Potentials of nano-ZnO as a Zinc Source for Correcting Zinc Deficiency and Zinc Biofortification in Rice

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Abstract

Zinc deficiency in the human population is highly correlated to zinc deficiency in soils and in staples including rice. One way to correct zinc deficiency and increase grain zinc is to apply zinc fertilizers. This study aimed to establish the potentials of nano-ZnO and bulk ZnO, compared to $ZnSO_4$, as zinc fertilizer applied through the soil, foliar, and combined soil-foliar application. An optimization experiment for nano-ZnO was conducted and established 1% as the best concentration of nano-ZnO foliar spray. Comparison among treatments revealed that nano-ZnO resulted in a higher grain yield when applied as foliar spray than bulk ZnO and $ZnSO_4$. In terms of grain and total Zn uptake, nano-ZnO fared as well as $ZnSO_4$, and better than bulk ZnO when the application was done by foliar or combined soil+foliar application, but not when soil-applied due to reactions in the soil. The benefits of nano-ZnO were also shown to be supported by an indirect effect of P uptake which showed a significant positive correlation with grain yield. The most effective method to correct zinc deficiency and increase grain zinc in rice using nano-ZnO is foliar application alone or in combination with soil application.

Keywords: biofortification, nano fertilizer, zinc deficiency, zinc oxide

Introduction

Zinc deficiency in humans is seen as one of the problems under the term 'hidden hunger' which has been battled by international humanitarian organizations for years (Grebmer et al., 2014). In 2011, an estimated 1.1 billion people are at risk of zinc deficiency (Kumssa et al., 2015). Biofortification of staple foods, including rice, has been one of the solutions to address zinc deficiency in the human population (Soumitra et al., 2013).

As zinc deficiency in soils and rice is correlated with human zinc deficiency, research efforts are made to correct zinc deficiency and increase zinc content in staple foods, including rice. Rice grown in zinc-deficient soils has expectedly low grain zinc, which is a disadvantage in addition to reduced yield. Marginal deficiency, with signs that are not yet noticeable, can cause as much as 20% yield loss (Alloway, 2008), 2008), while a severe deficiency can result in complete crop failure(Dobermann and Fairhurst, 2000). Such yield loss is attributed to the disrupted functioning of the plant's system due to the lack of zinc and its indirect effects. It is a functional component or a co-factor of several enzymes, zinc plays a key role in important physiological processes of the plant including gene expression (Prasad, 2012), proportioning of photosynthetic pigments (Kösesakal and Muammer, 2009); (Chen et al., 2008), carbohydrate metabolism, pollen formation and resistance to certain pathogens (Alloway, 2008). Zinc deficiency in rice is characterized by uneven, stunted plant growth and reduced tillering, chlorotic midribs with brown streaks in older leaves. In severe deficiency, tillering may stop, and crop duration may extend; and in most severe cases may result in crop failure (Dobermann and Fairhurst, 2000).

The application of zinc fertilizers is the most obvious way to correct zinc deficiency and biofortification of rice with zinc. However, reactions with the soil and the dynamics of lowland rice soils limit the effectivity of soil-applied fertilizers. For example, soil pH becomes neutral upon flooding, affecting the availability of Zn by the formation of insoluble Zn compounds (Hafeez et al., 2013). Also, the presence of carbonates can cause the precipitation of zinc as zinc carbonate or zinc hydroxycarbonate (Alloway, 2008) or adsorption to $CaCO_3$ (Dobermann and Fairhurst, 2000), making it unavailable to plants. Submergence, as is the practice for rice cultivation, brings about certain reactions that further reduce the availability and uptake of zinc by plants. Additionally, puddling with continuous submergence causes a decline in the soil available zinc (Bhaduri and Purakayastha, 2011). Therefore, zinc deficiency is often observed in young and newly transplanted rice (Dobermann and Fairhurst, 2000).

This is another concern of zinc management in rice and other cereal crops as well as the amount of zinc that accumulates in the grains. Efforts to increase zinc content in rice are either driven by the genetic makeup of rice, referred to as genetic biofortification, or by the management of zinc fertilization in rice production, referred to as agronomic biofortification (Cakmak, 2008). This approach offers a short-term and rapid solution to the problem. Many investigations have been conducted with the goal to improve the grain zinc content in rice. (Impa and Johnson-Beebout, 2012) emphasized the knowledge gaps that have impeded progress in rice biofortification as follows: (1) predicting Zn deficiency in rice soils; (2) the relationship between Zn-deficiency tolerance mechanisms and grain zinc accumulation; and (3) root zinc uptake mechanisms in contrasting soil environments. The authors arrive at this knowledge gap due to inconsistent results reported by researchers on the soil-related approach of zinc fertilization to rice. To overcome problems as-

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sociated with zinc dynamics in flooded soils (cultivation of rice in flooded soils), many researchers have investigated the potential of foliar delivery of zinc to plants. (Phattarakul et al., 2012) reported higher grain yield and increased zinc content in brown rice when zinc fertilization was done by foliar application irrespective of cultivars and environmental conditions. Other scientific findings also report that foliar application of zinc increases zinc content in brown rice (Ram et al., 2016) and polished rice (Wei et al., 2012) than soil application. (Mabesa et al., 2013) also tested the foliar application of zinc in different cultivars and reported that its effectiveness as an agronomic biofortification strategy was enhanced when applied to genotypes of strong Zn-remobilization capacity.

The most used and commercially available zinc sources are zinc sulfate and zinc oxide. (McBeath and McLaughlin, 2014) compared zinc sulfate and zinc oxide as zinc sources and concluded that zinc oxide had very low water solubility and slow dissolution rates compared to zinc sulfate, but this did not mean a lower ability to provide zinc to plants. An alternative to ZnO, coming with the advent of nanotechnology in fertilizer development, is ZnO nanoparticles (herein referred to as nano-ZnO). Nanofertilizers were engineered and designed such that problems in fertilizer use would be addressed (Solanki et al., 2015) . Having nano-sized dimensions, nano-ZnO has a higher specific area and reactivity compared to bulk zinc oxide (Milani et al., 2010). Due to its very small particle size of nano-ZnO, it is theoretically more bioavailable to plants (Milani et al., 2010). In corn, nano-ZnO improved growth, yield, and accumulation of zinc in corn kernels (Subbaiah et al., 2016). Nano-ZnO has also been observed to increase P uptake in mungbean, in addition to improving the crop's performance (Raliya et al., 2016). (Alharby et al., 2017) reported the potential of nano-ZnO as an anti-stress agent in crop production as it was demonstrated to relieve the stress of tomatoes in high salt culture. All these demonstrate the better performance of nano-ZnO than bulk ZnO, indicating its potential in improving rice performance and yield.

Application of zinc to rice can be done in several ways: seedbed application of $ZnSO_4$, seedling dipping into ZnO before transplanting (IRRI Rice Knowledge Bank), soil application, or foliar application. Recommendations to correct zinc deficiency in transplanted rice includes dipping seedling roots in 2-4% ZnO suspension; whereas, for direct-seeded rice, coating pregerminated seeds with ZnO before seeding (IRRI Rice Knowledge Bank). Soil applications of 25 kg $ZnSO_4$ ha^{-1} can have a correcting effect for up to five years (Alloway, 2008). (Ghoneim, 2016) recommended the soil application of 15 kg $ZnSO_4$ for increased grain yield, and increased grain and straw Zn, N, and K content. Likewise,(Umar Khan et al., 2003) recommended the soil application of 10 kg Zn/ha for higher grain yield. The application of organic manures along with zinc sulfate has the same benefit as applying twice the amount of zinc sulfate in rice. The benefit derived from the soil application of 25 kg $ZnSO_4$ ha^{-1} was more pronounced in the second year of cropping after the application was done. The benefit can be further increased with the cyclic incorporation of straw into the soil (Dwivedi and Srivastva, 2014). Foliar applications have also been investigated for more immediate benefits and lower fertilizer inputs. Foliar application of zinc after flowering increased grain zinc concentration, especially when repeated. A larger increase occurred in the husks. The high zinc concentration also had positive effects on seed germination (Boonchuay et al., 2013). Zinc solutions sprayed before seedling emergence at the rate of 1.1 – 2.2 kg Zn ha^{-1} had yields comparable to soil application of 11.2 kg Zn ha^{-1} (Slaton et al., 2005).

Since the use of nano-ZnO as zinc fertilizer in rice is relatively new, this study aimed to establish its potential in increasing grain yield and improving zinc content in grains.

Methodology

The experiment was conducted in pots at the screen house of the Agricultural Systems Institute of the University of Philippines – Los Baños in Laguna, Philippines from February until November 2018, with two successive pot experiments. Rice seeds of the variety NSIC Rc82, which was moderately tolerant to zinc deficiency, were used for this study. The soil used in this experiment, collected from a pre-determined rice field located in Sariaya, Quezon, contained 0.6 mg Zn kg $soil^{-1}$ (Table 1). The soil is loam, moderately acidic with a pH of 5.12 and with a medium level of CEC of 25.83 $cmol_c$ kg $soil^{-1}$. Problems with iron toxicity, which was evidenced by a high iron concentration (Table 1) has prompted the implementation of direct seeding and alternate wetting and drying irrigation scheme.

The first pot experiment optimized the concentration of nano-ZnO suspension for use as a foliar spray which tested the following concentrations: (0, 0.25%, 0.5%, 1.0%, and 1.5%). The second experiment consisted of three zinc fertilizer sources: bulk ZnO, nano-ZnO, and ZnSO4, applied through foliar, soil, and soil+foliar application. Foliar application for both pot experiments was done three times: once during active tillering (25 DAS), during the panicle initiation stage, and at 1 week after flowering (WAF). Soil application of zinc was done simultaneously with the first application of NPK. All treatments received 0.04 grams of Zn per plant. The effects of the treatments on grain and straw yield were evaluated. Zinc content in both grain and straw was analyzed by the dry ashing method followed by Atomic Absorption Spectrophotometer (AAS). Other chemical analyses performed were N, P, and K.

Both experiments were laid out following Randomized Complete Block Design (RCBD). All data were analyzed by Analysis of Variance (ANOVA) using Statistical Tool for Agricultural Research (STAR) version 2. A simple linear correlation analysis was done between grain yield and the uptakes of N, P, K, and Zn.

Results and Discussion

Optimized concentration of nano-ZnO foliar spray

An increasing trend was observed in grain yield as the concentration of foliar spray increased, with a peak at 1% at a value of 21.63 g pot-1 (Fig. 1a) and a decrease at 1.5% to 17.21 g pot-1 which was comparable to the yield of control

Table 1. Initial characteristics of the soil used in the experiment

Parameter	Method	Analysis
Soil texture	Hydrometer	Loam
pH	1:1 (soil:water)	5.12
CEC, cmolc kgsoil^{-1}	NH4OAc extraction	25.83
Total N, %	Kjeldahl	0.16
Available P, mg kg^{-1}	Bray 2	11.37
Exchangeable K, cmol$_c$kg soil^{-1}	NH_4OAc extraction, Flame photometer	0.80
Calcium, cmol$_c$ kgsoil^{-1}	NH_4OAc extraction, EDTA titration	11.02
Magnesium, cmol$_c$kg soil^{-1}	NH_4OAc method, EDTA titration	6.21
Zinc, mg kg^{-1}	DTPA extraction, AAS	0.60
Iron, mg kg^{-1}	DTPA extraction, AAS	841.35

(15.89 g pot-1). This finding agrees with Kulhare et al. (2017) who made comparisons of different concentrations of Zn-salts (including nano-ZnO) foliar spray applied to rice and concluded that 1% concentration had the best response in terms of grain yield. The reduction in yield at 0.5% may be attributed to the infestation of mites and stem borers leading to the low percentage of filled spikelets (data not shown), thus lowering grain yield. The percentage of damage by stemborer and mites was estimated to be about 30% of the whole experiment, but the damage was observed mostly on T3 (0.50% concentration) pots. The damage had caused a significant yield loss, enough to alter the effects of the treatments, since the experiment was done in pots. Whitehead observed in two or more panicles will result in a significant yield loss already. Mite infestation generally resulted in yellow or whitish specks on the leaves which in heavily infested leaves merge. Their effect on yield was indirect and therefore was not quantified.

Despite this, the deciding factor, which was the Zn uptake and grain Zn, both showed an increasing trend as the concentration of nano-ZnO foliar spray increased, peaking at 1% and decreasing at 1.5% of ZnO concentration. Partitioning of zinc uptake to grains was better when the concentration of nano-ZnO foliar spray was 1% as evidenced by the higher amount of zinc in grains with 1.03 mg pot-1 (Fig 1b), as compared to 1.5% with only 0.67 mg pot-1 – a value comparable to that of 0.5% (0.69 mg pot-1). In contrast, the trend for zinc content in straw was increasing up to 1.5% with the highest value of 8.43 mg pot-1 which was similar to that of 1% (8.08 mg pot-1), almost forming a plateau (Fig. 2b). These trends demonstrate a higher efficiency for Zn partitioning to grain in 1% concentration of nano-ZnO foliar spray, as evidenced by the higher grain Zn content and grain yield. This finding is in strong agreement with (Umar et al., 2021) and (Yang et al., 2021) who reported the effectivity of ZnO nanoparticles in the biofortification of grains maize and rice, respectively. Thus, the concentration of 1% was concluded to be the optimal concentration of nano-ZnO for use as a foliar spray.

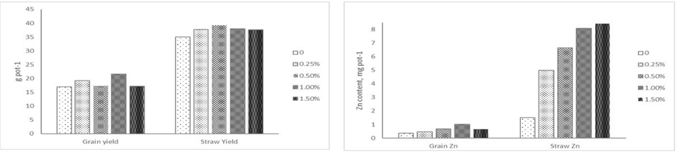

Figure 1. Influence of nano-ZnO concentration on (A) grain and straw yield, and (B) Zn content in grain and straw

Effect on yield

The application of zinc affected both the grain and straw yield of rice. The use of nano-ZnO generally resulted in a higher yield with a mean of 15.89 g pot^{-1}, which was 2.18% and 9.13% over bulk ZnO and $ZnSO_4$, respectively (Fig. 2). The advantage of nano-ZnO was observed when it was applied through the foliar application or combined soil and foliar application, but the difference was more pronounced and evident when it was applied through foliar alone. Similarly, the straw yield was significantly increased by the application of nano-ZnO which resulted in a mean of 42.11 g pot^{-1} which was 3.82% and 14.64% higher than $ZnSO_4$ and bulk ZnO, respectively. Again, this trend was very evident when the application done was by foliar application. These results demonstrate the effectivity of nano-ZnO as a foliar spray, given its nano-sized dimensions and consequently properties such as higher specific area and reactivity over its non-nano (bulk) counterpart – bulk ZnO (Milani et al., 2010). Its size would have addressed its solubility problem in providing Zn to plants, as (McBeath and McLaughlin, 2014) concluded that zinc oxide had very low water solubility and slow dissolution rates compared to $ZnSO_4$. Due to its small particle size, nano-ZnO is theoretically more bioavailable when applied to plants (Milani et al., 2010). This is in good agreement with numerous findings that foliar application of nano-ZnO improved crop growth, yield, and general performance of corn (Subbaiah et al., 2016), mungbean (Raliya et al., 2016), onion seed crop (Laware and Raskar, 2014). Other authors attribute its benefits to reduced stress due to less reactive oxygen species (in chickpea seedlings, (Burman et al., 2013), with ZnO nanoparticles serving as an ”anti-stress” agent in salt-stressed tomato (Alharby et al., 2017) and sunflower (Torabian et al., 2016).

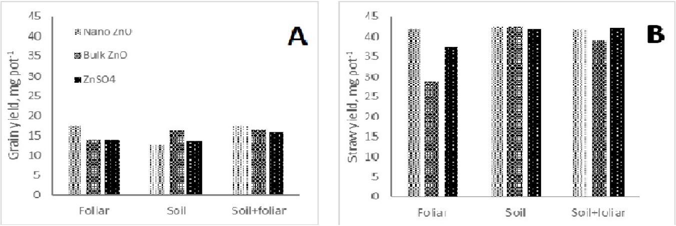

Figure 2. Influence of nano-ZnO, bulk ZnO and ZnSO4 applied by foliar, soil, and combined soil+foliar application on the (A) grain and (B) straw yield of rice

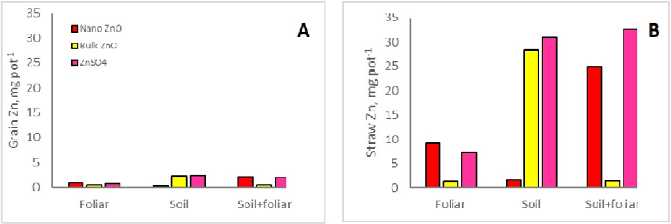

Figure 3. Influence of nano-ZnO, bulk ZnO and ZnSO$_4$ applied by foliar, soil, and combined soil+foliar application on the (A) grain and (B) straw zinc uptake of rice

On the other hand, the reported higher reactivity of nano-ZnO (Milani et al., 2015) would also explain the results observed in soil-applied zinc, with nano-ZnO resulting in the lowest yield of 12.54 g pot^{-1} which was lower by 28.47% than the yield of bulk ZnO (16.11 g pot^{-1}) and by 9.73% than ZnSO$_4$ (13.76 g pot^{-1}). This could mean that Zn from the nano-ZnO reacted to form other compounds being converted to plant-available Zn as was observed by (Liu and Lal, 2015). Another aspect of nano-ZnO that may have caused the yield reduction can be its reported toxicity to plants and soil microorganisms, especially in acidic and neutral soils (Shen et al., 2015); (Sheteiwy et al., 2021). However, there are still limited investigations in flooded soils and alternatively flooded soil conditions.

Effect on zinc uptake

Zinc uptake of rice in grain and straw showed a positive response to zinc fertilization (Fig. 3). The application of nano-ZnO resulted in a mean grain uptake of 1.17 mg pot^{-1}, placing it in between ZnSO$_4$ (1.80 mg pot^{-1}) and bulk ZnO (1.09 mg pot^{-1}) if ranked (ZnSO$_4$ >nano-ZnO >bulk ZnO) in terms of grain zinc uptake. When foliar was applied, Zn applied as nano-ZnO had a grain Zn uptake of 0.92 mg pot^{-1} which was higher by 14.57% and 91.07% compared to ZnSO$_4$ (0.801 mg pot^{-1}) and bulk ZnO (0.48 mg pot^{-1}), respectively. This trend was also observed in combined soil-foliar application with nano ZnO having a value of grain Zn uptake of 2.21 mg pot^{-1}, which was higher by 6.76% than ZnSO$_4$ (2.07 mg pot^{-1}) and as much as 350.95% than bulk ZnO (0.49 mg pot^{-1}). However, when the soil was applied, nano-ZnO resulted in much lower grain Zn uptake with only 0.38 mg pot^{-1}, which was found to be 84.95% and 83.45% lower than that of ZnSO$_4$ (2.53 mg pot^{-1}) and bulk ZnO (2.30 mg pot^{-1}). These results indicate the comparable effectivity of nano-ZnO with ZnSO$_4$ when applied as a foliar fertilizer with or without combination with soil application.

The reduction in grain zinc in treatments applied with zinc using nano-ZnO as a source by soil application during the early growth stage demonstrates the importance of the application of zinc during the reproductive and grain-filling stages of rice, particularly after flowering (Boonchuay et al., 2013), to the process of zinc loading to grains; and the possible reactions particular to nano-ZnO with the soil. This finding is also similar to those of (Mabesa et al., 2013) who reported that zinc application at the active tillering stage had no effect on grain zinc.

The same trends were apparent for straw zinc as well (Fig. 3b), with the highest value observed in the soil-foliar application of ZnSO$_4$ with a value of 32.62 mg pot^{-1} – a value that is comparable to soil application of the same zinc source (31.14 mg pot^{-1}) and soil-applied bulk ZnO with values of 28.29 mg pot^{-1}. Significantly lower than these was the value from the combined soil-foliar application of nano-ZnO with 24.81 mg. The foliar application of zinc increased the zinc content of the straw, with the highest increase observed when nano-ZnO (9.18 mg pot^{-1}) was used with a difference of 7.96 mg over the control (1.22 mg). This is higher than both bulk ZnSO$_4$ and bulk ZnO with 7.28 mg and 1.33 mg, respectively. This trend was also observed in the combined soil-foliar application, with ZnSO$_4$ and nano-ZnO having high comparable values of 32.62 mg pot^{-1} and 24.81 mg pot-1, respectively; and the bulk ZnO giving a far lower value of 1.43 mg pot^{-1}. Meanwhile, with a maximum advantage of 29.92 mg over control observed from ZnSO$_4$ (31.14 mg pot^{-1}), soil application of zinc increased the straw zinc of rice.

Among the zinc, sources tested, ZnSO$_4$ generally resulted in the highest straw zinc with a mean of 23.68 mg pot-1, which was higher by 11.8 mg than nano-ZnO with a mean of 11.88 mg pot^{-1}. When applied by combined soil-foliar or by foliar application, this difference was not as well demonstrated with differences of only 7.81 mg and 1.9 mg, respectively. A much larger difference between the two sources was observed in soil application (29.49 mg). Meanwhile, with a difference of 13.33 mg lower than ZnSO$_4$, bulk ZnO had a mean of 10.35 mg pot^{-1}. Low values were observed in plants applied with bulk ZnO, especially when done by foliar and soil-foliar application with 1.33 mg pot-1 and 1.43 mg pot^{-1}, respectively. Only when the soil was applied did bulk ZnO result in a high amount of Zn in straw with 28.29 mg pot^{-1}. On the other hand, among the different methods, application of zinc by soil or by foliar application generally resulted in high straw zinc with means of 20.36 mg pot^{-1} and 19.62 mg pot^{-1}, respectively; and foliar application to the lowest mean of 5.93 mg pot^{-1}. When nano-ZnO was used as the zinc source, the highest value observed was 24.81 mg pot^{-1}, resulting from its combined soil-foliar application, followed by 9.18 mg pot^{-1} from the foliar application and a much lower value of 1.65 mg pot^{-1} resulting from soil application. For both bulk ZnO, soil application resulted in its highest value of 28.29 mg pot-1, as compared to its foliar (1.33 mg pot^{-1}) and combined soil-foliar counterparts (1.43 mg pot-1), indicating it is best applied by soil application. Meanwhile, like nano-ZnO, $ZnSO_4$ also had its highest straw zinc when the application was done by combined soil-foliar (32.62 mg pot^{-1}) or soil application (31.14 mg pot^{-1}), and 7.28 mg pot^{-1} resulting from its foliar application.

The zinc content in straw would reflect the amount of zinc absorbed but was not remobilized and translocated to grains. In this study, the total zinc uptake reflects better, rather than grain zinc. The difference, following the order: soil-foliar,¿ foliar, in the values observed in nano-ZnO and $ZnSO_4$. Treatments between the foliar and the combined soil-foliar application implies the advantage of soil application over foliar application during the early growth stage (in this study 25 DAS) when the plants are still small and the leaf area for foliar reception is limited. Further increases in zinc uptake were caused by succeeding foliar applications. Succeeding applications are done during panicle initiation and after flowering when the leaf area is significantly larger and contributes greatly to the effectiveness of foliar application (Mabesa et al., 2013). However, taking their differences against the values of soil application does not confirm this assumption. On the other hand, a comparison between the three sources in soil application indicates a relative effectivity of bulk ZnO and $ZnSO_4$ to nano-ZnO, which may be caused by certain reactions particular to ZnO nanoparticles. ZnO nanoparticles were found to disintegrate rapidly in the presence of phosphate (Herrmann et al., 2014), resulting in the formation of zinc phosphate which has very low solubility.

Interaction with other nutrients

Simple linear correlation analysis was done between grain yield and N, P, K, Zn, and Fe uptakes as presented in Table 2. Overall correlation for N, P, and K uptake with grain yield was found to be moderately positive, but only P uptake showed a significant correlation. Meanwhile, the correlation between grain yield and both Zn and Fe uptake was very low and almost zero with $r = 0.043$ and 0.041, respectively. This explains the contrasting trends between grain yield and total Zn uptake under the different zinc sources. The advantage in grain yield of nano-ZnO over bulk ZnO and $ZnSO_4$ and the trends of the relationship between nutrient uptakes and grain yield showed that increases in the grain yield were a function of enhanced N, K and especially P uptake, rather than an effect of Zn uptake as directly affected by the treatments tested. This, along with the observed decrease in Zn uptake in plants with higher yield (those applied with nano-ZnO) and increase (Zn uptake) in plants with slightly lower yield (those applied with bulk ZnO and $ZnSO_4$), also demonstrated the antagonistic interaction of phosphorus and zinc within the plant as reported by (Mousavi et al., 2012) who stated that high concentration of phosphorus in the plant reduces Zn transport from roots to shoots. There were four mechanisms by which phosphorus can inhibit the absorption of zinc as discussed by (Alloway, 2008). One of those mechanisms was the enhancement of zinc adsorption to soil constituents including a hydrous oxide of iron and aluminum and changing soil pH, which may be considered relevant in this case because of the high concentration of iron and the low pH of the soil (Table 1). Moreover, alternate wetting and drying could have brought about intermittent changes in soil pH. Another explanation might be provided by (Herrmann et al., 2014) who reported that ZnO (nanoparticles) were degraded rapidly in the presence of phosphates, forming a compound of very low solubility (zinc phosphate), rendering both nutrients unavailable for plant uptake. This finding was further supported by a negative correlation found between P and Zn uptake with $r = -0.10$.

Conclusion

From the optimization experiment, it was identified that 1% nano-ZnO suspension applied as foliar spray resulted in the best yield response as indicated by a consistently increasing trend in grain yield, straw yield, harvest index, total zinc uptake, and Zn loading to grains. All datasets peaked at 1% concentration of ZnO foliar spray except for straw yield, which peaked at 0.5% ZnO concentration, indicating a possible higher grain yield if the plants were not infested with stemborers. Despite this, the deciding factor, which was the Zn uptake and grain Zn, both showed an increasing trend, peaking at 1% and decreasing at 1.5% of ZnO concentration. Thus, it was concluded that the optimum concentration of nano-ZnO suspension for use as a foliar spray in rice is 1%.

A significant positive response to zinc fertilization was observed in zinc uptake and grain zinc. The highest total Zn uptake has resulted from the application of $ZnSO_4$. Also, $ZnSO_4$ applied through soil application had better zinc loading to grains. Both nano-ZnO and $ZnSO_4$ were better than nano-ZnO in increasing zinc uptake and grain zinc in rice when delivery to plants was done by foliar application, except when they were soil-applied. This result demonstrates the effectivity of foliar application of micronutrients in soluble form ($ZnSO_4$), and the relative advantage of nano-ZnO over bulk ZnO given its smaller particle sizes which consequently resolved solubility issues of its bulk (ZnO) counterpart. On the other hand, soil application of nano-ZnO resulted in lower total and grain Zn uptake, compared to comparable values of bulk ZnO and $ZnSO_4$, implying that reactions (in the soil) particular to nano-ZnO have occurred. As explained by (Herrmann et al., 2014), ZnO (nanoparticles) rapidly disintegrates in the presence of phosphates, forming zinc phosphate which has very low solubility. This explanation was deemed relevant owing to the simultaneous soil application of N, P, K, and Zn at 25 DAS. Additionally, correlation analysis revealed that increases in yield observed in nano-ZnO were a function of Zn effects on N, K, and especially P uptake, rather than an effect of increased Zn uptake. The importance of the interaction of P and Zn within the plant and in the soil was demonstrated by these effects. As explained by (Alloway, 2008), this may have been due to the

Table 2. Simple linear correlation of grain yield with nutrient uptakes

	Computed *r*			
Parameter	[1]Overall	[2]Nano-ZnO	[2]Bulk ZnO	[2]ZnSO4
N uptake	0.562	0.977	0.999 *	0.998 *
P uptake	0.712 *	0.992	0.999 *	0.995
K uptake	0.466	0.979	0.998 *	0.998 *
Zn uptake	0.043	0.916	0.703	0.774

[1] Tabular *r= 0.632 at 5% level of significance with df = 8 and n = 10*

[2] Tabular *r= 0.997 at 5% level of significance with df = 1 and n = 3*

*Significant at a 5% level of significance

inhibitive action of high phosphorus on the translocation of Zn from roots to shoots due to several mechanisms. Meanwhile, a general comparison between methods revealed that foliar and combined soil-foliar application gave almost similar results, which were better than soil application. It is thus inferred that foliar application is more effective in correcting zinc deficiency and increasing zinc content in rice grains, given that soil application requires a greater amount of zinc fertilizer.

Given all these findings, the foliar application of nano-ZnO for the biofortification of rice grown in Zn-deficient soils is recommended.

Acknowledgment

This study was conducted under the funding of the DOST-SEI under the ASTHRDP Graduate Scholarship.

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