The impact of nano-ZnO foliar fertilizer on growth and yield of cultivated rice (*Oryza sativa* L.) varieties in Sri Lanka

S. Somaratne, S.R. Weerakoon*, N. Karthikeyan and D.S.P. Munasinghe

**Highlights**

- There was a significant effect of nano-ZnO-fertilizer on yield parameters than growth parameters of the three rice varieties.
- The varietal responses to the applied nano-ZnO-fertilizer varied and higher responses were observed in inbred *Bg94-1*.
- The performances between the two traditional varieties, *Pachchaperumal* and *Suwandel* were more or less similar.
- Weight of 100-seed of *Suwandel* treated with nano-ZnO 120 mg L\(^{-1}\) was prominent.
- Foliar application of nano-ZnO-fertilizers increases growth and specifically yield performances of rice varieties under consideration.
The impact of nano-ZnO foliar fertilizer on growth and yield of cultivated rice (Oryza sativa L.) varieties in Sri Lanka

S. Somaratne¹, S.R. Weerakoon¹*, N. Karthikeyan² and D.S.P. Munasinghe¹

¹Department of Botany, The Open University of Sri Lanka, Nawala, Sri Lanka.
²Department of Physics, The Open University of Sri Lanka, Nawala, Sri Lanka.

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Abstract: Nanotechnological improvements such as nano-fertilizers are important in agriculture due to higher capability of penetration into plants, nutrient use efficiency and reduced off-target wastage of fertilizers. Rice being the second most important cereal crop in the world, is the national staple food in Sri Lanka. Insufficiency of soil Zn in paddy fields led to a reduction in plant growth and yield. Present study was conducted to assess the effect of nano-ZnO-fertilizer and bulk ZnO on growth and yield performances of commonly cultivated traditional (Pachchaperumal, Suwandel) and inbred (Bg94-1) rice varieties in Sri Lanka. Nano-ZnO particles were synthesized by thermal decomposition route and characterized using powder X-ray diffraction and FE-SEM. Sizes of the nano-ZnO particles varied from 50 nm to 500 nm. Pots were arranged in randomized complete block design with twenty replicates per treatment and varied from 50 nm to 500 nm. Pots were arranged in randomized complete block design with twenty replicates per treatment and treated with distilled water, bulk ZnO 60 mg L⁻¹, nano-ZnO 60 mg L⁻¹ (Conc. 1), and nano-ZnO 120 mg L⁻¹ (Conc. 2). Growth related characters were measured at 30, 60, and 90 days after sowing (DAS). Yield characters were measured at the harvesting stage. Analysis of variance showed a significant effect of nano-ZnO-fertilizer on yield parameters than growth parameters of three rice varieties. Varietal responses to the applied nano-ZnO-fertilizer vary and higher responses were observed in nano-ZnO-fertilizers on Pachchaperumal and Suwandel were almost similar; however, weight of 100-seed of Suwandel treated with nano-ZnO 120 mg L⁻¹ was prominent. Findings of the study revealed, a better yield performance for three rice varieties with foliar application of nano-ZnO-fertilizers.

Keywords: Foliar application; growth and yield; nano-ZnO-fertilizer; rice.

INTRODUCTION

The continuous growth of the global population makes a significant impact on the environment, which in turn affects the capacity for food production directly via changes in land availability and suitability for agriculture (Rampe and Dietrich, 2014). However, at present, agriculture activities are encountering various global challenges such as climate changes, urbanization, non-sustainable use of resources, and environmental issues such as runoff, accumulation of pesticides and fertilizers (Chen and Yada, 2011). The fate of the greater part of the applied agrochemicals is uncertain as they may not entirely be utilized by the targeted plants (Fernandez and Brown, 2013) and the off-targeted chemicals could finally become environmental pollutants and result in undesirable environmental consequences. In addition, excessive application of chemical fertilizers leads to accumulation in soil and water, surpassing the natural levels and becoming pollutants (Nair et al., 2010).

The continuous cultivation of high-yielding varieties with the application of macronutrients in cropping systems causes deficiencies in micronutrients. Depletion of soil Zn is reaching deficient levels in most of the croplands and becoming a crisis in the world and almost all crops respond positively to the application of Zn (Welch, 2002). Among the essential plant micronutrients, Zn is one of the important determinants of crop productivity which is similar to that of major nutrients (Lal, 2009) and plays a very important role in plant metabolism influencing the activities of hydrogenase and carbonic anhydrase, stabilization of ribosomal fractions and synthesis of cytochrome (Tisdale et al., 1984). Further, Zn deficiency can also adversely affect the quality of harvested products; plant susceptibility towards injury by high light or temperature intensity, and infections by fungal diseases (Marchner, 1995; Cakmak, 2000).

The deficiencies of micronutrients are of critical importance for sustaining the high productivity of rice in Sri Lanka. Following the addition of Zn fertilizers to the soil, Zn transforms gradually from more active and available fractions into less available species such as precipitates (i.e. ZnCO₃) and adsorbs to oxide phases (e.g. Fe-, Al-oxides)(Ma and Uren, 2006). It has been well-established that higher Zn fertilizer efficiency can be achieved through sources of Zn in fertilizers with higher solubility (Mortvedt and Giordano, 1969; Shaver et al., 2007). On the other hand, foliar application of micronutrient fertilizers with Zn, B, Cu, Mn, and Fe has been shown suitable for field use with marked effectiveness and rapid plant response (Fernández et al., 2013). All aerial plant parts are covered by a hydrophobic cuticle that limits the bidirectional exchange of water, solutes, and gases between the plant and the surrounding environment except certain structures like stomata and lenticels (Fernandez et al., 2013).

*Corresponding Author’s Email: shyamaweerakoon@gmail.com

https://orcid.org/0000-0003-0975-2738

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Nano particles possess the greater ability to penetrate the plant cell membranes due to their small size than other foliar-applied conventional fertilizers. Also, foliar application of chemical fertilizers has the advantage of being absorbed into the plants since they directly contact the plant surface either leaves or stem, thus losses can be minimized and the expected results of chemical fertilizer application can be maximized (Fageria et al., 2009).

Nanofertilizers are synthesized or modified forms of traditional fertilizers or bulk materials or extractions of different vegetative parts of plants by different methods with the help of nanotechnology to improve soil fertility, productivity, and the quality of agricultural products (Salama, 2012). The reduced particle size of nanofertilizers has increased specific surface area and particles per unit area providing more opportunity to contact nanofertilizers to the crop plant that leads to high penetration and uptake of nutrients (Lin and Xing, 2007). Nanoparticles and nanocapsules provide an efficient means to distribute pesticides and fertilizers in a controlled fashion with high site-specificity thereby reducing collateral damage (Moore, 2006). Therefore, the usage of nanofertilizers is more efficient than conventional fertilizers, and also it reduces the wastage of chemicals to the environment (Naderi et al., 2011). The smaller size, higher specific surface area, and reactivity of nano-particulate-ZnO compared to Bulk ZnO may affect Zn solubility, diffusion, and hence availability to plants.

In Sri Lankan agricultural field, nanotechnology is at an emerging stage and it is under-utilized. The use of nanoparticles in plant productivity is unlimited. However, being an infant technology, there are ethical and safety issues associated surrounding nanotechnology. Therefore, careful evaluation of nanoparticles is required before adapting to the use of nano-fertilizers. The literature review revealed that studies on the application of nano-ZnO-fertilizers and evaluation of growth and yield performances of rice varieties grown in Sri Lanka are hardly found. The objectives of this study were to assess the effect of nano-ZnO-fertilizers on the growth and yield performances of selected inbred and traditional rice varieties cultivated in Sri Lanka.

**MATERIALS AND METHODS**

**Selection of rice varieties**

Three rice varieties; two traditional, *Pachchaperumal*, *Suwandel*, and one inbred, *Bg94-1* were selected for the study, based on their extent of cultivation, popularity among Sri Lankan farmers, and suitability for lowland paddy cultivation. The seed materials were obtained from Rice Research and Development Institute (RRDI), Bathalagoda, Sri Lanka.

**Synthesis of nano-ZnO**

Precursor chemicals zinc acetate \([\text{Zn(H}_2\text{C}_2\text{O}_4\text{)}_2\cdot2\text{H}_2\text{O}]\), NaOH and isopropyl alcohol (2-propanol) with high purity (>99.5 %) were used for the experiment. All the chemicals and reagents used in this study were of analytical grade. All aqueous solutions were prepared using deionized double distilled water. Nano-ZnO particles were synthesized via thermal decomposition routes to get the desirable surface morphologies such as rod and hexagonal shapes suitable for this study (Saravanan et al., 2011; Saravanan et al., 2012). Zinc acetate dihydrate was heated at 500 °C for 3 h in a muffle furnace. After that, the sample was ground for an hour to get the desirable surface morphologies suitable for the study (Saravanan et al., 2011; Saravanan et al., 2012; Rathore et al., 2019). Thermo Gravimetric Analysis (TGA) was carried out to determine the decomposition temperatures of zinc acetate dihydrate as well as the temperature for the synthesis of ZnO nanoparticles. The samples were analyzed with a heating rate of 10 °C/min in the air atmosphere.

**Germination of rice seeds and transplanting**

Rice seeds were germinated in a growth chamber and then one-week-old seedlings were transplanted. Five seedlings/replicate were planted per pot (diameter 25.00 cm, height 22.00 cm) and five pots per treatment in each variety were used. Planting pots were filled with soil mixture (ready-made potting mixture and paddy soil in 1:1 ratio). N, P, K fertilizer was added to the pot before rice transplanting. For each pot, urea 0.57 g, triple super phosphate 0.16 g, muriate of potash 0.5 g were added (Department of Agriculture, 2018).

The pot-level experiment was carried out in the polyvinyl house. The factorial experiment (rice variety and treatment) was employed in the experiment based on Completely Randomized Block Design (CRBD) with twenty replicates.

**Characterization of nano-ZnO fertilizer**

Characterization was carried out at the Sri Lanka Institute of Nanotechnology. The synthesized nano-ZnO fertilizer particles were characterized using Powder X-Ray Diffraction using Bruker D8 Focus X-Ray Diffractometer (PXRD) and the PXRD pattern was recorded using Cu-Kα radiation (1.54 Å), a voltage of 40 kV, a current of 40 mA, 20 range from 5° to 80°, an increment of 0.5° and a scan speed of 10.0 s/step. The analysis of ZnO samples coated with Au/Pd was carried out using a Field Emission Scanning Electron Microscope, (SEM Hitachi SU6600 Analytical Variable Pressure Field Emission) (FE-SEM) to characterize the structural properties such as structure and surface morphology.

The average crystallite size of the nano and bulk ZnO particles was obtained by the Scherrer equation (Scherrer, 1918).

\[
D = \frac{K\lambda}{\beta \cos \theta}
\]

where \(D\) = Crystallite size in nm; \(K\) = Scherrer Constant (0.9); \(\lambda\) = X-ray wavelength; \(\beta\) = Full Width at Half Maximum (FWHM) in radians; \(\theta\) = diffraction angle in radians.

**Characterization of rice leaf tissues**

Rice leaves were trimmed and transferred immediately after...
Preparation of suspensions

The suspensions of initially synthesized nano-ZnO-fertilizer in different concentrations were prepared by weighing particles and dispersing them in deionized water. Nano-ZnO suspension of 60 mg L⁻¹ (Conc.1), 120 mg L⁻¹ (Conc.2) were the test solutions and the distilled water was served as Control-1 and the bulk ZnO suspension of concentration 60 mg L⁻¹ was taken as the Control-2.

Application of nano-ZnO-fertilizer to rice plants

The prepared suspensions were applied on two stages on rice plants at 40 days after sowing (DAS) and 70 DAS, i.e., filling stage of grains. Pump sprayers were used to spray fertilizer (0.46 g m⁻²). The application of fertilizer was done 30 seconds per plant at a constant rate of spraying. The treatments were performed in the morning between 6:00 to 7:00 am. The average temperature was 27 °C with an average 76% of humidity. Water was applied three times per week directly to the pots (without holes). The factorial experiment (rice variety and treatment) was employed in the experiment based on Completely Randomized Block Design (CRBD) with twenty replicates.

Characterization rice plants

The following measurements were taken 30, 60, and 90 days after sowing (DAS). All the measurements were made according to the standard recommended by PGRC Rice Characterization Catalogue (1999). The heights of five plants per block were measured from the base of the shoot to the tip of the tallest leaf blade. The number of tillers and the number of leaves were counted and recorded for five plants in a block. The plant biomass were determined by destructive sampling of two rice plants per block and measuring initial weights of plants and then kept at 105 °C for 20–40 minutes followed by simple air-drying (Pathan et al., 2010). Samples were mounted onto the sample stub using carbon tapes and the images were taken after gold sputter coating for 15 seconds. Upper (adaxial) and lower (abaxial) surfaces of rice plant leaves of three rice varieties were observed by Hitachi SU6600 Analytical Variable Pressure FE-SEM at Sri Lanka Institute of Nanotechnology.

RESULTS

Nano-ZnO particle size was determined by PXRD pattern using Scherrer equation and accordingly, the average crystallite size of a nano-ZnO particle is 31 nm. The PXRD pattern given in Figure 1 indicates that the crystal structure of nano-ZnO particles is in hexagonal wurtzite structure (JCPDS Card No. 01-079-0208). Similarly, the PXRD pattern of Figure 2 indicates that the crystal structure of Bulk-ZnO particles is also in hexagonal wurtzite structure with slight changes in the unit cell parameters (JCPDS Card No. 00-079-0207).

Scanning Electron Microscope (SEM) analysis of nano ZnO

FE-SEM images of bulk-ZnO particles shown in Figure 3 B indicated different shapes and sizes. The shapes of the particles vary from spherical, rod, to more irregular shapes. The different shapes of the ZnO particles are not uniformly dispersed and the particles have minimum agglomeration resulting greater surface area to volume ratio. The sizes of these particles range from 100 nm to 500 nm. FE-SEM images of nano-ZnO particles shown in Figure 3A indicate varying shapes and sizes. The shapes of particles are spherical and rod. The different shapes of the ZnO particles are well dispersed and show minimum agglomeration with complete separation of particles from one another. The sizes of the nano-ZnO particles vary from 50 nm to 500 nm.

Scanning Electron Microscope (FE-SEM) analysis of leaf stomata

The plants after first fertilization showed no physiological changes such as drying of leaves, chlorosis, and patches on laminar or any other visually observable characters. Thus, nano-ZnO-fertilizer concentrations chosen for the
study were well-tolerated by all the rice varieties included. The FE-SEM images of abaxial and adaxial surfaces of the lamina of three different rice varieties included in the study are shown in Figures 4 A, B, and C and all abaxial images indicated an elongated slit-like aperture guarded by dumbbell-shaped cells. The hairs and silicon projections were abundant in the abaxial surface of the leaf and in certain instances, the apertures of the stomata were occluded with micro-hairs of microscopic silicon projections. The adaxial lamina surface of the rice varieties indicated that the frequency of occurrence of microscopic projections higher in traditional rice varieties - Pachchaperumal and Suwandel.

Analyses of parametric characteristics

Growth characteristics

The vegetative/growth characteristics of the rice varieties treated with de-ionized water (Control 1), bulk ZnO (Control 2) suspension, and two concentrations of 60 mg L⁻¹ (Conc. 1) and 120 mg L⁻¹ (Conc. 2) of Nano-ZnO-fertilizers are shown in Table 1. The comparison of the plant height (cm), biomass, and percentage dry weight for Bg94-1 across the treatments indicated that there was a difference in magnitudes of height, biomass, and percentage dry weight. However, the mean height of Pachchaperumal and Suwandel indicated less variation compared to the rice variety Bg94-1. However, Suwandel showed an increase in mean height with the nano-ZnO 60 mg L⁻¹. More prominently, Bg94-1, Pachchaperumal and Suwandel show a considerable increase of mean values in biomass and percentage dry weight, with both 60 and 120 mg L⁻¹ nano-ZnO-fertilizer concentrations (p ≤ 0.05).

Total chlorophyll and chlorophyll a and chlorophyll b of rice varieties treated with De-ionized water (Control 1), bulk ZnO (Control 2) suspension, and two concentrations of 60 mg/L (Conc. 1) and 120 mg L⁻¹ (Conc. 2) of Nano-ZnO-Fertilizers are shown in Table 2. The comparison of the total chlorophyll, chlorophyll a, and Chlorophyll b for Bg94-1 across the treatments indicated that there was a difference in magnitudes of the contents of total chlorophyll and chlorophyll b. However, the results suggest that different treatments i.e. bulk and Nano form, as well
Figure 4: FE-SEM images of Adaxial and Abaxial surfaces and stomata of A) Bg94-1 B) Pachchaperumal and C) Suwandel.

Table 1: Descriptive statistics of the parametric characteristics, plant height (cm), biomass, and percentage dry weight with treatments and rice varieties

| Rice Variety | Treatment     | Height/cm | Biomass     | Percentage Dry Weight |
|--------------|---------------|-----------|-------------|-----------------------|
| Bg94-1       | De-ionized water | 69.73(4.86)* | 3.33(0.61)* | 23.59(0.62)*          |
|              | Bulk - 60 mg L^{-1} | 72.06(4.07)* | 3.60(0.58)* | 21.94(1.32)*          |
|              | Nano - 60 mg L^{-1} | 71.04(4.26)* | 4.68(0.91)* | 23.75(0.94)*          |
|              | Nano-120 mg L^{-1} | 75.36(4.13)* | 5.92(1.03)* | 24.02(1.05)*          |
| Pachchaperumal | De-ionized water | 99.39(4.76)* | 1.49(0.25)* | 21.98(1.11)*          |
|              | Bulk - 60 mg L^{-1} | 108.35(5.62)* | 1.89(0.31)* | 24.50(1.54)*          |
|              | Nano - 60 mg L^{-1} | 105.08(5.76)* | 2.92(0.47)* | 26.53(1.75)*          |
|              | Nano-120 mg L^{-1} | 104.86(5.41)* | 4.21(0.75)* | 26.13(1.54)*          |
| Suwandel     | De-ionized water | 93.11(5.30)* | 1.14(0.26)* | 23.03(1.52)*          |
|              | Bulk - 60 mg L^{-1} | 88.68(4.44)* | 1.30(0.30)* | 23.99(1.50)*          |
|              | Nano - 60 mg L^{-1} | 96.10(5.17)* | 1.57(0.35)* | 25.69(1.71)*          |
|              | Nano-120 mg L^{-1} | 90.24(5.17)* | 1.90(0.46)* | 27.34(1.82)*          |

Mean values follow standard errors of mean within parenthesis. The similar letters in a column indicate the statistical significance at $p \leq 0.05$.
Table 2: Descriptive statistics of the parametric characteristics, total chlorophyll and chlorophyll a and chlorophyll b with treatment and rice variety

| Rice Variety | Treatment      | Total Chlorophyll | Chlorophyll a | Chlorophyll b |
|--------------|----------------|-------------------|---------------|---------------|
| **Bg94-1**   | De-ionized water | 35.654 (0.394)   | 26.617 (0.144) | 9.046 (0.280) |
|              | Bulk - 60 mg L⁻¹ | 41.828 (0.391)   | 26.859 (0.203) | 14.980 (0.581) |
|              | Nano - 60 mg L⁻¹ | 42.694 (1.059)   | 26.638 (0.161) | 16.067 (0.902) |
|              | Nano-120 mg L⁻¹  | 42.553 (1.418)   | 26.647 (0.174) | 15.918 (1.248) |
| **Pachchaperumal** | De-ionized water | 43.531 (0.183)   | 26.743 (0.067) | 16.801 (0.118) |
|              | Bulk - 60 mg L⁻¹ | 42.185 (0.762)   | 26.330 (0.102) | 15.867 (0.663) |
|              | Nano - 60 mg L⁻¹ | 44.659 (0.222)   | 27.217 (0.268) | 17.455 (0.476) |
|              | Nano-120 mg L⁻¹  | 42.922 (1.128)   | 26.356 (0.330) | 16.578 (0.803) |
| **Suwandel** | De-ionized water | 40.284 (0.895)   | 25.938 (0.114) | 14.357 (1.003) |
|              | Bulk - 60 mg L⁻¹ | 43.168 (1.055)   | 27.487 (0.175) | 15.692 (1.194) |
|              | Nano - 60 mg L⁻¹ | 42.501 (1.736)   | 25.596 (0.608) | 16.917 (1.131) |
|              | Nano-120 mg L⁻¹  | 43.100 (1.977)   | 25.617 (0.624) | 17.495 (1.354) |

The mean value follows the standard error of mean within the parenthesis. The similar letters in a column indicate the statistical significance at p ≤ 0.05.

Table 3: Descriptive statistics of the yield/Reproductive characteristics, percentage grain filling weight of 100 seeds and Harvest Index based on treatment and rice variety

| Rice Variety | Treatment      | Percentage Grain filling | Weight of 100 seeds | Harvest Index |
|--------------|----------------|--------------------------|---------------------|---------------|
| **Bg94-1**   | De-ionized water | 80.21 (1.46)  | 1.89 (0.01)   | 5.99 (0.82) |
|              | Bulk - 60 mg L⁻¹ | 82.71 (1.30)  | 1.96 (0.01)   | 3.67 (0.84) |
|              | Nano - 60 mg L⁻¹ | 86.93 (1.03)  | 2.24 (0.08)   | 13.71 (4.73) |
|              | Nano-120 mg L⁻¹  | 88.54 (0.72)  | 2.10 (0.01)   | 4.78 (0.98) |
| **Pachchaperumal** | De-ionized water | 85.30 (1.71)  | 1.51 (0.01)   | 7.14 (2.18) |
|              | Bulk - 60 mg L⁻¹ | 82.97 (0.93)  | 1.54 (0.01)   | 4.10 (0.17) |
|              | Nano - 60 mg L⁻¹ | 87.51 (0.99)  | 1.65 (0.01)   | 4.93 (0.66) |
|              | Nano-120 mg L⁻¹  | 89.89 (0.70)  | 1.74 (0.01)   | 8.92 (1.96) |
| **Suwandel** | De-ionized water | 82.58 (1.24)  | 0.83 (0.01)   | 2.97 (0.34) |
|              | Bulk - 60 mg L⁻¹ | 83.51 (1.32)  | 0.88 (0.01)   | 4.35 (0.90) |
|              | Nano - 60 mg L⁻¹ | 85.73 (0.93)  | 1.05 (0.02)   | 4.43 (0.44) |
|              | Nano-120 mg L⁻¹  | 88.00 (0.78)  | 1.07 (0.02)   | 4.46 (0.59) |

The mean value follows the standard error of mean within parenthesis. The similar letters in a column indicate the statistical significance at p ≤ 0.05.

as concentrations, bring a varying effect on the chlorophyll a of the rice varieties. The comparison of mean values by a, b, and c within each column implies that the means with the same lower case letter are not significantly different (p ≤ 0.05).

**Yield characteristics**

There was a prominent difference in magnitudes of the yield/ reproductive parameters across the four treatments, de-ionized water, bulk ZnO, Nano-ZnO 60 mg L⁻¹ and 120 mg L⁻¹ (Table 3). The three rice varieties have increased mean values of % grain filling, the weight of 100 seeds and Harvest Index with the nano-ZnO, 60 mg L⁻¹ and 120 mg L⁻¹. The percentage grain filling of the three rice varieties gradually increases with the four treatments. However, the weight of 100 seeds and the Harvest Index of Bg94-1 showed a conspicuous increase with the concentration of nano-ZnO 60 mg L⁻¹ (p ≤ 0.05). The increase in mean values of the weight of 100 seeds and Harvest Index of Pachchaperumal and Suwandel with the treatments were different from the Bg94-1, however, similar between the two.

According to Table 3, the magnitude of the difference is inferior than Bg94-1. The comparison of mean values by a, b and c within each column imply that the means with the same lower case letter are not significantly different (p > 0.05).

**Analyses of variances (ANOVA)**

**Growth and yield parameters**

The plants after first fertilization showed no physiological
changes such as drying of leaves, chlorosis, and patches on laminar or any other visually observable characters. Thus, nano-ZnO-fertilizer concentrations chosen for the study well-tolerated by all the rice varieties included. The summary of the one-way ANOVA performed on the plant height, biomass, percentage dry weight, total chlorophyll, chlorophyll a, and chlorophyll b for *Bg94-1, Pachchaperumal* and *Suwandel* are summarized in Table 4 and according to the data summarized, *Bg94-1* showed no significant difference between total chlorophyll and chlorophyll b across the treatments. Both *Pachchaperumal* and *Suwandel* showed significant differences with chlorophyll a. The biomass of *Pachchaperumal* was significant (p < 0.05). However, the Plant Height, Biomass, percentage dry weight, Chlorophyll a of *Bg94-1* were found to be insignificant. Plant height, percentage dry weight, total chlorophyll, and chlorophyll b of *Pachchaperumal* and plant height, biomass, percentage dry weight, total chlorophyll, and chlorophyll b of *Suwandel* were insignificant (p > 0.05).

Statistically significance: * = significant at p ≤ 0.05; not significant NA = p > 0.05.

The mean of percentage grains per panicle of three rice varieties, *Bg94-1, Pachchaperumal* and *Suwandel* varieties with the nano-ZnO 60 mg L⁻¹ and 120 mg L⁻¹ revealed that the *Bg94-1* and *Pachchaperumal* possess over 40% of grains per panicle with nano-ZnO 60 mg L⁻¹ (Conc. 1) and nearly 20% of increase of grains per panicle of *Suwandel* with nano-ZnO 60 mg L⁻¹ (Figure 3). The percentage increase of grains per panicle of *Bg94-1* was ca. 70% with Nano-ZnO 120 mg L⁻¹ and *Pachchaperumal* showed over 50% at the same concentration of nano-ZnO-fertilizer. *Suwandel* also had ca. 20% increased percentage Grains per Panicle at a concentration of 120 mg L⁻¹ of nano-ZnO-fertilizer.

The standardized mean values of the percentage weight of 100 seeds of *Bg94-1* showed over 10%, with nano-ZnO 60 mg L⁻¹ (Conc. 1) (Figure 5). *Pachchaperumal* showed about 10% increase in the percentage weight of 100 seeds with nano-ZnO 120 mg L⁻¹ (Conc. 2). The mean values of percentage grain filling of *Bg94-1* and *Pachchaperumal* showed increased with nano-ZnO 120 mg L⁻¹ (Conc. 2). *Suwandel* possesses 20% of the increased percentage weight of 100 seeds with nano-ZnO 60 mg L⁻¹ (Conc. 1) and nano-ZnO 120 mg L⁻¹ (Conc. 2).

The size of nano-ZnO-fertilizer and bulk-ZnO-fertilizer is lower than the stomata aperture diameter which facilitates the higher infiltration of fertilizer into the plant cells. It is evident that the size of the bulk-ZnO-particles is greater than that of nano-ZnO-particles. The characteristic

| Rice Variety | Growth Character | Sum of Squares | df | Mean Square | F   | Sig. |
|--------------|------------------|----------------|----|-------------|-----|------|
| *Bg94-1*     | Plant Height/cm  | 519.853        | 3  | 173.284     | 0.307| NA   |
|              | Biomass          | 75.038         | 3  | 25.013      | 2.131| NA   |
|              | Percentage Dry Weight | 47.447 | 3  | 15.816      | 0.851| NA   |
|              | Total Chlorophyll| 409.675        | 3  | 136.558     | 13.232| *    |
|              | Chlorophyll a    | 0.463          | 3  | 0.154       | 0.436| NA   |
|              | Chlorophyll b    | 401.434        | 3  | 133.811     | 16.008| *    |
|              | Height/cm        | 1242.036       | 3  | 414.012     | 0.473| NA   |
|              | Biomass          | 79.666         | 3  | 26.555      | 6.244| *    |
|              | Percentage Dry Weight | 230.269 | 3  | 76.756      | 1.888| NA   |
|              | Total Chlorophyll| 39.417         | 3  | 13.139      | 2.262| NA   |
|              | Chlorophyll a    | 6.218          | 3  | 2.073       | 3.527| *    |
|              | Chlorophyll b    | 15.446         | 3  | 5.149       | 1.295| NA   |
|              | Height/cm        | 963.469        | 3  | 321.156     | 0.423| NA   |
|              | Biomass          | 5.956          | 3  | 1.985       | 0.910| NA   |
|              | Percentage Dry Weight | 195.468 | 3  | 65.156      | 1.346| NA   |
|              | Total Chlorophyll| 65.892         | 3  | 21.964      | 0.829| NA   |
|              | Chlorophyll a    | 29.089         | 3  | 9.696       | 4.027| *    |
|              | Chlorophyll b    | 69.802         | 3  | 23.267      | 1.399| NA   |

The summary of the one-way ANOVA performed on the Yield/Reproductive characteristics, percentage Grain filling, Weight of 100 seeds and Harvest Index for *Bg94-1, Pachchaperumal* and *Suwandel* rice variety are summarized in Table 5. It is apparent that Bg94-1 showed significant differences between % Grain filling, Weight of 100 seeds and Harvest Index across the treatments (p ≤ 0.05). The percentage Grain filling and Weight of 100 seeds of *Pachchaperumal* and *Suwandel* was significant. However, the Harvest Index of both *Pachchaperumal* and *Suwandel* found to be insignificant (p > 0.05).
features of nano-ZnO-particles such as uniform distribution of different shapes, low level of agglomeration and fewer irregular particles increase the chance of diffusion in the plant through stomatal pore than bulk-ZnO-particles. The fusion of particles caused agglomeration and thus results in lesser surface area to volume ratio and reduced reactivity. Agglomeration could also result in blocking of the stomatal pore when the nano-ZnO-fertilizer diffuse through the stomata cavity. Similarly, the shape of the particles affects diffusing into plant. Spherical and rod shapes of ZnO particles in nanoscales enable the diffusion through the stomatal pore. The higher frequency of irregular particle shapes in the bulk-ZnO-fertilizer has a lower probability of passing the stomatal pore.

The plant surface images of FE-SEM revealed that there was no prominent port of entries other than stomatal pores for the sprayed fertilizer. Therefore, it can be stated that the bulk and nano-ZnO- fertilizer particles entered the plant through stomatal pores. The dumbbell-shaped rice stomatal guard cells resulted in elongated stomatal pores. According to the FE-SEM images of upper (adaxial) and lower (abaxial) surfaces of Bg94-1, Pachchaperumal and Sauwandel leaf surfaces (Figure 4), the distribution of stomata is not uniform and arranged in parallel rows along the leaf blade. The width of the stomata of Bg94-1, Pachchaperumal and Sauwandel ranged from 0.6 μm to 1.0 μm. The length of the stomata of Bg94-1, Pachchaperumal and Sauwandel ranged from 5.0 μm to 7.0 μm. The surface structures, hairs, silicon depositions

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**Table 5**: Summary of the one–way ANOVA performed on percentage grain filling, weight of 100 seeds and Harvest Index for Bg94-1, Pachchaperumal and Sauwandel rice varieties for nano-ZnO 60 mg L$^{-1}$ and 120 mg L$^{-1}$

| Rice variety | Yield Character | Sum of Squares | df | Mean Square | F       | Sig. |
|--------------|----------------|----------------|----|-------------|---------|------|
| **Bg94-1**   | Percentage Grain filling | 525.584        | 3  | 175.195     | 10.808  | *    |
|              | Weight of 100 seeds | 0.831          | 3  | 0.277       | 12.030  | *    |
|              | Harvest Index     | 371.890        | 3  | 123.963     | 3.353   | *    |
| **Pachchaperumal** | Percentage Grain filling | 316.306        | 3  | 105.435     | 6.699   | *    |
|              | Weight of 100 seeds | 0.414          | 3  | 0.138       | 97.818  | *    |
|              | Harvest Index     | 85.868         | 3  | 28.623      | 2.102   | NA   |
| **Sauwandel** | Percentage Grain filling | 211.152        | 3  | 70.384      | 4.941   | *    |
|              | Weight of 100 seeds | 0.523          | 3  | 0.174       | 58.686  | *    |
|              | Harvest Index     | 9.322          | 3  | 3.107       | 1.411   | NA   |

Statistically significance: * = significant at p ≤ 0.05; not significant NA = p > 0.05.

**Figure 5**: Variation of mean of percentage grains per panicle of rice varieties at the concentration of 60 mg L$^{-1}$ of nano-ZnO and 120 mg L$^{-1}$.
and other physical barriers present in the leaf surface check the entrance of particles of both bulk and nano-ZnO into the plants. However, due to the miniature size of the nano-ZnO-particles, entry of nano-ZnO-fertilizer could not be blocked by these structures. The presence of these structures is often found in higher frequency on the lower (adaxial) surface and prominently in both leaf surfaces of Pachchaperumal and Suwandel. Therefore, the study on leaf surface characters affirmed that the pore sizes are larger than the nanoparticles.

The results of the number of tillers, leaves, panicles, and grains per plant revealed that these parameters vary with the foliar ZnO fertilization. The increased number of tillers and leaves after the first fertilization resulted in more surface area for the second fertilization which collectively promoted higher counts of panicles and grains and other yield parameters. Foliar fertilization provides direct contact of fertilizers to the plants which results rapid response to the applied fertilizer instead of soil application (Fernandez et al., 2013). Rice plants of three varieties were well-tolerated the nano-ZnO-fertilizer concentrations which were indicated with no signs of toxicity symptoms. The foliar-applied fertilizers should possess the ability to pass the leaf surface barriers for an easy entry into the plant. Stomata were found to have recognized importance for the exchange of agents such as gasses, vapors, herbicides, pesticides, fertilizers applied to plants from the environment (Fernandez et al., 2013).

Results of the study revealed that the responses of three rice varieties Bg94-1, Pachchaperumal and Suwandel varied across the four treatments of de-ionized water bulk ZnO, nano-ZnO 60 mg L\(^{-1}\) and 120 mg L\(^{-1}\). According to the Table 1 and 2, it is evidenced that the nano-ZnO-fertilizer has a considerable effect on yield/reproductive characteristics over the growth characteristics. Figure 3 and 4 depicted that Bg94-1 had over 50% of increase in grains per panicle with nano-ZnO-fertilizer which indicated that the inbred rice, Bg94-1 showed higher responses for nano-ZnO-fertilizer than the traditional rice Pachchaperumal and Suwandel. These results were in accordance with previously reported data which showed the importance of Zn in activating plant enzymes carbohydrate metabolism (Cakmak, 2002) which has led to an increase in seed weight, percentage grain filling and grains per panicle.

Further, Zn is required for chlorophyll synthesis and Zn deficiency in plants affects photosynthesis due to the altered pathway/s of synthesis of photosynthetic pigments (Kosesakal and Unal, 2009). The present study also evidenced that the effect of ZnO on chlorophyll synthesis (Table 2). Moreover, higher responses to Bulk and nano, ZnO-fertilizer on total chlorophyll and chlorophyll b were observed for Bg94-1.

The plant height, biomass, and percentage dry weight of three rice varieties poorly responded to the fertilizer treatments. Though, Zn has an important role in protecting plant cells from damage by reactive species such as oxygen, the responses in plant growth such as height, biomass and percentage dry weight could not simply be explained by the Zn deficiency alone (Upadhyaya, 2015).

![Figure 6](image_url)

**Figure 6:** Variation of the standardized mean of percentage grain filling and percentage weight of 100 seeds of rice varieties across treatments of nano-ZnO 60 mg L\(^{-1}\) and 120 mg L\(^{-1}\).
CONCLUSIONS

Nano-ZnO-fertilizer has a greater impact on yield/reproductive parameters than the growth parameters. Comparatively, the inbred rice variety, Bg94-1 indicated better responses to the nano-ZnO-fertilizer than Pachchaperumal and Suwandel. The laminar surface structures played an important role in the entry of foliar-applied Bulk and nano-ZnO-fertilizer into the rice plants. However, further studies are required to address the possible blocking effects in laminar surface structures. The optimization of varietal-specific time of application and concentration of nano-ZnO-fertilizers are required to have desired results. The nano-ZnO-particles were successfully fabricated by thermal decomposition route, which is a simple, fast, and cost-effective method. The size of nano-particles can be further improved by optimizing the synthesizing protocols.

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DECLARATION OF CONFLICT OF INTEREST

There are no conflicting interests.

REFERENCES

Alloway, B.J. (2008). Zinc in soils and crop nutrition. Brussels, Belgium: International Zinc Association.

AOAC (1990). Official Methods of Analysis, 15th edition.

K. Helrich (ed.), Association of Official Analytical Chemists Inc., Arlington, Virginia, USA. Pp. 40-41.

Brennan, R.F. (2005). Zinc application and its availability to plants (Doctoral dissertation, Murdoch University).

ISSN 2224-3186/ Vol.7, No.14, 2017.

Cakmak, I. (2000). Tansley Review No. 111 Possible roles of zinc in protecting plant cells from damage by reactive oxygen species. The New Phytologist 146(2): 185-205. DOI: 10.1046/j.1469-8137.2000.00630.x.

Chen, H. and Yada, R. (2011). Nanotechnologies in agriculture: new tools for sustainable development. Trends in Food Science and Technology 22: 585–594. https://doi.org/10.1016/j.tifs.2011.09.004.

Disante, K.B., Fuentes, D. and Cortina, J. (2011). Response to drought of Zn-stressed Quercussuber L. seedlings. Environmental and Experimental Botany 70(2-3): 96-103. https://doi.org/10.1016/j.envexpbot.2010.08.008.

Duhan, J.S., Kumar, R., Kumar, N., Kaur, P., Nehra, K. and Duhan, S. (2017). Nanotechnology: The new perspective in precision agriculture. Biotechnology Reports 15: 11-23. https://doi.org/10.1016/j.btre.2017.03.002.

Fageria, N.K., Barbosa, M.P., Filho, A. Moreira, C. and Guimaraes, M. (2009). Foliar Fertilization of Crop Plants. Journal of Plant Nutrition 32(6): 1044-1064, DOI: 10.1080/01904160902872826.

Fernández, V. and Brown, P.H. (2013). From plant surface to plant metabolism: the uncertain fate of foliar-applied nutrients. Frontiers in Plant Science 4: 289. https://doi.org/10.3389/fpls.2013.00289.

Hiscox, J.D. and Israelstam, G.F. (1979). A method for the extraction of chlorophyll from leaf tissue without maceration. Canadian Journal of Botany 57(12): 1332-1334. https://doi.org/10.1139/b79-163.

Kasim, W.A. (2007). Physiological consequences of structural and ultra-structural changes induced by Zn stress in Phaseolus vulgaris L. Growth and photosynthetic apparatus. International Journal of Botany 3(1): 15-22.

Kumar, V. and Yadav, S.K. (2009). Plant-mediated synthesis of silver and gold nanoparticles and their applications. Journal of Chemical Technology & Biotechnology: International Research in Process, Environmental and Clean Technology 84(2): 151-157. https://doi.org/10.1002/jctb.2023.

Lal, R. (2009). Soil degradation as a reason for inadequate human nutrition. Food Security, 1(1): 45-57. https://doi.org/10.1007/s12571-009-0009-z.

Lin, D. and Xing, B. (2007). Phytotoxicity of nanoparticles: inhibition of seed germination and root growth. Environmental Pollution 150(2): 243-250. https://doi.org/10.1016/j.envpol.2007.01.016.

Marschner, H. (1995). Mineral nutrition of higher plants. 2nd. Edn. Academic Pres. ISBN 978-0-12-384905-2.

Moore, M.N. (2006). Do nanoparticles present ecotoxicological risks for the health of the aquatic environment?, Environment International 32(8): 967-976. https://doi.org/10.1016/j.envint.2006.06.014.

Mortvedt, J.J. and Giordano, P.M. (1969). Availability to corn of zinc applied with various macronutrient fertilizers. Soil Science 108(3): 180-187.

Naderi, M., Danesh, Shahraki, A.A. and Naderi, R. (2011). Application of nanotechnology in the optimization of formulation of chemical fertilizers. Iran Journal of Nanotechnology 12: 16-23.

Nair, R., Varghese, S.H., Nair, B.G., Maekawa, T., Yoshida, Y. and Kumar, D. S. (2010). Nano particulate material delivery to plants. Plant Science 179(3): 154-163. https://doi.org/10.1016/j.plantsci.2010.04.012.

Pathan, A.K., Bond, J. and Gaskin, R.E. (2010). Sample preparation for SEM of plant surfaces. Materials Today 12: 32-43. https://doi.org/10.1016/S1369-7012(10)70143-7.

Plant Genetic and Recourses Centre, Rice (Oryza Sativa L.) Characterization Catalogue. (1999).

Prasad, R., Bhattacharyya, A. and Nguyen, Q.D. (2017). Nanotechnology in sustainable agriculture: recent developments, challenges, and perspectives. Frontiers in Microbiology 8: 1014. https://doi.org/10.3389/fmicb.2017.01014.

Rathore, R., Harinkhere, D and Kaurav, N. (2019). Synthesis and characterization of ZnO nanoparticles by thermal decomposition method. AIP Conference Proceedings. 020198-1 – 020198-3. https://doi.org/10.1063/1.5098752.

Rempe, D.M. and Dietrich, W.E. (2014). A bottom-up control on fresh-bedrock topography under landscapes. Proceedings of the National Academy of Sciences 111(18): 6576-6581. https://doi.org/10.1073/
Salama, H.M. (2012). Effects of silver nanoparticles in some crop plants, common bean (*Phaseolus vulgaris* L.) and corn (*Zea mays* L.). *International Research Journal of Biotechnology* 3(10): 190-197.

Saravanan, R., Karthikeyan, N., Govindan, S., Narayanan, V. and Stephen, A. (2012). Photocatalytic degradation of organic dyes using ZnO/CeO$_2$ nanocomposite material under visible light. *In Advanced Materials Research* 584: 381-385. https://doi.org/10.4028/www.scientific.net/AMR.584.381.

Saravanan, R., Shankar, H., Prakash, T., Narayanan, V. and Stephen, A. (2011). ZnO/CdO composite nanorods for photocatalytic degradation of methylene blue under visible light. *Materials Chemistry and Physics* 125(1-2): 277-280. https://doi.org/10.1016/j.matchemphys.2010.09.030.

Scherrer, P. (1918). Bestimmung der Grösse und der inneren Struktur von Kolloidteilchen mittels Röntgenstrahlen, Nachrichten von der Gesellschaft der Wissenschaften, Göttingen, Pp 98–100. https://doi.org/10.1007/978-3-662-33915-2_7.

Sharma, V.K., Yngard, R.A., and Lin, Y. (2009). Silver nanoparticles: green synthesis and their antimicrobial activities. *Advances in Colloid and Interface Science* 145(1-2): 83-96. https://doi.org/10.1016/j.ics.2008.09.002.

Shaver, T.M., Westfall, D.G. and Ronaghi, M. (2007). Zinc fertilizer solubility and its effects on zinc bioavailability over time. *Journal of Plant Nutrition* 30(1): 123-133. https://doi.org/10.1080/01904160601055145.