

## Yield performances of rice varieties (*Oryza sativa*) under nano-CuO and nano-ZnO micronutrient fertilizers

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**Abstract.** Sandanayake CLT, Weerakoon SR, Karthikeyan N, Somaratne S. 2022. Yield performances of rice varieties (*Oryza sativa*) under nano-CuO and nano-ZnO micronutrient fertilizers. *Nusantara Bioscience* 14: 95-103. Rice (*Oryza sativa* L.) is a primary staple food in the world. There is an extreme need for higher rice yields to meet the ever-increasing demand with the rise in population despite the hampering impact of climatic changes. Nanotechnology plays a potential role in food security and introduces nano fertilizers as an alternative to conventional fertilizers. Therefore, the present research was carried out to determine the effects of nano micronutrient fertilizers, nano-CuO, and nano-ZnO on the yield of selected rice varieties, *Bg360*, *BW364*, *Kaluheenati*, and *Kuruluthuda*. Sol-gel and thermal decomposition methods synthesized nano-fertilizers used in the study. Nano-ZnO, nano-CuO, and nano-CuO-ZnO composite treatments were applied as a foliar spray at concentrations of 30 mg L<sup>-1</sup> (T1), 60 mg L<sup>-1</sup> (T2), and 120 mg L<sup>-1</sup> (T3) and double deionized water served as the control (T0). The synthesized nano-fertilizers were applied during the growth stage [at 48-58 days after sowing (DAS)] and grain filling stage [100-105 DAS], while the plants were provided with appropriate levels of N, P, and K fertilizers, as recommended by Department of Agriculture. A Complete Randomized Block Design was employed with three blocks and five replicates in each block. The Yield parameters were recorded at the harvesting stage. Descriptive statistics such as mean and standard error of mean and inferential statistics were performed on the data obtained. ANOVA with interaction terms was performed to assess the significant differences between the treatments. The descriptive analyses show an increase in the yield of the rice varieties under the application of nano-fertilizers. ANOVA suggests a significant effect ( $p \leq 0.05$ ) of nano-CuO and nano-ZnO on the yield of rice varieties used in the study. Both traditional and inbred rice varieties indicated yield response to applied nano-fertilizers.

**Keywords:** Micronutrients, nano-fertilizer, *Oryza sativa*, traditional and inbred rice varieties

### INTRODUCTION

Rice is a semi-aquatic annual plant of the family Poaceae, the second-largest commodity produced worldwide (Hiyasmin et al. 2015). Population growth is one of the main factors leading to increased demand (Nurhasanah et al. 2016). Rice is the single most important crop and the main staple diet in Sri Lanka, according to the Department of Agriculture (2020), accounting for 34 percent (0.77 million hectares) of the total planted area. Sri Lanka's current annual rough rice production is 2.7 million tons, which meets the bulk of the country's needs. Rice demand is expected to expand at a rate of 1.1 percent per year, while rice output will need to grow at 2.9 percent per year to keep up. Increasing the cropping intensity and national average yield can achieve these production targets.

A widespread nutrient deficiency worldwide has been reported in agricultural lands, significantly decreasing crop yield. In Sri Lanka, the yields of many annual crops are much less than the potential and the world average due to an inadequate supply of mineral nutrients (Kumaragamage et al. 2011). Sri Lanka has three agroecological zones, the wet zone (>2,000 mm), the intermediate zone (1,000-2,000 mm), and the dry zone (<1,000 mm) (Bandara and Silva 2000a; 2000b). Paddy production is high in the

intermediate zone and the dry zone. In dry and intermediate zones, soils are deficient in Zn and Cu (Bandara and Silva 2000a; 2000b). Moreover, poorly drained rice-growing soils of a mid-country wet zone of Sri Lanka are also deficient in zinc (Nagarajah et al. 1983).

With the green revolution, the micronutrients of agricultural lands have decreased due to the significant increase in crop yields. Hence, global crop production and food security depend highly on fertilizers input to agricultural lands. Studies have found a substantial increase in crop production due to the application of micronutrients (Zain et al. 2015). It is reported that after the green revolution era in India, nitrogen fertilizer usage in the form of urea has increased by 29% (Duhan et al. 2017). However, such large-scale use of chemical fertilizers is unsustainable in the long run (Solanki et al. 2015; Mapa 2020).

It is predicted that the world population will exceed 9 billion by 2050. Total food consumption will have to rise by 50%-70% if these people are adequately fed (Jaggard et al. 2010). Such an increase in food demand cannot be met without extensive application of fertilizers. Yet, the effectiveness of conventional fertilizers is limited by their low nutrient use efficiency (Guo et al. 2018). Nanotechnology manipulates matter on an atomic and

molecular scale (Qureshi et al. 2018). Nano fertilizers are nanostructured formulations of plant nutrients that can be applied to plants (Raliya et al. 2017). There are three ways to deliver agrochemicals into plants; seed treatments, soil amendment, and foliar sprays (Mahil and Kumar 2019). Nano fertilizers are rising as a competent substitute for conventional fertilizer application methods and can significantly supply micronutrients to plants (Sekhon 2014). Nanoparticles can be given to plants at specific times and concentrations; hence they are considered SMART (Specific, Measurable, Achievable, Relevant, and Time-oriented) delivery systems (Shaoyu Lu 2016).

According to a recent study, foliar application of Nano-ZnO fertilizers increases growth and specifically yield performances of rice varieties *Suwandal*, *Pachchaperumal*, and *Bg94-1* (Somaratne et al. 2021). Though nano fertilizers are prominent in countries such as China, India, Iran, etc., using Nanofertilizers in Sri Lankan paddy cultivation is inconspicuous. Since the yield of traditional rice remains low and there is a market for traditional rice due to its medicinal and nutritional value, this study was mainly focused on the possibility of increasing the rice yield of inbred and traditional rice varieties. This study also studies the possibility of introducing Nano fertilizers as an alternative to conventional micronutrient fertilizers. The yield performances of two traditional and two inbred rice varieties under the treatment of Nano-CuO and Nano-ZnO SMART fertilizers were assessed.

## MATERIALS AND METHODS

### Selection, germination, and transplant of rice varieties

Four rice varieties of the three-and-half-month category of rice varieties were chosen for the study. Inbred varieties *Bg360* (Keeri samba) and *BW364* (Red Nadu) and traditional varieties *Kalu Heenati* and *Kuruluthuda* were chosen based on the duration of cultivation, suitability for lowland paddy cultivation, and popularity among the Sri Lankan farmers, consumers, and rice distributors. Seed materials were collected from Rice Research and Development Institute (RRDI), Batalagoda, and the Regional Rice Research and Development Center (RRRDC), Bombuwela.

The seeds were germinated in plastic trays in a growth chamber at the Botany research laboratory of The Open University of Sri Lanka. After 14 days, the seedlings were transplanted in pots (22 cm x 22 cm) containing paddy soil. The recommended levels of macronutrients by the Department of Agriculture, nitrogen, phosphorous, and potassium, were added to the pot soils before seedling transplant. The pot-level experiment was conducted under greenhouse conditions in the Open University premises, Nawala. These pots consisting of five (5) rice plants were arranged in a Complete Randomized Block Design (CRBD). Five replicates of each treatment were carried out for each rice variety.

### Synthesis of nano-CuO and nano-ZnO

Copper and Zinc nanoparticles were prepared using the metal acetates of the respective elements. Two different routes were followed to get the desirable surface morphologies suitable for this investigation (Saravanan et al. 2012). nano-CuO was prepared via thermal decomposition, and Nano-ZnO was prepared via a Sol-gel method.

Thermogravimetric analysis was carried out to confirm the decomposition temperatures of the precursor molecules by SDTQ600 Thermo Gravimetric Analyzer at the Sri Lanka Institute of Nano Technology under the following conditions; temperature range ambient to 800°C, Ramp 10°C/mins, Purge gas was compressed air.

Precursor chemicals Copper acetate [ $\text{Cu}(\text{CH}_3\text{COO})_2 \cdot \text{H}_2\text{O}$ ] and Zinc acetate [ $\text{Zn}(\text{CH}_3\text{COO})_2 \cdot 2\text{H}_2\text{O}$ ], NaOH, and isopropyl alcohol (2-propanol) with high purity (> 99.5 %) were purchased. Analytical grade chemicals and reagents were used in this study, and deionized double distilled water was used to prepare all aqueous solutions.

### Nano fertilizer characterization

Nanoparticles were characterized with XRD (X-Ray diffraction) and SEM/TEM (Scanning Electron Microscopy/Transmission Electron Microscopy) to confirm their physical properties, viz. structure, morphology, and elemental composition. In addition, prepared Nano-CuO and Nano-ZnO particles were characterized with a Bruker D8 Focus X-ray diffractometer. Samples were compressed onto the sample holder, and the X-ray diffraction patterns were recorded under the following conditions: Cu-K $\alpha$  radiation (1.54Å), a voltage of 40 kV, a current of 40 mA, 2 $\theta$  range from 10° to 20° and a scan speed of 1 sec/step.

The average crystallite size of the nanoparticles was calculated using the Scherrer equation.

$$D = \frac{k \lambda}{\beta \cos \theta} \quad \text{Eq. 1}$$

Where; k: Scherrer's constant,  $\lambda$ : wavelength of X-rays,  $\theta$ : Bragg's diffraction angle,  $\beta$ : full width at half maximum, and D: crystallite size (Scherrer 1918; Mohammadian et al. 2018).

To confirm the synthesized nanoparticles' physical properties, viz. structure, and surface morphology, Scanning Electron Microscopy was performed by Hitachi SU6600 Analytical Variable Pressure FE-SEM (Field Emission Scanning Electron Microscope) at the Sri Lanka Institute of Nanotechnology.

### Nano fertilizer suspension preparation and application

Nanoparticle suspensions of different concentrations were prepared by weighing the prepared nano-CuO and nano-ZnO particles and suspending them in the double-deionized water. Nano-CuO, nano-ZnO, and the Nanocomposite of CuO and ZnO Fertilizers were used in spray applications at a concentration of 0, 30 and 60, and 120 mg L<sup>-1</sup> (F0, F1, F2, and F3) and double deionized water were served as control. The treatments were carried out in two stages as a foliar spray application. During

bearing [at 48-58 Days after sowing (DAS)] and at the stage of grain filling [(100-105 DAS)]. The application was made using pump sprayers for 30 seconds per plant at a constant spraying rate. The first application was made 50 DAS on 26 July 2020 at 07.00 am. (Temperature was 24°C, in the absence of the wind). The second application was at 100 DAS on 14 September 2020. (Temperature was 25°C, in the absence of the wind).

### Measurement of yield parameters

The yield parameters were taken during the harvest. First, the number of panicles per plant was counted and recorded (Plant Genetic Resource Center 1999). Then, after the harvesting, the length of panicles was measured from the tip of the panicle to the base. Next, the number of grains per panicle was counted and recorded for each panicle. Finally, 100- filled grains were weighed using the electrical balance and recorded.

### Statistical analysis

Descriptive statistics, i.e., mean and standard error of the mean, were calculated and tabulated. The data were subjected to univariate analysis of variance (3-way ANOVA) to assess the interaction between the rice varieties, fertilizer treatment, and DAS. The significance level of the results,  $p \leq 0.05$ , was considered statistically significant. Non-parametric characters were subjected to the  $\chi^2$  test. Statistical analysis was carried out by using SPSS ver. 20.0.

## RESULTS AND DISCUSSION

### Thermogravimetric analysis (TGA)

The TGA thermogram of copper acetate monohydrate at the temperature range of 50-800°C is shown in Figure 1. The initial weight loss of 9.190% at 150°C is due to dehydration, yielding anhydrous copper acetate  $[\text{Cu}(\text{CH}_3\text{COO})_2]$ . A weight loss of 52.75% resulted in the range of 250-300°C, indicating acetate decomposition and the formation of copper oxide. There was no further decomposition beyond 350°C. A nano-CuO yield of 5g was obtained from 14.06g of Copper acetate monohydrate.

The TGA thermogram of zinc acetate dihydrate at the temperature range of 50-800°C is shown in Figure 2. According to the figure, there is an initial weight loss of 16.52% at 100°C, which is due to dehydration, yielding anhydrous zinc acetate  $[\text{Zn}(\text{CH}_3\text{COO})_2]$ . A weight loss of 63.47% resulted in the range of 250-350°C, indicating acetate decomposition and the formation of zinc oxide, and there was no further decomposition beyond 350°C. A nano-ZnO yield of 5g was obtained from 22.7g of zinc acetate dihydrate.

### X-ray diffraction

The XRD of the prepared CuO nanoparticles and ZnO nanoparticles are shown in Figures 3 and 4, respectively. First, the highest XRD peak was used to calculate the crystallite size, and the peak width and height were extracted with ORIGIN ver.9.5 software. Then the

crystallite size was calculated using Scherrer's equation (Equation 1). The calculated crystallite size of the Nano-CuO particles was 26 nm, and that of the Nano-ZnO particles was 31 nm.

### Scanning Electron Microscopic (SEM) analysis

FE-SEM (Field Emission SEM) was carried out to observe the surface morphologies of the synthesized nanoparticles. FE-SEM images of synthesized Nano-CuO and Nano-ZnO particles are shown in Figure 5 and Figure 6, respectively. Nano-CuO particles also showed agglomeration and varied particle shape from rod to spherical. The scale is one  $\mu\text{m}$  divided into 10; therefore, the particle size of CuO nanoparticles is confirmed to be included in the nano range. ZnO nanoparticles also showed agglomeration, which results in a greater surface-area-to-volume ratio. In addition, the shape of the nanoparticles ranges from spherical to hexagonal. The scale is 500 nm divided into 10; therefore, the size of the ZnO nanoparticles is in the nano range.

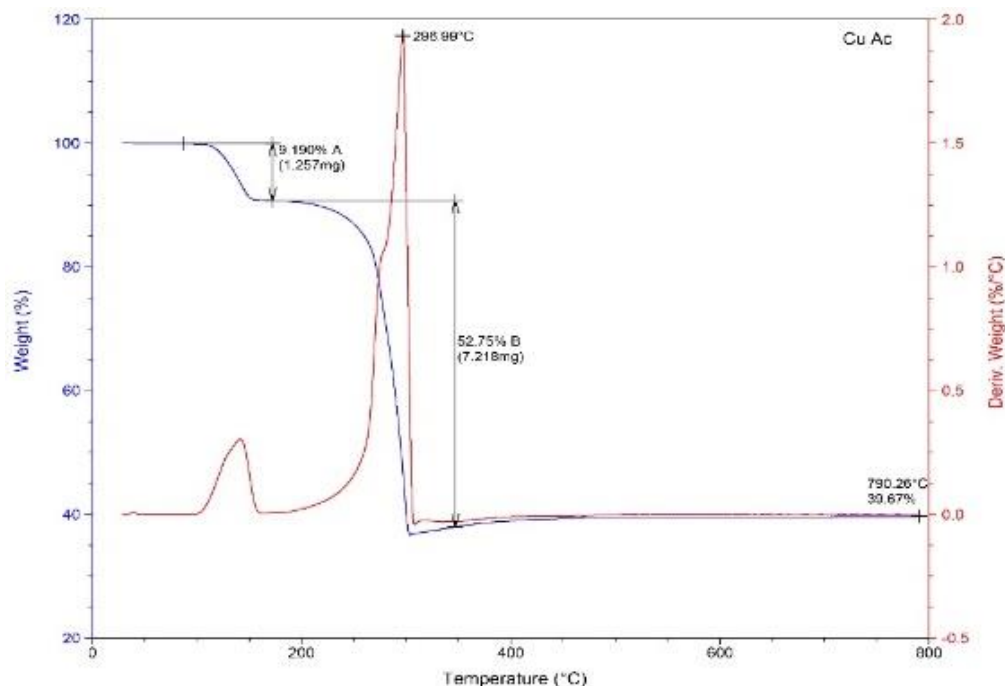
### Descriptive statistical analysis of yield parameters

Standardized Mean values of the number of panicles per plant in four rice varieties were plotted against the nano fertilizer treatment and graphed (Figure 7). As per the descriptive analysis, the applied nano fertilizers affected the yield attributes. The mean number of panicles per plant in *Bg360*, *Bw364*, *Kaluheenati* and *Kuruluthuda* was highest in CuO 60  $\text{mgL}^{-1}$ , ZnO+CuO 60  $\text{mgL}^{-1}$ , and ZnO60  $\text{mgL}^{-1}$ . Comparatively, the number of panicles was higher in inbred varieties than in traditional varieties.

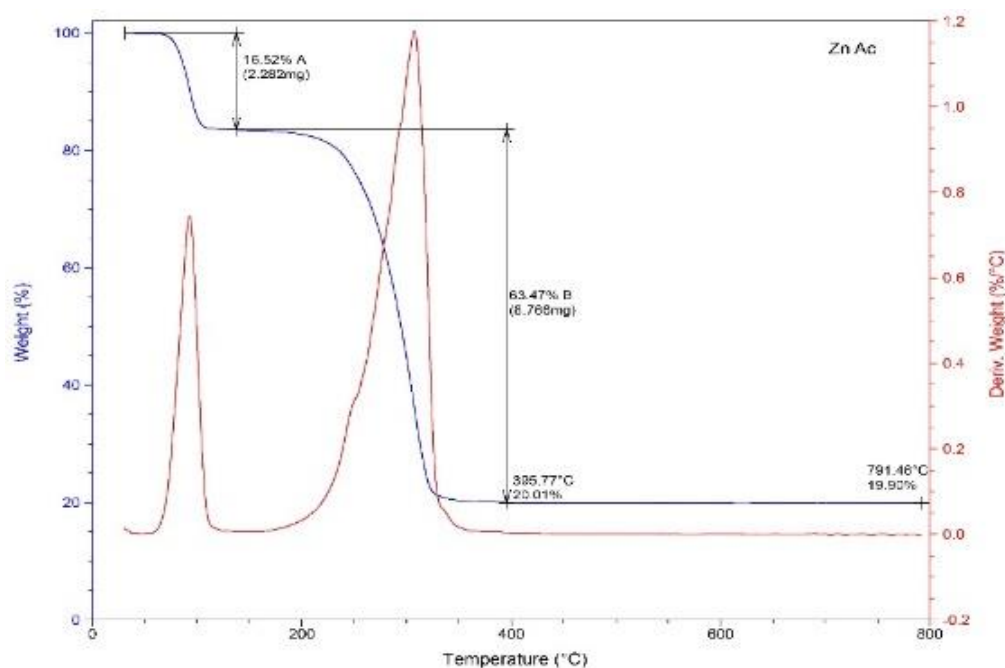
Standardized Mean values of the panicle length of four different rice varieties against the nano fertilizer treatment were graphed (Figure 8). The selected rice varieties showed the highest mean panicle length when treated with 60 $\text{mgL}^{-1}$  of Nano-CuO, Nano-ZnO, and the NanoCuO+Nano-ZnO. In addition, the mean panicle length was comparatively higher in the two traditional varieties than in the inbred varieties (Figure 8).

Standardized Mean values of the number of grains per panicle of four different rice varieties against the nano fertilizer treatment were graphed (Figure 9). According to the graph, the selected rice varieties showed the highest mean number of grains per panicle when treated with 60 $\text{mgL}^{-1}$  of Nano-CuO, Nano-ZnO, and NanoCuO+Nano-ZnO. The traditional variety, *Kuruluthuda*, showed the highest number of grains per panicle out of the four studied varieties.

Standardized Mean values of the weight of 100 grains of selected rice varieties were plotted against the nano fertilizer treatment (Figure 10). Results on the highest weight of 100 grains were reported in the rice plants of the selected varieties, treated with 60 $\text{mgL}^{-1}$  of Nano-CuO, Nano-ZnO, and the NanoCuO+Nano-ZnO. The mean weight of 100 grains in the two traditional varieties was more significant than in the studied inbred varieties. Between *Bg360* and *Bw364*, the latter showed a higher mean weight of 100 grains, as *Bg360* has small grains compared to the other varieties.



**Figure 1.** Two-step decomposition of Copper (II) Acetate monohydrate to form Nano-CuO



**Figure 2.** Two-step decomposition of Zinc (II) Acetate dihydrate to form ZnO

### Inferential statistical analysis of Yield parameters

The Inferential Statistical Analysis shows the number of panicles per plant (Table 1) of the four different rice varieties subjected to univariate variance analysis. The results suggest a significant ( $p \leq 0.05$ ) effect of the element, concentration, and variety, on the number of panicles per plant. The interactions between the subjects were insignificant in the number of panicles per plant. The panicle length of the four different rice varieties was subjected to univariate analysis of variance (Table 1). The results suggest a significant ( $p \leq 0.05$ ) effect on the panicle

length of the element, concentration, variety, and interactions between the subjects. The number of grains per panicle of the four rice types was significantly affected by the variety, element, concentration, and interactions between those factors ( $p \leq 0.05$ ) (Table 1). The weight of 100 grains of the four distinct rice cultivars was also subjected to ANOVA (Table 1). The result suggests that the weight of 100 grains is significantly ( $p \leq 0.05$ ) affected by the element, concentration, and variety. Also, there were substantial interactions between subjects.

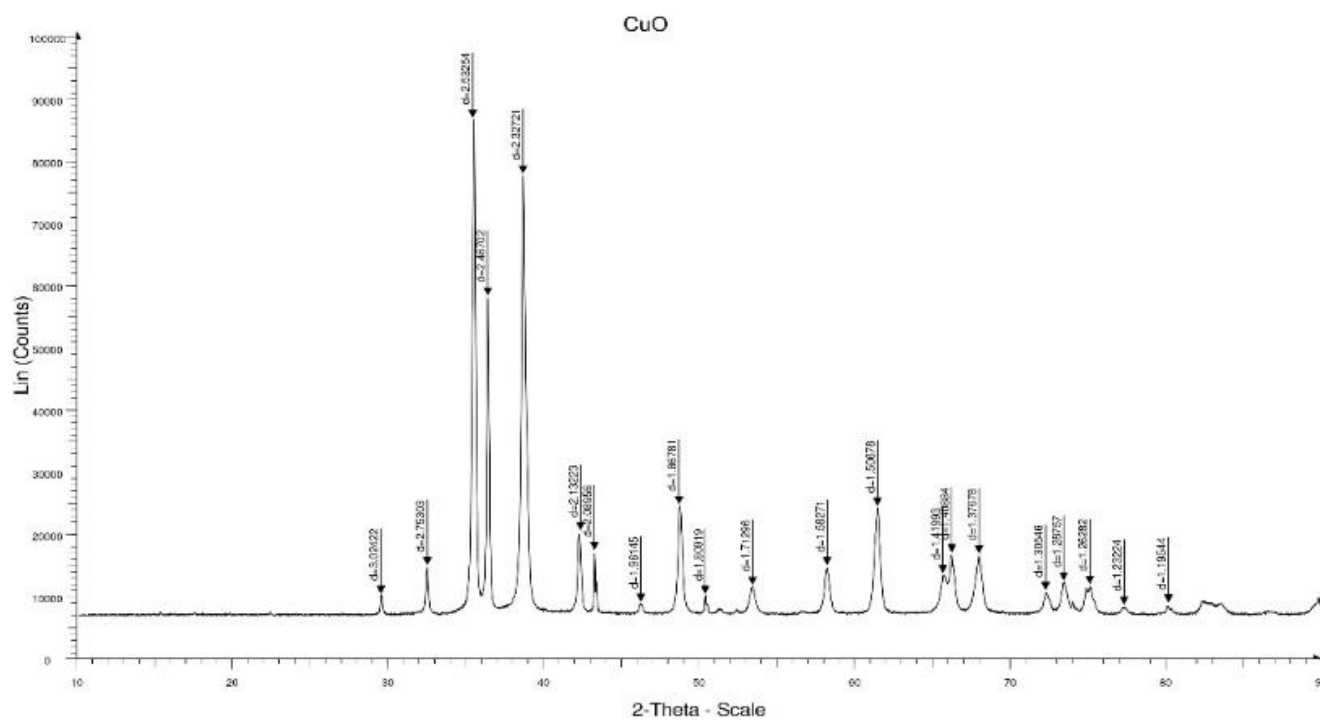


Figure 3. X-ray diffraction of CuO nanoparticles

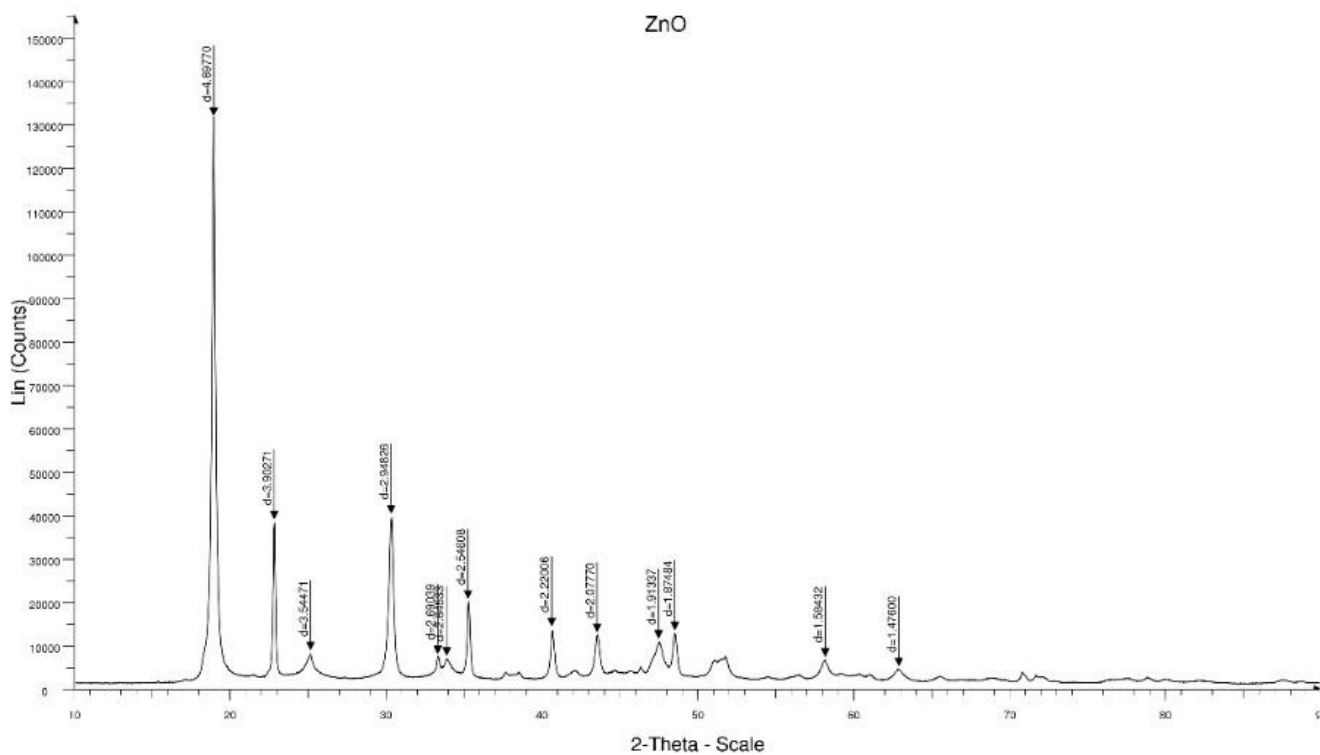


Figure 4. X-ray diffraction of ZnO nanoparticles

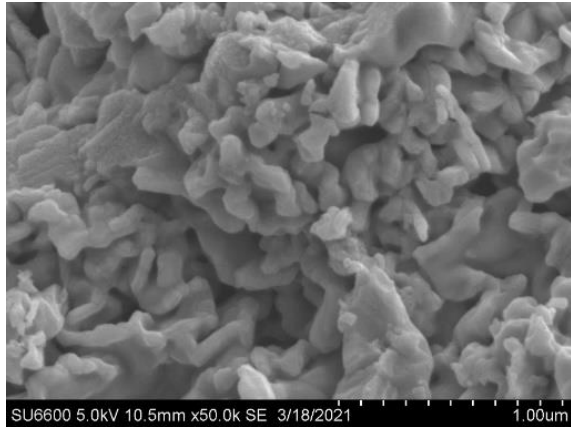


Figure 5. SEM of Synthesized CuO nanoparticles

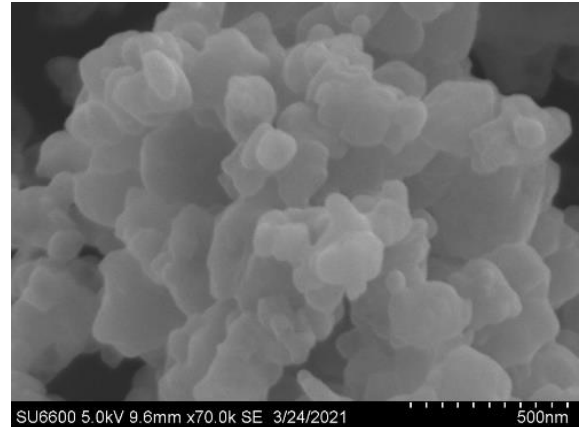


Figure 6. SEM of Synthesized ZnO nanoparticles

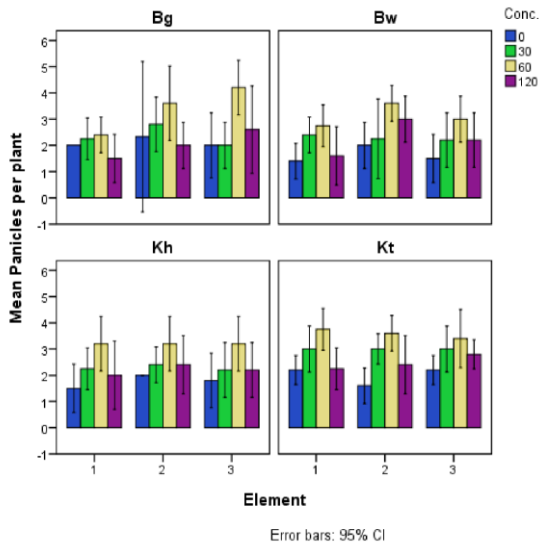


Figure 7. Mean number of panicles per plant with Different Nano fertilizer treatments in 4 different Rice varieties (1-Nano-CuO, 2- Nano-ZnO, 3-NanoCuO+Nano-ZnO)

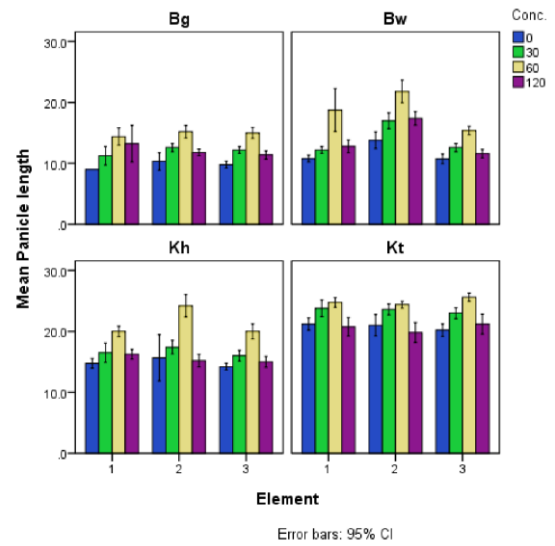


Figure 8. Mean Panicle length with Different Nano fertilizer treatments in 4 different Rice varieties (1-Nano-CuO, 2- Nano-ZnO, 3-NanoCuO+Nano-ZnO)

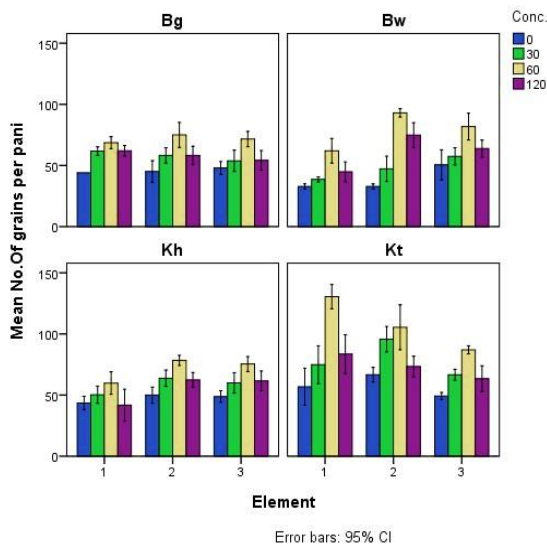


Figure 9. Mean Grains per panicle Different Nano fertilizer treatments in 4 different Rice varieties (1-Nano-CuO, 2- Nano-ZnO, 3-NanoCuO+Nano-ZnO)

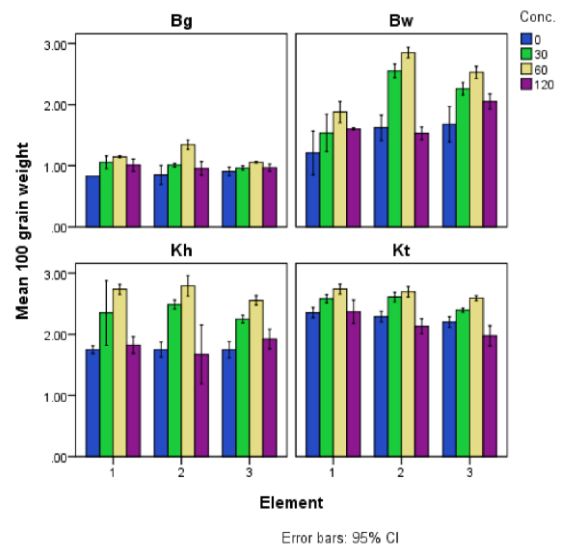


Figure 10. Mean of the weight of 100 grains with Different Nano fertilizer treatments in 4 different Rice varieties (1-Nano-CuO, 2- Nano-ZnO, 3-NanoCuO+Nano-ZnO)

**Table 1.** Summary of ANOVA of the yield parameters

Character	Source	df	Mean Square	Sig.
Panicles per plant	Corrected Model	46	2.011	.000
	Intercept	1	1241.169	.000
	Variety	3	2.019	.017
	Element	2	2.033	.032
	Concentration	3	17.066	.000
	Variety * Element	6	.899	.162
	Variety * Concentration	9	.373	.757
	Element * Concentration	6	.508	.511
	Variety * Element * Concentration	17	.517	.579
	Error	175	.577	
	Total	222		
Corrected Total	221			
Panicle length	Corrected Model	46	98.102	.000
	Intercept	1	55760.442	.000
	Variety	3	989.964	.000
	Element	2	65.592	.000
	Concentration	3	295.929	.000
	Variety * Element	6	23.871	.000
	Variety * Concentration	9	9.859	.000
	Element * Concentration	6	3.999	.000
	Variety * Element * Concentration	17	3.885	.000
	Error	175	.909	
	Total	222		
Corrected Total	221			
Number of Grains per panicle	Corrected Model	46	1538.026	.000
	Intercept	1	810479.680	.000
	Variety	3	6896.193	.000
	Element	2	1458.432	.000
	Concentration	3	10347.634	.000
	Variety * Element	6	1531.335	.000
	Variety * Concentration	9	655.769	.000
	Element * Concentration	6	161.972	.003
	Variety * Element * Concentration	17	288.872	.000
	Error	175	46.813	
	Total	222		
Corrected Total	221			
Weight of 100 grains	Corrected Model	46	1.843	.000
	Intercept	1	716.189	.000
	Variety	3	17.338	.000
	Element	2	.361	.000
	Concentration	3	4.630	.000
	Variety * Element	6	.641	.000
	Variety * Concentration	9	.344	.000
	Element * Concentration	6	.271	.000
	Variety * Element * Concentration	17	.073	.000
	Error	175	.015	
	Total	222		
Corrected Total	221			

## Discussion

Zn, Mn, B, Fe, Cu, and Mo are the most important micronutrients for higher plants and occupy a significant portion as they are essential for increasing plant growth. Their importance increases due to their role in plant nutrition and increasing soil productivity. Certain mineral nutrients are gaseous and enter plants through leaf stomata (Solanki et al. 2015). Moreover, copper holds extreme

importance in plant life. Copper is involved in cellular activities such as photosynthesis and mitochondrial respiration, carbon and nitrogen metabolism, oxidative stress protection, and cell wall synthesis. Copper act as a catalytic metal in many oxidase enzymes, such as cytochrome-c-oxidase. Copper also assists iron utilization in chlorophyll synthesis (Hänsch and Mendel 2009). Copper deficiency causes reduced pollen viability,

resulting in the formation of sterile spikelets and the development of unfilled grains (Dobermann and Fairhurst, 2000). Zinc is a crucial element in plants and is involved in Chlorophyll production, maintaining membrane activities, cytochrome, nucleotide synthesis, seed development, and stalk maturation. Under Zn deficiency, the time to reach crop maturity increases, and tillering, leaf blade area, and chlorophyll content decrease. Tillering can be halted entirely if the deficiency is severe (Dobermann and Fairhurst 2000).

Nano fertilizers can supply micronutrients to plants (Mahil and Kumar 2019). Although soil application is the most common method to supply essential nutrients to plants, higher plants can absorb mineral nutrients when applied as foliar sprays at inappropriate concentrations (Fageria et al. 2009). It has been shown that the foliar application of nano-scale nutrients increases nutrient uptake, and the aerosol spray facilitates the even distribution of the nanoparticles (Mahil and Kumar 2019). Rico et al. (2011) report that the foliar sprayed nanoparticles get transported via apoplastic, simplistic pathways, or plasmodesmata from one cell to another. Once entering the cytoplasm of a cell, these nanoparticles reach different organelles and participate in cellular metabolic processes (Solanki et al. 2015). The nanoparticle synthesis processes employed in this study, the Sol-gel method, and thermal decomposition are very effective in preparing fine nanoparticles.

The selected traditional rice varieties, *Kuruluthuda* and *Kaluheenati*, are popular among Sri Lankan farmers and consumers due to their taste and distinct medicinal properties. On the other hand, two selected inbred varieties *Bg360* and *Bw364*, are vastly cultivated in Sri Lanka due to the high yield and consumer demand. The findings of this study reveal that the foliar spray application of the Cu and Zn micronutrients in the form of nano fertilizer suspension can increase the yield of traditional and inbred rice varieties. That could be a viable option for reducing the excessive use of bulk fertilizers, which cause various environmental issues. According to the crop forecast of the Department of Agriculture in Sri Lanka, at the end of August 2020, rice cultivated was 465,191 ha, and the expected paddy production was 1.84 million metric tons. This paddy production can be increased with the application of nano fertilizers. The current study's findings support those of a previous study (Widanapathirana et al. 2018), which found that foliar application of Nano-ZnO-fertilizers improves rice's growth and yield performances varieties; *Suwandal*, *Pachchaperumal*, and *Bg94-1*. According to a study by Upadhyaya et al. (2015), the application of Zinc nanoparticles on rice has shown a significant physiological effect. Another study on the foliar application of Super-micro plus, an Iranian commercial nano fertilizer that contains 8% of zinc and 0.65% copper, has shown a significant effect on rice growth and yield (Jassim et al. 2019).

In conclusion, the selected rice varieties, *Bg360*, *BW364*, *Kalu Heenati*, and *Kuruluthuda*, exhibited a significant increase in the number of panicles per plant, panicle length, number and grains per panicle, and the 100-

grain weight in response to the application of nano-fertilizers of CuO and ZnO. In addition, the yield increases were noticeable in both traditional and inbred rice cultivars. In preparing nano-CuO and nano-ZnO, thermal decomposition and sol-gel techniques proved fruitful. Further, studies involving the application of nanotechnology in Sri Lankan agriculture are still in their early stages, and in-depth research is required to optimize the concentration and the time of application in a varietal-specific manner.

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