RESEARCH ARTICLE

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

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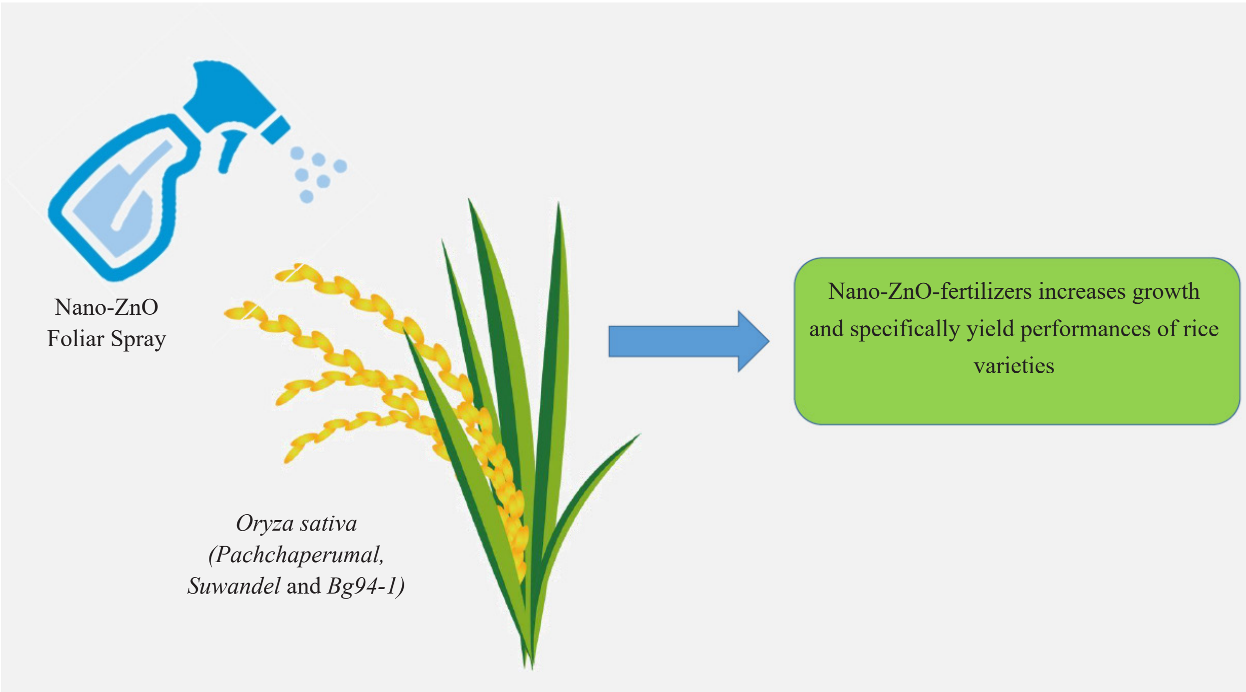

Highlights

- 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.

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RESEARCH ARTICLE

The impact of nano-ZnO foliar fertilizer on growth and yield of cultivated rice (*Oryza sativa* L.) varieties in 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 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 *Bg94-1*. Effect of 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 (Marschner, 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. $ZnCO_3$) 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).

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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 nano-fertilizers has increased specific surface area and particles per unit area providing more opportunity to contact nano-fertilizers to the crop plant which 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 thus reducing collateral damage (Moore, 2006). Therefore, the use of nano-fertilizers 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 [$Zn(H_3C_2O_2)_2.2H_2O$], 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 airy 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 (readymade 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, 2θ 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).

$$D = \frac{K\lambda}{\beta \cos\theta} \qquad \text{.........................(1)}$$

where D = Crystallite size in nm; K = Scherrer Constant (0.9); λ = X-ray wavelength; β = Full Width at Half Maximum (FWHM) in radians; θ = diffraction angle in radians

Characterization of rice leaf tissues

Rice leaves were trimmed and transferred immediately after separation from the mother plant. The leaf tissues of three rice varieties were chemically fixed by absolute methanol (Sigma-Aldrich) 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.

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 at 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 biomasses were determined by destructive sampling of two rice plants per block and measuring initial weights of plants and then kept at 105 ˚C in an oven for a constant weight (AOAC, 2012). The final weights were taken as biomass weights.

The chlorophyll content was determined by the method described by Hiscox and Israelstam (Hiscox *et al.*, 1978). Leaf samples of 1.0 g were taken from the third leaf from the top of the plant from each block of each variety and incubated in test tubes at 65 ˚C until the tissue became colourless (Hiscox *et al.*, 1978). Subsequently, chlorophyll a and b were extracted with dimethyl sulphoxide (DMSO) (Sigma- Aldrich). Then, the absorbance (A) measurements were taken using a UV-Visible spectrophotometer (Camspec M108) at 645 nm and 663 nm. Chlorophyll contents were determined using standard equations 2, 3, and 4 given below.

$$\text{Total Chlorophyll} = 20.2(A_{645}) + 8.02(A_{663}) \quad (2)$$

$$\text{Chlorophyll a} = 12.7(A_{663}) - 2.69(A_{645}) \quad (3)$$

$$\text{Chlorophyll b} = 22.9(A_{645}) - 4.68(A_{663}) \quad (4)$$

At the harvesting stage, the number of panicles per plant and the number of grains per panicle, and 100-seed weight were counted and measured.

Statistical analyses

Descriptive statistics *i.e.*, mean, and standard error of mean were calculated and tabulated. The data were subjected to the three-way analysis of variance to access the interaction between the rice variety, treatment, stage of application of treatment (DAS) (rice variety × treatment × DAS). Subsequently, data were subjected to mean separation making allowance for interaction if any, and the effect of treatments was assessed using ANOVA. Comparison of means was performed using Tuckey's Honest Significant Difference. The differences at $p \leq 0.05$ were considered as a statistically significant effect of the treatment. The non-parametric agro-morphological characters were analyzed using χ- test. Data analysis was carried out using the SPSS version 20.0 statistical package.

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

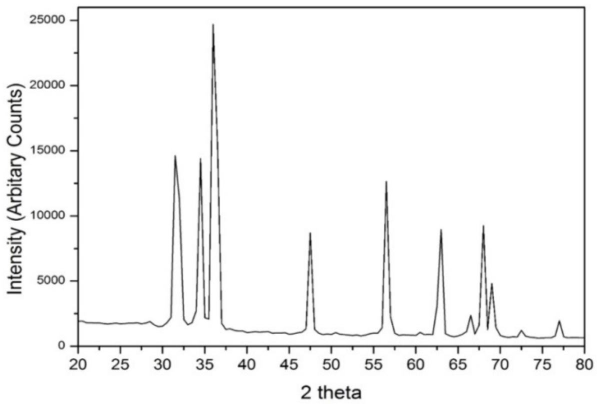

Figure 1: PXRD of Nano-ZnO particles.

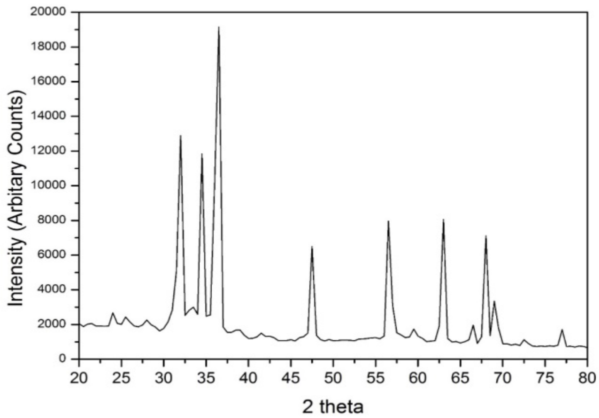

Figure 2: PXRD of Bulk -ZnO particles.

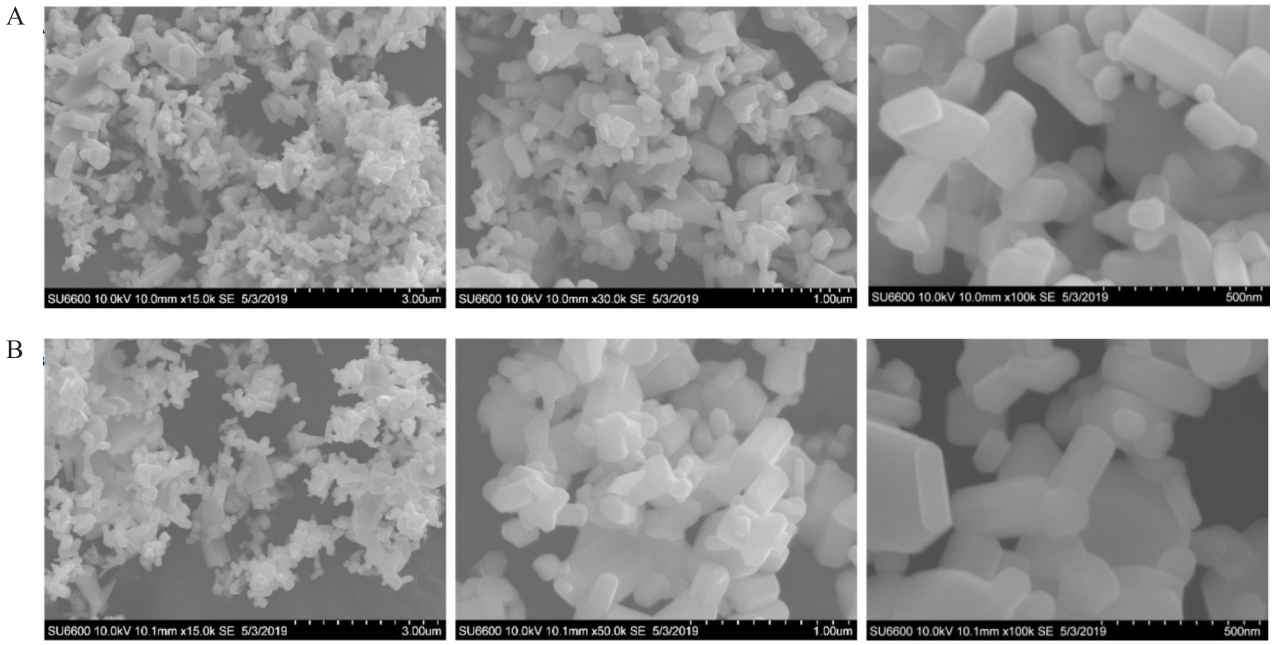

Figure 3: FE-SEM images of A) Nano ZnO particles and B) Bulk ZnO particles.

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 ($p \leq 0.05$).

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

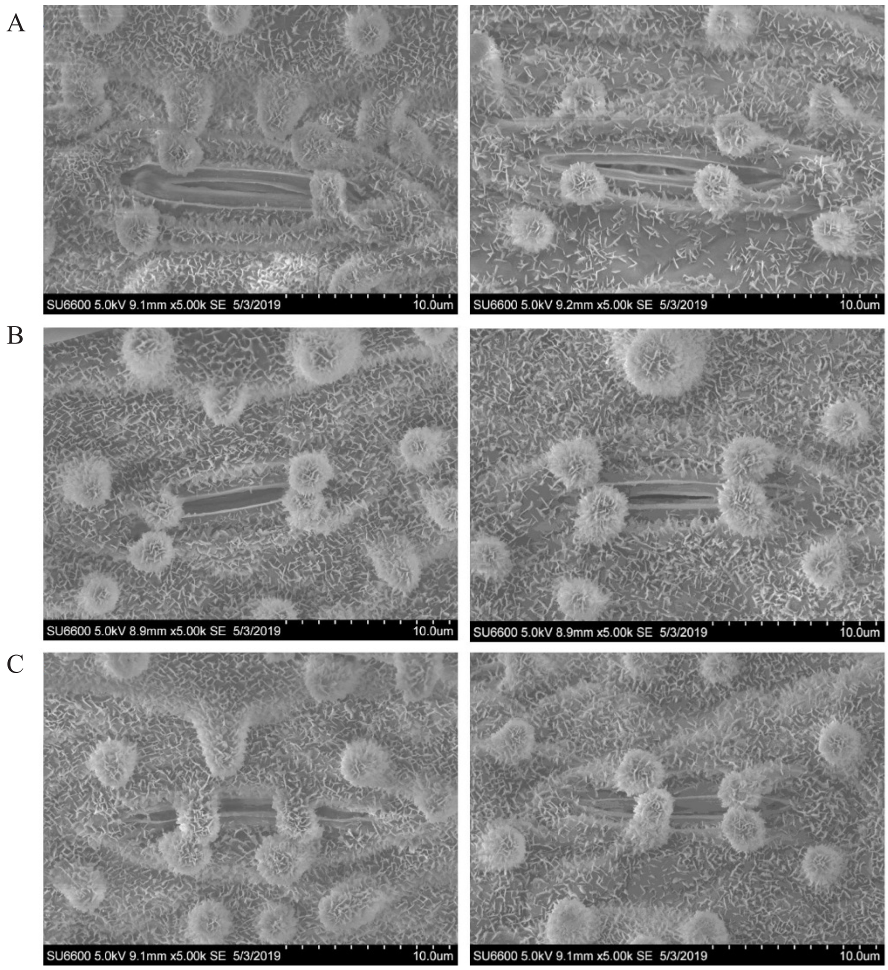

Figure 4: FE-SEM images of Adaxial and Abaxial surfaces and stomata of A) *Bg94-1* B) *Pachchaperumal* and C) *Suwandel.*

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)^{a}	3.33(0.61)^{a}	23.59(0.62)^{a}
	Bulk0 - 60 mg L^{-1}	72.06(4.07)^{a}	3.60(0.58)^{a}	21.94(1.32)^{a}
	Nano - 60 mg L^{-1}	71.04(4.26)^{a}	4.68(0.91)^{a}	23.75(0.94)^{a}
	Nano-120 mg L^{-1}	75.36(4.13)^{a}	5.92(1.03)^{a}	24.02(1.05)^{a}
Pachchaperumal	De-ionized water	99.39(4.76)^{a}	1.49(0.25)^{a}	21.98(1.11)^{a}
	Bulk - 60 mg L^{-1}	108.35(5.62)^{a}	1.89(0.31)^{a}	24.50(1.54)^{a}
	Nano - 60 mg L^{-1}	105.08(5.76)^{a}	2.92(0.47)^{ab}	26.53(1.75)^{a}
	Nano-120 mg L^{-1}	104.86(5.41)^{a}	4.21(0.75)^{b}	26.13(1.54)^{a}
Suwandel	De-ionized water	93.11(5.30)^{a}	1.14(0.26)^{a}	23.03(1.52)^{a}
	Bulk - 60 mg L^{-1}	88.68(4.44)^{a}	1.30(0.30)^{a}	23.99(1.50)^{a}
	Nano - 60 mg L^{-1}	96.10(5.17)^{a}	1.57(0.35)^{a}	25.69(1.71)^{a}
	Nano-120 mg L^{-1}	90.24(5.17)^{a}	1.90(0.46)^{a}	27.34(1.82)^{a}

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)[a]	26.617 (0.144)[a]	9.046 (0.280)[a]
	Bulk0 - 60 mg L^{-1}	41.828 (0.391)[b]	26.859 (0.203)[a]	14.980 (0.581)[b]
	Nano - 60 mg L^{-1}	42.694 (1.059)[b]	26.638 (0.161)[a]	16.067 (0.902)[b]
	Nano-120 mg L^{-1}	42.553 (1.418)[b]	26.647 (0.174)[a]	15.918 (1.248)[b]
Pachchaperumal	De-ionized water	43.531 (0.183)[a]	26.743 (0.067)[ab]	16.801 (0.118)[a]
	Bulk - 60 mg L^{-1}	42.185 (0.762)[a]	26.330 (0.102)[a]	15.867 (0.663)[a]
	Nano - 60 mg L^{-1}	44.659 (0.222)[a]	27.217 (0.268)[b]	17.455 (0.476)[a]
	Nano-120 mg L^{-1}	42.922 (1.128)[a]	26.356 (0.330)[a]	16.578 (0.803)[a]
Suwandel	De-ionized water	40.284 (0.895)[a]	25.938 (0.114)[ab]	14.357 (1.003)[a]
	Bulk - 60 mg L^{-1}	43.168 (1.055)[a]	27.487 (0.175)[a]	15.692 (1.194)[a]
	Nano - 60 mg L^{-1}	42.501 (1.736)[a]	25.596 (0.608)[b]	16.917 (1.131)[a]
	Nano-120 mg L^{-1}	43.100 (1.977)[a]	25.617 (0.624)[b]	17.495 (1.354)[a]

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

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

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

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 ($p \leq 0.05$).

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} ($p \leq 0.05$). 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 ($p \leq 0.05$). 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 $p \leq 0.05$; 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

Table 4: Summary of the one –way ANOVA perfumed on plant height, biomass, percentage dry weight, total chlorophyll, chlorophyll a and chlorophyll b for *Bg94-1, Pachchaperumal* and *Suwandel* rice varieties for nano-ZnO 60 mgL^{-1} and 120 mgL^{-1} .

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	*
Pachchaperumal	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
Suwandel	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 Bg94-1showed significant differences between % Grain filling, Weight of 100 seeds and Harvest Index across the treatments ($p \leq 0.05$). 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 ($p > 0.05$).

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 *Suwandel* 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
Suwandel	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 $p \leq 0.05$; not significant NA = $p > 0.05$.

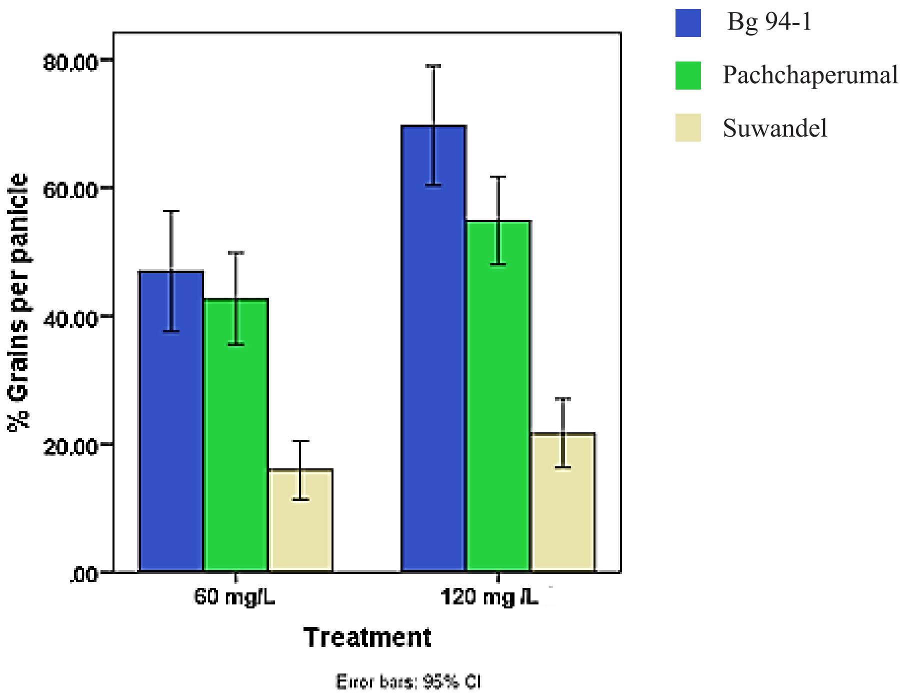

Figure 5: Variation of mean of percentage grains per panicle of rice varieties at the concentration of 60 mg L^{-1} of nano-ZnO and 120 mg L^{-1}.

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 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).

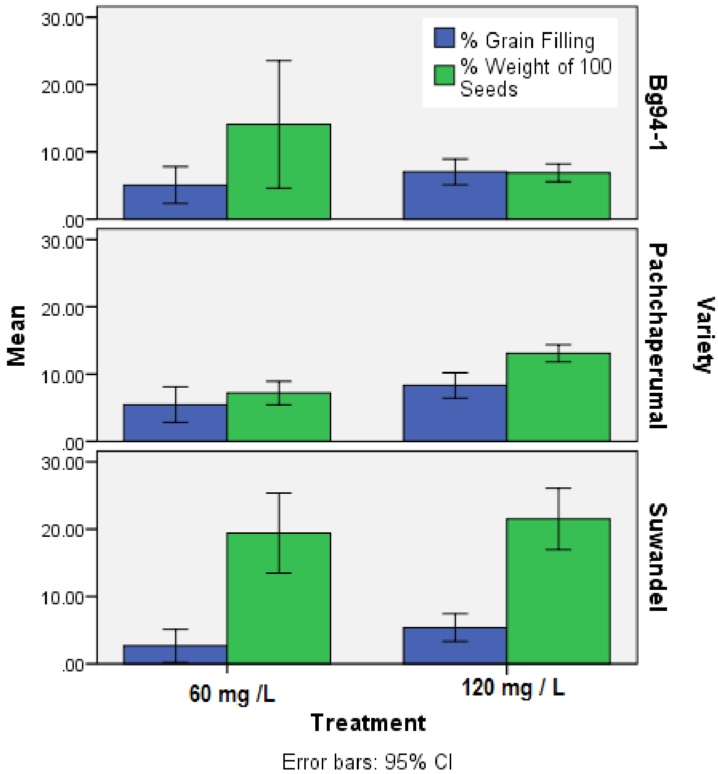

Figure 6: Variation of the standardized mean of percentage grain filling and percentage weight of 100 seeds of rice varieties across treatments of nano-ZnO 60 mg L^{-1} and 120 mg L^{-1}.

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.

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