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Impact of ZnO NPs on *In Vitro* Germination and Growth Characteristics of Rice (*Oryza sativa*)

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***Abstract* — A key feature of contemporary nanotechnology is the successful utilization of nanotechnological concepts in agriculture, for applications such as enhancement of the efficiency of crop production through improved seed germination & growth, smart fertilizers, smart pesticides etc. Effect of different nanoparticles (NPs) such as TiO_2, Ag, Si, Au, Cu, Zn and ZnO on seed germination have been studied in literature for crops such as canola, mung beans, onions, fenugreek and watermelon. Current study focuses on investigating the impact of ZnO NPs on the seed germination and growth of rice (*Oryza Sativa*). ZnO is a non-toxic NP and has the potential to boost the yield and growth of food crops. According to literature, ZnO NPs have been used to enhance the seed germination in different plants such as mung beans, peanut and black gram. Herein, ZnO NPs were synthesized by a wet chemical method by mixing ethanolic solutions of NaOH and Zn $(CH_3COO)_2{\cdot}2H_2O$.The morphology of the synthesized ZnO NPs was studied using Scanning Electron Microscopy (SEM) and synthesized ZnO NPs exhibited a spherical shape with a diameter ranging from 65 nm to 95 nm, with an average diameter of (73±2) nm. The impact of ZnO NPs on germination and growth of rice was studied under different NP concentrations (0-2000 mg/L) for Sudu samba and traditional rice varieties, Suwandel and Madathawalu. Further, the growth characteristics were investigated by measuring the root length and the shoot length of rice seeds. A significant enhancement of seed germination was observed in all three rice varieties after treating with ZnO NPs. Sudu Samba seeds showed a 7.3 % enhancement of seed germination (at 500 mg/L ZnO) while Suwandel and Madathawalu showed an enhancement of 20 % and 17% respectively. This can be attributed to the ability of ZnO NPs to penetrate through the cell wall and release targeting genes to specific cellular organelles to boost the cell division. As Zinc is an enzymatic component, it has the ability to influence secretion of indole acetic acid (IAA) which important to regulate plant growth and when the level of IAA is increased, it causes an effective response in seed germination. Interestingly, no significant difference in root length and shoot length was observed for Madathawalu while, a negative effect was observed for Sudu samba and Suwandel at 7 days. Further investigations with varying ZnO concentrations and other types of traditional Sri Lankan rice varieties are currently ongoing to gain more insight into the phenomena observed above.**

Keywords* — *Zinc oxide nanoparticles, germination, seedling growth, traditional rice

I. INTRODUCTION

Nanotechnology is an interdisciplinary research area that deals with the atomic and molecular scale of materials between 1 and 100nm scale. Owing to the unique properties of materials at nanoscale, its utilization has been researched in almost every field [1]. In agriculture, concepts of nanotechnology has successfully been utilized for applications such as enhancement of the efficiency of crop production through improved seed germination & growth, smart fertilizers, smart pesticides, nanosensors and nanobionics [2]. Several germination studies have been conducted in literature using nanoparticles (NPs) such as TiO_2, Ag, Si, Au, Cu, Zn and ZnO to enhance seed germination and crop growth [2]. For example, TiO_2 NPs have been experimented on canola and broad beans, Ag NPs have been experimented on fenugreek, watermelon and jasmine rice, whereas Si NPs have been experimented on sunflower. Further, $Mg(OH)_2$ NPs have been tested on corn and ZnO NPs have been experimented on peanuts and mung beans [3]-[4]. According to these studies, an increase in germination up to 75% has been observed when canola seeds (*Brassica napus*) were embedded with TiO_2 NPs (2000 mg/L). Additionally, the incorporation of TiO_2 NPs has also resulted in larger radicle and plumule growth of canola seedling at 1200 mg L^{-1}, 1500 mg L^{-1} [5]. Furthermore, an enhancement of germination and root lengths have been observed in TiO_2 treated broad bean (*Vicia faba*) seeds compared to the controlled seed [3]. Silver NPs on fenugreek (*Trigonella foenum-graecum*) has shown an enhanced germination percentage, where the highest germination (of 78%) has been observed at a AgNP concentration of 20µg mL^{-1} [6]. Acharya *etal* have investigated the seed germination, growth and yield of triploid and diploid watermelon (*Citrullus lanatus*) using AgNP, $AgNO_3$ and TNE (Turmeric Oil

Emulsion) and higher germination rates, lower mean germination times and higher final emergence percentages have been reported after treating with NPs [7]. Further, Jasmine rice (*Oryza sativa*) treated with Ag NPs have exhibited significantly higher germination percentages, vigor index and lengths of roots, shoots and seedling biomass compared to $AgNO_3$ primed seeds, while sunflower (*Helianthus annuus*) treated with Si NPs (0.2 and 0.4 mM) have shown a substantial reduction in days to 50% germination, mean germination time, improved root length, mean daily germination, seedling vigour index and final germination percentage [8], [9]. Also, corn (*Zea mays*) treated with $Mg(OH)_2$ NPs have shown enhanced seed germination (100%) and seedling growth at 500 ppm [10], whereas tomato (*Solanum lycopersicum*) seeds treated with Ag NPs have shown significantly high germination rates and seedling growth compared to untreated seeds [11]. Interestingly, these seeds have shown significant improvement in germination at only a specific concentration. At higher or lower concentrations apart from that concentration had caused different negative impacts on the seed germination, further growth and crop yield as well [6]. For example, Hojjat *etal* have reported a negative effect of Ag NPs on the germination of fenugreek at higher concentrations [6], [10]. Therefore, maintaining the effective and optimum concentration levels is enormously important for better results and maintaining the ecological balance.

Zinc Oxide NPs can be named as one of the most widely used NPs in the current context, owing to their antimicrobial, antifungal, optical and electrical properties. More importantly, ZnO NPs have the potential to boost the yield and the growth of food crops [9]. For example, N.Jayarambabu *etal,* have reported a higher degree of germination (of 84.75%) of mung beans (*Vigna radiata*) in the presence of ZnO NPs, with an increase in the root length by 29.2%, shoot length by 9.36% and seedling length by 2.51 % at 125 ppm of ZnO NPs [12]. Further, T.N.V.K.V Prasad *etal* have reported a 100% seed germination on peanut, while K.Raja *etal* have observedma 4 % increase of seed germination, 16.3 % increase in root length, 18.1 % increase in shoot length and 25.2 % increase in seedling vigor, over control in black gram (*Vigna mungo*) when treated with ZnO nanoparticles at 1100 mg kg^{-1} [4] [13].

II. OBJECTIVES

The objective of the current study is to investigate the effects of ZnO NPs on the process of *in vitro* seed germination and seedling growth of rice (*Oryza sativa*), specifically focusing on traditional rice varieties such as Suwandel, Madathawalu, Kalu heenati and Pachchaperumal, which are considered to be rich in nutrients such as proteins, minerals and antioxidants, compared to other varieties.

III. METHODOLOGY

A. Materials

Zinc acetate dehydrate [Zn $(CH_3COO)2.2H_2O$] (Molecular Weight 219.50 g.mol^{-1}, Minimum Assay 99.5%, Sisco Research Laboratories Pvt Ltd, India), sodium hydroxide (NaOH) (Molecular weight 40 g.mol^{-1}, Minimum Assay 97%, S D Fine-Chem Limited, India) and methanol (CH_3OH) (Sigma Aldrich, USA) were used to synthesis the ZnO NPs from the wet chemical method. Seeds of rice (*Oryza sativa*), Sudu samba, Suwandel, Madathawalu were gathered from local farmers.

B. Synthesis of ZnO NPs

The synthesis was adapted from "Germination and Growth Characteristics of Mung-bean Seeds (*Vigna radiata* L.) affected by Synthesized Zinc Oxide Nanoparticles [12]. ZnO NPs were synthesis as stated in literature via a chemical method. 0.1M zinc acetate was dissolved in methanol and stirred for 30 minutes at 50^0C. 0.2M sodium hydroxide was dissolved in methanol with stirring and it was scaled up to 1:2 by adding 100ml methanol. Sodium hydroxide was added drop wise to the zinc acetate solution that was kept at 50^0C. During the addition of sodium hydroxide solution, 50ml methanol was added to zinc acetate solution to prevent methanol evaporation, followed by another 50 ml at the end. A white milky solution was obtained and stirring continued for 80 minutes at 50^0C. The solution was aged for 17 hrs at 27^0 C temperature. After that the solution was centrifuged for 10 minutes for volume reduction. The volume reduction solution was washed three times using centrifuge. The sample was then placed in oven at 70^0C for 90 minutes. The observed white powder was collected to a ceramic crucible. The crucible was placed in a muffle furnace and heat treated at 400^0C for 2 hours. Finally, the powder was grinded using mortar and pestle.

C. Characterization of ZnO NPs

The characterization of the ZnO NPs, presented herein, was performed at an accredited research institute, Sri Lanka Institute of Nanotechnology at Homagama, Sri Lanka. The morphology of particles was determined using Scanning Electron Microscopy (SEM) (Hitachi SU6600 Scanning Electron Microscope, Europe).

D. Seed Preparation

The seeds were first checked for their viability by suspending them in distilled water. The seeds which are settled to the bottom were selected for further study. For in vitro cultures 50 seeds were tested for each sample. The seeds were soaked under different NP concentrations (0 – 1000 mgL^{-1}). The control (0 mgL^{-1}) seeds were treated with 10 mL distilled water. The seeds were kept in the prepared medium for two days and then kept on a wetted paper towel for a day. Sudu samba and traditional rice varieties, Suwandel and Madathawalu seeds were experimented in the research.

E. Seed Germination Test

The seed germination rate {SGR- Equation (1)}, relative root growth {RRG - Equation (2)} and germination index {GI - Equation (3)} were calculated using the following equation [12].

Seed Germination Rate $$\mathrm{SGR} = \frac{\mathrm{SS}}{\mathrm{SC}} \times 100\% \quad (1)$$

Relative Root Growth $$\mathrm{RRG} = \frac{\mathrm{RS}}{\mathrm{RC}} \times 100\% \quad (2)$$

Germination Index $$\mathrm{GI} = \frac{\mathrm{SGR}}{\mathrm{RRG}} \times 100\% \quad (3)$$

Where SS is the number of seed germinated in ZnO NPs sample, SC is number of seed germinated in control, RS is the average root length in ZnO NPs sample and RC is average root length in control.

F. Root Length and Shoot Length

Root length was taken from the seminal root of the seed. Shoot length was measured from the beginning of the mesocotyl to the end of the second leaf. The root and shoot length were measured with the help of a thread and scale.

IV. RESULTS AND DISCUSSION

A. Characterization of ZnO NPs

Morphology of the synthesized ZnO NPs was studied using Scanning Electron Microscope (SEM) images (Fig. 1). According to the SEM analysis, synthesized ZnO NPs exhibited a spherical shaped with a diameter ranged from 65 nm to 95 nm, with an average diameter of (73±2) nm.

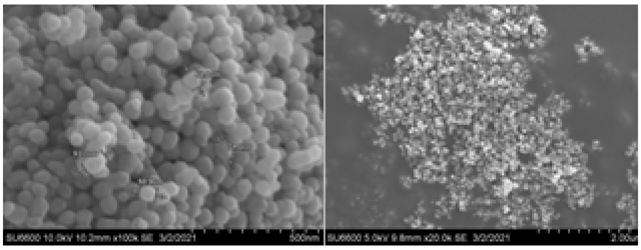

Fig. 1. Scanning electron microscopy (SEM) images of zinc oxide nanoparticles (ZnO NPs) at different scales of 500 nm, 2µm from left to right

B. Seed Germination

Table 1 summarizes the seed germination percentages of ZnO NPs embedded Sudu samba, Suwandel and Madathawalu. As can be seen in Table 1 a significant enhancement in percent germination was observed in ZnO NPs treated samples compared to the control, at lower concentrations. For Sudu samba, the highest germination was observed at 500 mg/L, which was 88 %. This was an enhancement of 7.3% compared to the control which showed germination percentage of 82%. Interestingly, seeds treated with 1000 mgL^{-1} ZnO NPs showed the least germination, and it was even lower than the control. A similar behavior was observed with Suwandel where the sample treated with 200 mgL^{-1} ZnO NPs showed the highest germination of 96%, while the control showed a seed germination percentage of 70 (Fig. 2). The seeds which were treated with 1000 mgL^{-1} and 2000 mgL^{-1} ZnO NPs showed the least germination and the seeds treated with 500 mg/L exhibited a germination percentage of 84 %. Overall, the dynamics of seed germination were most intensive in the media with ZnO NPs at concentration of 200 mg/L. Further, Madathawalu showed the highest germination of 14% at the 500 mgL^{-1} ZnO NPs while the control showed 12%. And this was a 17 % enhancement in the percent germination. Overall form these preliminary data, it can be stated that the elements of seed germination have generally escalated in the media with ZnO NPs at the convergence of difference concentration of ZnO NPs.

Table 1. . Seed germination percentage of different seeds types on different concentration of ZnO NPs at second day

Seed Type	ZnO NPs Concentration (mgL^{-1})	Germination Percentage at 2nd day
Sudu samba	0	82 %
	500	88 %
	1000	78%
Suwandel	0	70 %
	200	96 %
	500	84 %
	1000	60 %
	2000	66 %
Madathawalu	0	12 %
	500	14 %

Fig. 2. Suwandel germination on 200 mgL^{-1} ZnO NPs at Day 2

C. Seedling Growth

The seedling growth was observed for a period of 7 days. As can be seen in Table 2 and 3 the control has exhibited the highest root length and shoot length in Sudu samba and Suwandel. Interestingly, no significant difference in root length and shoot length was observed for Madathawalu while, the above-mentioned negative effect was observed for Sudu samba and Suwandel at 7 days.

Table 2. Seedling growth characteristics of Sudu samba within 7 days

Concentration (mgL^{-1})	Germination Percentage (%)	Root Length(mm)	Shoot Length(mm)	Relative Root Growth	Germination Index
0	92	12.1 ± 5.0	30.7±10.9	100.0	92.0
500	90	6.1 ± 2.3	29.8 ± 7.4	50.3	179.0
1000	84	7.4 ± 2.6	28.5±10.9	61.2	137.3

Table 3. Seedling growth characteristics of Suwandel within 7 days

Concentration (mgL^{-1})	Germination Percentage (%)	Root Length(mm)	Shoot Length(mm)	Relative Root Growth	Germination Index
0	96	17.7 ± 8.6	38.9 ± 9.8	100.0	96.0
200	98	15.3 ± 6.7	37.0±10.4	86.7	113.1
500	100	10.3 ± 4.8	33.7±11.9	58.2	171.7
1000	96	10.9 ± 4.3	36.0± 9.7	61.7	155.5
2000	96	10.8 ± 4.1	35.0±11.2	61.0	157.3

Herein, a significantly positive impact on early seed germination was observed when rice seeds were treated with ZnO NPs. Interestingly, different concentrations of ZnO NPs have affected on different seeds types in different ways. The experiment results showed that ZnO NPs can be used to enhance the seed germination and early seedling growth of rice seeds. However, it is crucial to find the optimum concentration of ZnO NPs for individual rice varieties as there can be side effects on the growth of the seeds at higher concentration.

This observation of enhanced germination in the presence of ZnO NPs can be attributed to their ability to pass through the cell wall and release targeting genes to specific cellular organelles. Furthermore, NPs have the ability to stimulate or inhibit of seed germination or seedling growth, activation of genes involved in metabolism and induction of photosynthesis or reactive oxygen species. Specifically, ZnO NP has the potential to pass through the cell wall and release targeting genes to specific cellular organelles, enhance the cell division while zinc is an enzymatic component influencing the secretion indole acetic acid (IAA) which is a phytohormone(auxin) which significantly regulates plant growth. By increasing the level of IAA, zinc gives a positive response in seed germination.[1], [14]-[15]. Moreover, the early seed germination and seedling growth might have happened due to the water uptake by the ZnO NPs treated seeds and also it caused to reduce the adverse effects of heat, drought and salt stresses [1]. Additionally, the antifungal and antibacterial action of ZnO NPs can also be useful herein to control the spread of infections by a variety of plant pathogens and thereby enhancing the crop productivity.

Fig. 3. Abnormal seed germination due to the high concentration of ZnO NPs

Interestingly, at higher concentrations of ZnO NPs, some negative effects such as slow germination rate, lower seedling growth such as root growth, shoot growth and distorted root growth were observed (Fig.3). For example, Suwandel showed only a 66% of seed germination at 2000 mg/L while 200 mgL^{-1} sample showed a 96% germination, whereas the control seeds showed 70% seed germination. According to literature, similar phenomena have been observed where different types of NPs have shown positive impacts on the seed germination of crops such as fenukgreek (*Trigonella foenum-graecum*), sunflower (*Helianthus annuus*), corn (*Zea mays*), watermelon (*Citrullus lanatus*), peanut (*Arachis hypogaea*), jasmine rice (*Oryza sativa*) and chickpea (*Cicer arietinum*) at lower concentrations. For example, S. S. Hojjat etal have shown that Ag NPs enhance the germination and seedling growth of fenugreek by 78 % at 20μg mL^{-1} concentration. However, at higher concentrations, Ag NPs have shown adverse effects. The higher concentrations of AgNPs (>20μg mL^{-1}) strongly inhibited both the shoot and root growth (especially as moisture weight), with a more marked inhibition of the shoot growth than the root growth[6]. Also, M. Sedghi *etal* have observed slightly adverse effects such as reduction in the residual fresh and dry weight (respectively 0.36g and 0.090g) at 1g.L^{-1} with soybean (*Glycine max*) compared to the control as a consequence of increasing the concentration of ZnO NPs [16].

V. CONCLUSION

In this study, ZnO NPs were synthesized via a wet chemical method and the impact of ZnO NPs on the germination and the growth of rice (*Oryza sativa*) was studied using different types of rice samples including traditional Sri Lankan rice spices such as Suwandel and Madathawalu. Synthesized ZnO NPs were characterized by Scanning Electron Microscope to determine the morphology and the size. And ZnO NPs were found to be spheres with a diameter in the range of 65nm to 95 nm. According to the results, the application of ZnO NPs had significantly enhanced the seed germination of rice where Sudu Samba seeds showed a 7.3 % enhancement of seed germination (at 500 mg/L ZnO) while Suwandel and Madathawalu showed an enhancement of 20 % and 17% respectively. This can be attributed to enhanced cell division supported by ZnO NPs, a critical nutrient in the cell division and growth in plants. Further, according to preliminary data, it was also observed that lower concentration of ZnO NPs leads to best germination percentage. Sudu samba seeds treated with 500 mgL^{-1} showed 88% germination while seeds treated with 1000 mg/L resulted a germination percentage of 78 % at the second day. Suwandel seeds treated with 200 mgL^{-1} showed 96% germination and at concentrations above 500 mg/L, percentages of germination were below 70 %, which was the germination percentage of the control at second day. According to preliminary data, it can be concluded that ZnO NPs have exhibited a positive impact on *the in vitro* germination of Rice (*Oryza sativa)* for Sudu samba, Suwandel and Madathawalu. However, it is significantly important to focus on the optimum and effective concentration of ZnO NPs for higher seed germination, as well as for early plant growth which is essential in achieving higher crop productivity.

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