZnO nanoparticles showed a negative effect on the shoot length when compared to control. When we compare the results from root length to the ZnO nanoparticles concentration, the highest growth of root i.e. 2.4 cm was observed with lowest concentration of ZnO nanoparticles i.e. 20mg/L. The results from shoot and root fresh weight to the ZnO nanoparticles concentration, the highest fresh weight of shoot and root i.e. 0.135 and 0.29 cm respectively was observed with 40mg/L concentration of ZnO nanoparticles The results from shoot and root dry weight to the ZnO nanoparticles concentration, the highest dry weight of shoot and root i.e 0.0127 and 0.0041 cm respectively was observed with 40mg/L concentration of ZnO nanoparticles. The highest Germination index was recorded as 786.2 at 20mg/L concentration with 100% Seed germination rate.

Best root and shoot growth is observed at a concentration of 20 mg/L. The reduction in growth observed at a concentration of 150 mg/L. The different concentration of ZnO nanoparticles showed a positive result on seed germination rate. The different concentration showed enhanced effect on the germination of seed and development of seedling in the cowpea seeds. During storage, due to this capability, they are more proned for deterioration.

## IV. CONCLUDING REMARKS AND FUTURE PERSPECTIVE

The nanotechnology is a technical area which has prompted intense application of Nano Particles in plant biology. The nanoparticles results showed a positive effect on the seed germination. However, it actually depends on Nano Particles concentration, type, shape, genotype of the plant. This research showed that Zinc Oxide nano particles could stimulate invitro germination of cowpea at 20mg/L conc. with no affect on growth and development of seedlings. The findings presented reveal that the nano particulate form of zinc oxide has a positive effect on the early seedling growth of V. radiata.

**Fig. 1: Control Cowpea Seeds**

**Fig. 2: Germinated Cowpea Seeds (at a Concentration of 100mg/L)**

From the results, the explants which was treated with various concentrations of ZnO nanoparticles have showed different response in terms of shoot length, root length, Fresh weight of root and shoot, dry weight of root and shoot, Seed germination (%), Germination index (Table 1). Each of the parameter is measured or determined by its own module (Table 2)

**Table-1: Measurement of Growth Characteristics Of Cowpea At Different Concentration Of Zno Nps (Control, 150mg/L, 100mg/L, 60mg/L, 40mg/L, 20mg/L) Within 2 Weeks**

| Treatment | Germination index | Seed germination (%) | Seedling growth (cm) | | Fresh weight (g) | | Dry weight (g) | |
|---|---|---|---|---|---|---|---|---|
| | | | Root length | Shoot length | Root | Shoot | Root | Shoot |
| Control | 580.45 | 85 | 1.5 | 14.6 | 00.21 | 0.118 | 0.0038 | 0.0099 |
| 150mg | 568.6 | 80 | 1.2 | 14.2 | 00.19 | 0.116 | 0.0031 | 0.0072 |
| 100mg | 671.35 | 90 | 1.7 | 14.9 | 00.23 | 0.121 | 0.0039 | 0.0121 |
| 60mg | 755.95 | 100 | 1.9 | 15.1 | 00.25 | 0.126 | 0.0041 | 0.0125 |
| 40mg | 781.05 | 100 | 2.1 | 15.6 | 00.29 | 0.135 | 0.0042 | 0.0129 |
| 20mg | 786.2 | 100 | 2.4 | 15.9 | 00.27 | 0.130 | 0.0041 | 0.0127 |

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**Table-2: Parameters and Their Measurements**

| Factors | Unit | Measuring device |
|---|---|---|
| Root length | Centimeter (cm) | Scale |
| Shoot length | Centimeter (cm) | Scale |
| Germination index | $\frac{Germinated\ seeds}{Total\ seeds} \times 100$ | Formula |
| Fresh weight | Grams | Electronic Balance |
| Dry weight | Grams | Electronic Balance |

**Fig. 3: Germinated Cowpea Seeds (at a Concentration of 20mg/L)**

**Fig. 4: Germinated Cowpea Seeds (at a Concentration of 40mg/L)**

**Fig. 5: Germinated Cowpea Seeds (at a Concentration of 60mg/L)**

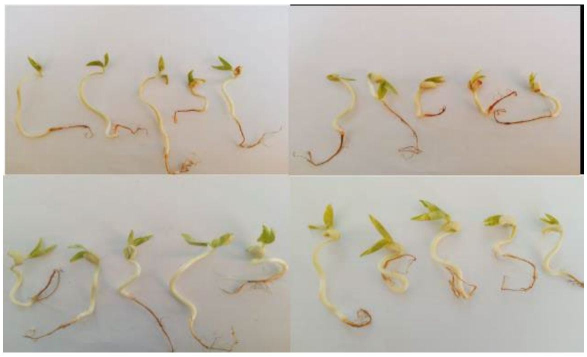

**Fig. 6: Germinated Cowpea Seeds (at a Concentration of 100mg/L)**

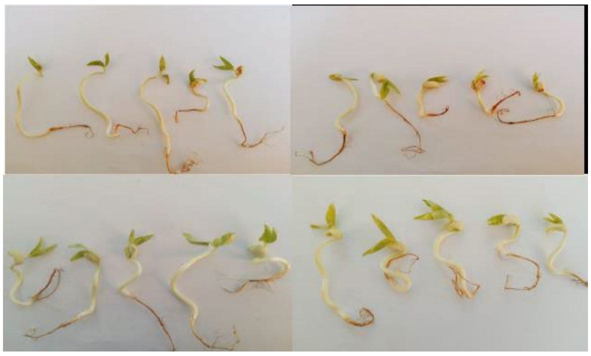

**Fig. 7: Germinated Cowpea Seeds (at a Concentration of 150mg/L)**

**Fig. 8: Germinated Cowpea Seeds (control)**

## DECLARATION STATEMENT

Authors are required to include a declaration of accountability in the article, counting review-type articles, that stipulate the involvement of each author. The level of detail differs; Some subjects yield articles that consist of isolated efforts that are easily voiced in detail, while other areas function as group efforts at all stages. It should be after the conclusion and before the references.

| | |
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| Availability of Data and Material/ Data Access Statement | Not relevant. |
| Anthors Contributions | Ala Devi Manogna collection and isolation of cowpea seeds from the seeds allocated in the particular region of city Kakinada and further importance of cowpea seeds.<br>Yeneti Yeswanth kumar estimation and production of Zno Nano particles and interactions.<br>Aravind Mahipani estimation of root and shoot lengths after and before reactions with Zno nanoparticles.<br>Jaya Sahitya kosuri in estimation of dry weight and fresh weight analysis. |

## REFERENCES


1. H. Xiong, A. Shi , B. Mou , J. Qin ,D. Motes and W. Lu , "Genetic Diversity and Population Structure of Cowpea" Vignaunguiculata L. Wal,. PLoS ONE 2016, vol.11, no. 8 https://doi.org/10.1371/journal.pone.0160941


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*Published By:*
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2. BC. Miller, ES. Oplinger, R. Rand, J. Peters, G. Weeıs, "Effect of planting date and plant population on sunflower performance" Argon.J, vol. 76, pp. 511-515.1984. https://doi.org/10.2134/agronj1984.00021962007600040002x
3. J. G. Vandemark, A. M. BrickOsorno, D. J. Kelly and A. C. Urrea, "Edible Grain Legumes" University of Nebreska-Lincoln Panhandle Research and Extension Center, 2014 https://doi.org/10.2135/cssaspecpub33.c5
4. AH. Arnall, Future technologies, today's choices nanotechnology, artificial intelligence and robotics; a technical, political and institutional map of emerging technologies. A Report for the Greenpeace Environmental Trust, London: Department of Environmental Science and Technology Environmental Policy and Management Group, Faculty of Life Sciences, Imperial College London, University of London, 2003.
5. LL. Bergeson, "The Regulatory Implications of Nanotechnology", Environmental Quality Management, vol. 14, no. 1, pp. 71 – 82, 2004. https://doi.org/10.1002/tqem.20027
6. V. Ghormade, MV. Deshpande and KM. Paknikar, "Perspectives for nano biotechnology enabled protection and nutrition of plants, Biotechnology Advances, vol. 29, pp. 792–803, 2011. https://doi.org/10.1016/j.biotechadv.2011.06.007
7. R. Raliya , V. Saharan , C. Dimkpa , P. Biswas, "Nanofertilizer for precision and sustainable agriculture": current state and future perspectives, J Agric Food Chem, 2017, vol. 66, no. 26, pp. 6487–6503. https://doi.org/10.1021/acs.jafc.7b02178
8. Duhan JS, Kumar R, Kumar N, Kaur P, Nehra K, Duhan S, "Nanotechnology: the new perspective in precision agriculture Biotechnol" Rep, vol. 15, pp. 11–23, 2017. https://doi.org/10.1016/j.btre.2017.03.002
9. M. Bundschuh, J. Filser and Lüderwald "where do we come from, where do we go to?", Environ SciEur, Nanoparticles in the environment, vol. 30, no. 6, 2018. https://doi.org/10.1186/s12302-018-0132-6
10. Siddiqui, Dr. H. Manzer, Al-whaibi, Mohamed, Mohammad, Firoz, Al-Khaishany and Mutahhar, Role of Nanoparticles in Plants, 2015. https://doi.org/10.1007/978-3-319-14502-0_2
11. Rudani, Komal, Prajapati, Kalavati, Patel and Vishal, THE IMPORTANCE OF ZINC IN PLANT GROWTH -A REVIEW vol. 5, pp. 2349-4077, 2018
12. A. Sharma, B. Patni, D. Shankhdhar and SC. Shankhdhar , "Zinc - an indispensable micronutrient", PhysiolMolBiol Plants, vol. 19, no. 1, pp. 11-20, 2013 https://doi.org/10.1007/s12298-012-0139-1
13. A. Tymoszuk and J. Wojnarowicz, "Zinc Oxide and Zinc Oxide Nanoparticles Impact on In Vitro Germination and Seedling Growth in Allium cepa L. Materials", Basel, vol. 13, no. 12, pp. 2784, 18 Jun 2020. https://doi.org/10.3390/ma13122784
14. Maity, Aniruddha, Natarajan, Nagappan, Pastor, Dunna, Vijay, Gupta, Chandan, Wasnik and Vinod, "Nanoparticles influence seed germination traits and seed pathogen infection rate in forage sorghum (Sorghum bicolour) and cowpea (Vignaunguiculata)", Indian Journal of Experimental Biology, vol. 56, 2018.
15. R. KOÇAK, M. OKCU, K. Haliloglu, A. Alayli, H. Nadaroglu, "Determination of the Effect of Magnesium Oxide Nanoparticles (MgO-NP) on in Vitro Culture of Cowpea (VignaUnguiculata L. Walp)", Research Square, 2021. https://doi.org/10.21203/rs.3.rs-1088469/v1
16. I J. Pemberton and G R Smith, "Inheritance of ineffective nodulation in cowpea", Crop Science, vol. 30, pp. 568-571, 1990. https://doi.org/10.2135/cropsci1990.0011183X003000030020x
17. Islam, M. T., & Lelin, P. (2023). Tweeting Climate Strike: A Netnographic Study of Fridays for Future's Narratives in the USA and Bangladesh. In Indian Journal of Mass Communication and Journalism (Vol. 3, Issue 2, pp. 1–8). https://doi.org/10.54105/ijmcj.b1058.123223
18. Sojourner Cultural Adaptation Toward National Culture Change. (2019). In International Journal of Recent Technology and Engineering (Vol. 8, Issue 3S2, pp. 616–619). https://doi.org/10.35940/ijrte.c1201.1083s219
19. Sreenivasan, A., S, A. M., Dhanya, M., & Raj, R. (2019). Safety Culture App: An innovation in Safety Performance System at the Aviation Industry. In International Journal of Engineering and Advanced Technology (Vol. 9, Issue 2, pp. 1214–1216). https://doi.org/10.35940/ijeat.b3620.129219
20. Zambare, Dr. A. V., Kulkarni, Prof. D. A., & Shirtode, Mr. P. (2023). Storage Study of Custard Apple Seed Powder. In Indian Journal of Food Engineering (Vol. 3, Issue 1, pp. 1–6). https://doi.org/10.54105/ijfe.a1009.123123
21. Patra, P. M., Pandey, D., R, Aswini., Krishnan, C., & Saha, P. (2022). DNA Based Molecular Detection Methods of Biological Adulterations in Commercial Teas: Successes and Obstacles. In International Journal of Basic Sciences and Applied Computing (Vol. 8, Issue 8, pp. 1–11). https://doi.org/10.35940/ijbsac.h0474.048822