SILICON AND ZINC IMPROVES GRAIN YIELD AND NUTRIENT STATUS IN RICE WHEN SUPPLIED DURING THE DIFFERENT GROWTH STAGES

Mehrdad Sheykhzadeh, Hamidreza Mobasser^{*}, Elyas Rahimi Petrodi, Mohammad Rezvani

Department of Agronomy, Qaemshahr Branch, Islamic Azad University, Qaemshahr, Iran *Corresponding author. E-mail: drmobasser.neg@gmail.com

ABSTRACT

Silicon (Si) and zinc (Zn) has been recognized as beneficial elements to improve rice (Oryza sativa L.) grain yield. The present study was aimed at investigating the effects of silicon and zinc foliar application at different growth stages on yield components, grain yield, and concentration of silicon and zinc in rice grain. The experiment design was a factorial two-factor randomized complete block design (RCBD) with three replications. Treatments included the fertilizers foliar application at 3 levels (Potassium silicate, Silicon nanoparticles, Zinc Oxide nanoparticles) and rice growth stages at 5 levels (T1: early tillering + middle tillering + late tillering + full heading stage; T2: middle tillering + late tillering + full heading stage; T3: late tillering + full heading stage; T4: late tillering; T5: full heading stage). The results indicated that the application of Si and Zn nanoparticles was superior to potassium silicate in terms of improving the yield components, yield and nutrients concentration in rice grain. Application of Si and Zn nanoparticles increased the grain yield by 7.4 and 4.5%, respectively, above the potassium silicate. Foliar application of nutrients in both T1 and T2 stages led to improved grain yield (7716 and 7700 kg.ha⁻¹, respectively). The highest Si concentration in grain was obtained by foliar application of nano-Si at the T1 and T2 stages (3.97 and 3.83%, respectively), while the maximum Zn concentration in grain was observed with the nano-Zn application at the T2 stage (21.67 mg.kg⁻¹). In general, we suggest applying Si and Zn nanoparticles at the middle tillering + late tillering + full heading stages to improve the yield and rice grain fortifying.

Keywords: rice, yield and yield components, nanoparticle, plant nutrition.

INTRODUCTION

Rice (Oryza sativa L.) is one of the most widely consumed cereal foods in the world (Choi et al., 2015) and a primary food source for 50% of the world's population (Fitzgerald et al., 2009). Among nutrients, silicon and zinc play a key role in improving plant nutrition and increasing rice growth, so that deficiency of these nutrients reduces growth and consequently decreases yield (Sainz et al., 1998; Jeer et al., 2017). Increasing the rice grain yield by application of silicon and zinc can be attributed to the availability of nutrients needed by the plant and improving the process of absorption and transport of nutrients to the shoots and grain (Kheyri et al., 2019a). Absorption of silicon in the plant increases resistance to pests and diseases (Hossain et al., 2007), tolerance to abiotic stresses (Liang et al., 2005) and improves the quantitative and qualitative yield of the plant (Kamenidou et al., 2010). Silicon plays an important role in increasing the sink size and strength (Lavinsky et al., 2016).

Zinc deficiency is one of the limiting factors of rice production worldwide and also a widespread nutritional disorder that affects human health (Rehman et al., 2012). In fact, reducing the concentration of zinc in the grain reduces its bioavailability for humans and may lead to zinc deficiency in susceptible human populations (Hussain et al., 2012). Mahmoud-Soltani et al. (2020) stated that zinc application significantly increased the tillers number per hill, 1000-grain weight and dry matter yield of rice.

Foliar application of nutrients not only increases yield but also reduces the amount economical optimum of chemical fertilizers applied in soil (Bhuyan et al., 2012). Radhika et al. (2013) found that the foliar application of micronutrients at three growth stages (15 days after transplanting + maximum tillering and panicle initiation) significantly increased the yield components and grain yield of rice. Shaygany et al. (2012) reported that foliar application of nutrients at different growth stages (transplanting + tillering + heading stage) produced maximum rice grain yield. Lavinsky et al. (2016) demonstrated that the application of 2 mM silicon at the reproductive stage (panicle initiation to full heading) increased the filled grains number per panicle and grain yield of rice.

Nanofertilizers have a higher use efficiency compared to conventional chemical fertilizers and release nutrients at the critical growth stages, which has a significant impact on the growth characteristics of plants (Mazaherinia et al., 2010). Foliar application of nano-Si can have better benefits than conventional silicon fertilizers (Wang et al., 2015). Kheyri et al. (2018a) found that the use of silicon by nanoparticles foliar application significantly increased the silicon uptake in rice grain and straw compared to the soil application of calcium silicate and control.

The aim of present study was to investigate the effects of foliar application of potassium silicate and nanoparticles of silicon and zinc at different growth stages on yield components, grain yield and nutrients uptake in rice grain.

MATERIAL AND METHODS

The field experiment was conducted during the crop years 2018 and 2019 at the farmer's field in Babol, Mazandaran Province, Iran (36°34'N, 52°44'E; -2 m asl). The soil physical and chemical properties of the experimental site for both crop years are shown in Table 1.

Table 1. Soil Physical and chemical properties of the experimental site

| Years | 2018 | 2019 |
|-----------------------------------|------|------|
| Soil texture | Clay | Clay |
| Sand, % | 30 | 27 |
| Silt, % | 26 | 33 |
| Clay, % | 44 | 40 |
| Ph | 7.48 | 7.26 |
| EC, Ds.m ⁻¹ | 2.33 | 1.27 |
| Organic matter, % | 5.64 | 2.73 |
| Total N, % | 0.32 | 0.21 |
| Available P, mg kg ⁻¹ | 11 | 6 |
| Available K, mg kg ⁻¹ | 320 | 238 |
| Available Zn, mg kg ⁻¹ | 0.96 | 0.89 |

The experiment design was a factorial two-factor randomized complete block design (RCBD) with three replications. Treatments included the fertilizers foliar application at 3 levels (Potassium silicate, Silicon nanoparticles, Zinc Oxide nanoparticles) and rice growth stages at 5 levels (T1: early tillering + middle tillering + late tillering + full heading stage; T2: middle tillering + late tillering + full heading stage; T4: late tillering; T5: full heading stage). Rice seeds (*Oryza sativa* cv. Shiroudi) were used as plant material for the

present study. Shiroudi cultivar has high yield and good cooking quality. The rice seeds were pregerminated by soaking in water for 24 h and incubated for 48 h in the dark. Germinated seeds were sown in nursery beds. The nursery land was prepared one week before sowing. Land preparation was performed by puddling, harrowing and leveling the soil in the field. The experimental site was divided into three equal replications. Each replicate had 15 plots (5 m \times 2 m). Three rice seedlings were transplanted per hill at a spacing of 25×25 cm.

All the experimental plots received P_2O_5 as triple superphosphate, 100 kg.ha⁻¹, and K₂O as potassium chloride, 50 kg.ha⁻¹, before the seedlings were transplanted. Each plot received urea-N 180 kg.ha⁻¹, 1/3 as a basal fertilizer, 1/3 as topdressing at the panicle initiation stage, and 1/3 as topdressing at the full heading stage. Potassium silicate (produced

by AgriTecno Company) was sprayed at a rate of 5 per thousand (Ghasemi et al., 2014) and nanoparticles of silicon and zinc (produced by the US Research Nanomaterials, Inc) was applied at a concentration of 50 mg¹ (Kheyri et al., 2019b). The properties of the nanoparticles used in this study are presented in Table 2.

| Nanoparticles | Purity percentage (%) | Particles size (nm) | True density (g cm ⁻³) | $\frac{SSA}{(g m^{-2})}$ | Color |
|------------------|-----------------------|---------------------|---------------------------------------|--------------------------|-------------|
| SiO ₂ | >99% | 20-30 | 2.4 | 180-600 | White |
| ZnO | >99% | 10-30 | 5.606 | 20-60 | Milky white |

Table 2. The properties of the nanoparticles used in this study

To control weeds, Butachlor herbicide was applied at a concentration of 3.5 L ha⁻¹ 1 week after transplanting, and the manual weeding as needed during the experiment. In order to control *Chilo suppressalis*, diazinon insecticide (5% Granule) was used at a rate of 30 kg ha⁻¹ at the late tillering and heading stages.

Harvesting was done manually with sickles after leaving the border area. The total number of tillers per hill was evaluated from 12 hills per plot. The panicle length and total number of spikelets per panicle were determined by measuring 20 panicles in each plot. The Percentage of filled spikeletes per panicle was obtained from the ratio of the number of filled spikeletes per panicle to the total number of spikeletes per panicle, and was expressed as a percentage. The 1000-grain weight was recorded by counting and weighing 10 samples of 100 seeds. The grain yield (GY) and biological yield (BY) were measured through harvesting all hills from an area of 4 m² (2 m \times 2m) in the middle of each plot. The moisture content of the grains at the time of measuring the 1000-grain weight and the GY was 14%. HI was calculated according to the following formula (Yoshida, 1981): Harvest index (%) = Grain yield / Biological yield \times 100. Measurements of zinc and silicon concentrations in rice grain were performed according to the methods described by Emami et al. (1996) and Fallah et al. (2004), respectively.

A randomized complete block design (RCBD) with factorial arrangement combined over the years was used for data analysis, and means between treatments were compared with the Duncan's multiple range test (DMRT) using MSTAT-C Software. A probability of $p \le 0.05$ was considered significant. Figures were drawn using Excel 2007 software.

RESULTS AND DISCUSSION

Yield components

The results of combined analysis of variance revealed that total number of tillers per hill, total number of spikelets per panicle, Percentage of filled spikelets per panicle, and 1000-grain weight were affected by foliar application of fertilizers and rice growth stages. The interaction effect of foliar application and rice growth stages was significant only on total number of spikelets per panicle. Panicle length was not affected by any of the experimental treatments (Table 3).

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| S.O.V. | df | Panicle length | Total number of tillers per hill | Total number of spikelets per panicle | Percentage of filled spikelets per panicle | 1000-grain weight |
|------------------------|----|---------------------|--|---|--|----------------------|
| Year (y) | 1 | 0.178 ^{ns} | 3.21 ^{ns} | 46.94 ^{ns} | 0.28 ^{ns} | 4.90 ^{ns} |
| R (Y) | 4 | 0.878 | 4.94 | 265.64 | 0.51 | 2.52 |
| Foliar application (F) | 2 | 0.078 ^{ns} | 89.41** | 1818.68** | 351.68** | 52.14** |
| $Y \times F$ | 2 | 3.478 ^{ns} | 7.34 ^{ns} | 33.08 ^{ns} | 10.81 ^{ns} | 2.63 ^{ns} |
| Growth Stages (G) | 4 | 0.628 ^{ns} | 163.96** | 1266.87** | 212.15** | 38.32** |
| $Y \times G$ | 4 | 0.428 ^{ns} | 0.63 ^{ns} | 1.08 ^{ns} | 2.25 ^{ns} | 0.12 ^{ns} |
| $F \times G$ | 8 | 4.494 ^{ns} | 3.83 ^{ns} | 158.37^{*} | 8.98 ^{ns} | 1.46 ^{ns} |
| $Y \times F \times G$ | 8 | 2.728 ^{ns} | 1.09 ^{ns} | 23.05 ^{ns} | 3.28 ^{ns} | 1.06 ^{ns} |
| Error | 56 | 5.842 | 5.16 | 72.69 | 9.86 | 2.89 |
| CV (%) | - | 8.81 | 12.80 | 6.33 | 3.41 | 6.66 |

Table 3. Multivariate analysis of variance for year (Y), foliar application (F) and growth Stages (G) as well as their interactions on yield components of rice

* Significant at the 0.05 probability level. ** Significant at the 0.01 probability level. ns non-significant.

Panicle length

In this study, the simple and interaction effects of experimental treatments were not significant on panicle length (Table 3), which is consistent with the results of Mahmoud-Soltani et al. (2020).

Total number of tillers per hill

Among the experimental treatments, foliar application of ZnO nanoparticles caused the largest increase in total number of tillers per hill (19.73 tillers). By foliar application of ZnO nanoparticles, the total number of tillers per hill significantly increased compared to foliar application of SiO2 nanoparticles and potassium silicate by about 15.7% and 14.5%, respectively (Table 4). The results showed that zinc application had better effects than silicon application in terms of tillers number per hill. Zinc has an effective role on tiller production in plants by affecting chemical processes such as nucleotide synthesis, auxin metabolism and enzymes activity (Mahmoud-Soltani et al., 2020). Some researchers have reported that the tillers number per hill increased by the application of ZnO nanoparticles (Kheyri et al., 2019b) and zinc sulfate (Ghasemi et al., 2014).

In terms of foliar application at the different growth stages, the maximum total number of tillers per hill (21.8 tillers) was recorded by foliar application at the early tillering + middle tillering + late tillering + full heading stage. In this study, by reducing the number of foliar application stages, especially foliar application at the late tillering and full heading stage, the tillers number per hill decreased by about 31.8% and 32%, respectively (Table 4). Foliar application at different growth stages was more effective than foliar spray during one, two or three stages of plant growth in terms of which producing tiller, indicates the importance of plant nutrition during growth and development. Radhika et al. (2013) found that foliar application of micronutrients at three different times (15 days after transplanting, maximum tillering and panicle initiation) produced the highest tillers number per hill. Kheyri et al. (2019b) reported that foliar application of nano-Zn at different rice growth stages resulted in a significant increase in the number of fertile tillers per hill.

 Table 4. Mean comparison of effects the fertilizers foliar application and growth stages on total number of tillers/hill, percentage of filled spikelets/panicle and 1000-grain weight of rice

| Experimental treatments | Total number of tillers per hill | Percentage of filled spikelet per panicle | 1000-grain weight (g) |
|-------------------------|----------------------------------|---|--------------------------|
| Foliar application | | | |
| Potassium silicate | 16.87 b | 88.70 c | 25.60 b |
| Nano-Si | 16.63 b | 95.53 a | 24.17 c |
| Nano-Zn | 19.73 a | 91.73 b | 26.80 a |
| Growth stages | | · · · · · | |
| T1 | 21.83 a | 94.83 a | 24.11c |
| T2 | 19.50 b | 94.72 a | 24.00 c |
| Т3 | 17.67 c | 93.89 a | 25.56 b |
| T4 | 14.89 d | 88.33 b | 27.00 a |
| T5 | 14.83 d | 88.17 b | 26.94 a |

Means in columns followed by the same letter(s) are not significantly different at $P \leq 0.05$.

T1: early tillering + middle tillering + late tillering + full heading; T2: middle tillering + late tillering + full heading; T3: late tillering + full heading; T4: late tillering; T5: full heading.

Total number of spikelets per panicle

The results showed that the maximum total number of spikelets per panicle was obtained by foliar application of potassium silicate at the early tillering + middle tillering + late tillering + full heading (151.5 panicle) and also by foliar application of silicon nanoparticles at the middle tillering + late tillering + full heading (153.3 panicle), although there was no statistically significant difference with the application of nano-Si at the early tillering + middle tillering + late tillering + full heading stage (Figure 1).

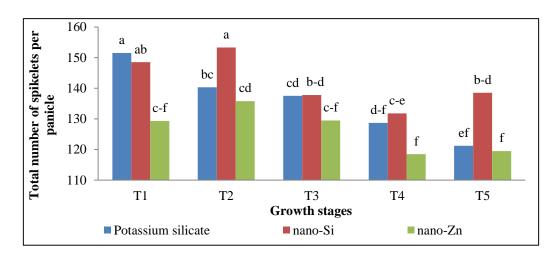


Figure 1. Interaction between fertilizers foliar application and growth stages on total number of spikelet per panicle of rice (T1: early tillering + middle tillering + late tillering + full heading; T2: middle tillering + late tillering + full heading; T3: late tillering + full heading; T4: late tillering; T5: full heading)

In general, at different growth stages of rice, the foliar application of nano-Si had better impacts compared to nano-Zn application in terms of number of spikelets per panicle. Ghasemi et al. (2014) reported that foliar application of silicon has positive effects in increasing the total number of spikelets per panicle of rice, which is consistent with the results of this research. It seems that the reason for reducing the total number of spikelets per panicle by the application of zinc nanoxide is the decrease in the allocation of environmental factors to each tiller due to the increase in the tillers number per hill. The present result showed that at different growth stages, the application of nano-Si had a higher or similar efficiency than potassium silicate in terms of number of spikelets per panicle. The greater effectiveness of nanoparticles compared to conventional particles can be attributed to the high adsorption efficiency and specific surface area of these particles (Prasad et al., 2012).

Percentage of filled spikelets per panicle

In this study, the highest and lowest percentages of filled spikelets per panicle were obtained using nano-Si (95.53 spikelets) and potassium silicate (88.70 spikelet), respectively (Table 4). The results of this study indicate that nanoparticles were able to create more favorable conditions for increasing the percentages of filled spikelets per panicle. Nanoparticles have very small dimensions and high specific surface area, so they are distributed in the plant with higher speed and homogeneity, which ultimately leads to an increase in effective parameters in yield components (Nair et al., 2010). Among the nanoparticles used in this experiment, nano-Si had better effects compared to nano-Zn in terms of filled spikelets per panicle. Silicon plays a key role in increasing the number of rice grains (Lavinsky et al., 2016). Jawahar et al. (2015) Stated that silicon fertilizer significantly increases the grains number per panicle by enhancing carbohydrate uptake in spike. Silicon increases photosynthesis by increasing chlorophyll content, improving Rubisco enzyme activity, and increasing leaf number and area, resulting in increased carbohydrate and assimilation in panicle (Savvas and Ntatsi, 2015). A significant increase in the number of grains per panicle by increasing silicon application has been reported in the results of other studies (Kheyri et al., 2019b; Cuong et al., 2017).

Among the different growth stages, the highest percentages of filled spikelets per panicle was obtained by foliar application at the early tillering + middle tillering + late tillering + full heading (94.83%), middle tillering + late tillering + full heading (94.72%) and late tillering + full heading

(93.89%) (Table 4). The highest percentages of filled spikelets per panicle were obtained by foliar application at different rice growth stages. This result indicates the importance of nutrition at different growth and development stages, especially late tillering and full heading stage to increase the production of filled spikelets per panicle. Radhika et al. (2013) reported that the maximum number of filled grains per panicle was obtained by foliar application of micronutrients at three growth stages days rice (15 after transplanting + maximum tillering and panicle initiation). The application of macro and micro elements at different growth stages, especially at the late vegetative growth and early reproductive growth, plays an important role in improving photosynthesis, accumulation of photosynthetic materials and finally its transfer to seeds (Mahmodi et al., 2019). Lavinsky et al. (2016) found that the application of 2 mM silicon at the reproductive stage (panicle initiation to full heading) increased the filled grains number per panicle of rice.

1000-grain weight

The maximum 1000-grain weight was obtained by foliar application of nano-Zn (26.8 g), while by using the potassium silicate and nano-Si, the grain weight was reduced by about 4.5 and 9.8%, respectively (Table 4). The results of this study indicated that each of the yield components may affect each other, so that by increasing or decreasing the total number of spikelet per panicle, the 1000-grain weight decreased or increased, respectively. The grain number per panicle, tillers number, plant dry matter, as well as panicle length plays an important role in determining the 1000-grain weight of rice (Mahmodi et al., 2019). However, zinc also plays an important role in the accumulation of photosynthetic materials in grains during the grain filling stage (El-azeem et al., 2014). Application of zinc improves the 1000-grain weight by reducing source limitation, increasing photosynthetic materials and better transferring these materials to the grain (Mahmoud-Soltani et al., 2020). Fischer and Kohn (2006) found that the 1000-grain weight increased significantly with the use of nano-Zn.

In this study, the highest 1000-grain weight was observed only during one stage of foliar application at the late tillering (27 g) and full heading (26.94 g) (Table 4). Mahmodi et al. (2019) stated that the grain shell size and final grain size are determined two weeks before flowering and three weeks after flowering, respectively. Mahmoud-Soltani et al. (2020) reported that the highest 1000-seed weight was obtained by foliar application at the heading stage or ripening phase. Shaygany et al. (2012) observed that the maximum 1000-grain weight was obtained during two crop years by foliar application of nutrients at different growth stages (transplanting + tillering + heading).

Grain yield (GY), biological yield (BY), and harvest index (HI)

The results of combined analysis of variance revealed that GY was affected by foliar application of fertilizers and rice growth stages. The effect of foliar application was significant on BY. The HI was not affected by any of the experimental treatments (Table 5).

Table 5. Multivariate analysis of variance for year (Y), foliar application (F) and growth Stages (G) as well as their interactions on grain yield (GY), biological yield (BY) and harvest index (HI) of rice

| S.O.V. | df | Grain yield | Biological yield | Harvest index |
|--------------------------------|----|------------------------|-------------------------|---------------------|
| Year (y) | 1 | 82749.3 ^{ns} | 5376311.2 ^{ns} | 8.040 ^{ns} |
| R (Y) | 4 | 188927.3 | 2338075.8 | 17.906 |
| Foliar application (F) | 2 | 2486228.9** | 11089491.7* | 6.642 ^{ns} |
| $\mathbf{Y} \times \mathbf{F}$ | 2 | 54221.4 ^{ns} | 53611.2 ^{ns} | 0.365 ^{ns} |
| Growth Stages (G) | 4 | 1145787.8** | 5970452.8 ^{ns} | 2.036 ^{ns} |
| $\mathbf{Y} \times \mathbf{G}$ | 4 | 10981.7 ^{ns} | 106953.9 ^{ns} | 0.544 ^{ns} |
| $F \times G$ | 8 | 90643.2 ^{ns} | 2790679.4 ^{ns} | 6.521 ^{ns} |
| $Y\times F\times G$ | 8 | 277052.2 ^{ns} | 3186645.6 ^{ns} | 3.033 ^{ns} |
| Error | 56 | 207814.2 | 2895830.5 | 6.696 |
| CV (%) | - | 6.11 | 9.07 | 6.48 |

* Significant at the 0.05 probability level. ** Significant at the 0.01 probability level. ns non-significant.

Grain yield

The results of the present study showed that maximum GY was obtained by foliar application of nano-Si (7733 kg.ha⁻¹) and nano-Zn (7498 kg.ha⁻¹) (Table 6). In this study, the reason for increasing GY by foliar application of nanoparticles can be related to increasing yield components such as total number of spikelet per panicle and percentage of filled spikelet per panicle by nano-Si application and also total number of tillers per hill and 1000-grain weight by nano-Zn application. Kheyri et al. (2019b) reported that the increase in grain yield by application of silicon and zinc can be due to the improvement of silicon and zinc uptake in plant tissue and to the increase in yield attributes, especially the fertile tillers number per hill. There is a positive and significant

linear correlation between rice grain yield with the Si and Zn concentration in grain (Khevri et al., 2018b). Moaveni and Kheiri (2011) stated that nanoparticle fertilizers are superior to conventional fertilizers in terms of grain yield production. Kheyri et al. (2019b) found that the foliar application of silicon and zinc as nanoparticles caused a significant increase in rice grain yield compared to the control. Application of Silicon improves grain yield by increasing leaf growth, improving water use efficiency, reducing cuticle transpiration (Ahstiani et al., 2012), increasing growth, yield components and improving nutrient uptake (Pati et al., 2016). Zinc increases grain yield by increasing the photosynthetic capacity of the plant and providing the photosynthetic material needed for plant growth (Seghatoleslami and Forutani, 2015). Panam et al. (2016) reported that nano-Zn due to its high specific surface area and high solubility plays an important role in providing micronutrient for the plant and ultimately increasing yield.

Among the growth and development stages, the lowest grain yields were recorded by foliar application during one stage of plant growth at late tillering (7209 kg.ha⁻¹) and full heading (7201 kg.ha⁻¹). By increasing the number foliar application of stages, especially foliar application during three and four stages of plant growth, GY increased by about 6.5 and 6.7%, respectively (Table 6). This result showed that in order to achieve maximum rice grain yield, nutrients should be provided to the plant at different growth stages. Shaygany et al. (2012) reported that the highest rice grain yield was obtained by foliar application of nutrients at different growth stages (transplanting + tillering + heading stage), which is consistent with the results of this study. Lavinsky et al. (2016) stated that the application of 2 mM silicon in the reproductive stage (panicle initiation to full heading) increased rice grain yield compared to no silicon consumption by 45%. Abbasi et al. (2019) found that foliar application of zinc during different plant growth stages through nutrient supply increases vegetative growth, improves photosynthetic system, increases chlorophyll content, and ultimately increases yield.

Biological yield

The maximum BY was obtained by foliar application of nano-Si (19213 kg.ha⁻¹) and nano-Zn (18986 kg.ha⁻¹), while by foliar application of calcium silicate, BY was reduced by about 6% (Table 6). In this research, the maximum BY was obtained by nanoparticles foliar application. The small size of nanoparticles causes more and faster absorption of nutrients, and consequently increases the efficiency of nanofertilizers 2011). (Peyvandi et al., Therefore, nanofertilizers have a higher use efficiency compared to conventional chemical fertilizers (Mazaherinia et al., 2010). Improving rice growth by the use of silicon and zinc nanoparticles has been reported by Kheyri et al. (2019a, b). The silicon application increases photosynthesis and plant yield by vertical holding of leaves and receiving better light (Gottardi et al., 2012). Application of zinc leads to increased dry matter yield by increasing auxin biosynthesis, improving carbonic anhydrase enzyme activity, reducing the sodium accumulation in the plant tissues and increasing the nitrogen and phosphorus uptake (Mahmoud-Soltani et al., 2020).

| <i>Table 6.</i> Mean comparison of effects the fertilizers foliar application and growth stages |
|---|
| on grain yield (GY), biological yield (BY) of rice |

| Experimental treatments | Grain yield (kg ha ⁻¹) | Biological yield (kg ha ⁻¹) |
|-------------------------|---------------------------------------|--|
| Foliar application | | |
| Potassium silicate | 7160 b | 18065 b |
| Nano-Si | 7733 a | 19213 a |
| Nano-Zn | 7498 a | 18986 a |
| Growth stages | | |
| T1 | 7716 a | ns |
| T2 | 7700 a | ns |
| T3 | 7492ab | ns |
| T4 | 7209 b | ns |
| T5 | 7201 b | ns |

Means in columns followed by the same letter(s) are not significantly different at $P \le 0.05$. T1: early tillering + middle tillering + late tillering + full heading; T2: middle tillering + late tillering + full heading; T3: late tillering + full heading; T4: late tillering; T5: full heading

Harvest index

Harvest index was not significant under any of the simple or interaction effects of the experimental treatments, which is consistent with the results obtained by Cuong et al. (2017).

Concentrations of silicon and zinc in grain

The results of combined analysis of variance revealed that silicon and zinc concentrations in rice grain were affected by simple effect of experimental treatments, as well as the interaction between foliar application and growth stages (Table 7).

 Table 7. Multivariate analysis of variance for year (Y), foliar application (F) and growth Stages (G) as well as their interactions on silicon and zinc concentrations of rice

| S.O.V. | df | Silicon concentration in grain | Zinc concentration in grain |
|--------------------------------|----|--------------------------------|-----------------------------|
| Year (y) | 1 | 0.036 ^{ns} | 2.147 ^{ns} |
| R (Y) | 4 | 0.358 | 1.218 |
| Foliar application (F) | 2 | 16.430** | 503.154** |
| $\mathbf{Y} \times \mathbf{F}$ | 2 | 0.265 ^{ns} | 0.126 ^{ns} |
| Growth Stages (G) | 4 | 6.344** | 39.524** |
| $\mathbf{Y} \times \mathbf{G}$ | 4 | 0.082 ^{ns} | 0.198 ^{ns} |
| $F \times G$ | 8 | 1.512** | 26.023** |
| $Y\times F\times G$ | 8 | 0.017 ^{ns} | 0.892 ^{ns} |
| Error | 56 | 0.132 | 1.801 |
| CV (%) | - | 15.25 | 11.94 |

^{*} Significant at the 0.01 probability level. ^{ns} non-significant.

Silicon concentration in grain

Foliar application of nano-Si at the early tillering + middle tillering + late tillering + full heading caused the highest concentration of silicon in the grain (3.9%), although there was no significant difference with the nano-Si application at the middle tillering + late tillering + full heading stage (3.8%) (Figure 2). In general, foliar application of silicon nanoparticles at all growth stages was superior to calcium silicate in terms of silicon accumulation in the rice grain. The rate of adsorption, transport and accumulation of nanoparticles is higher than normal particles

due to the smaller diameter of nanoparticles (Torabian and Zahedi, 2013). Yazdpour et al. (2014) found that the highest silicon uptake in rice grains was obtained during two crop years by nano-Si foliar application, which is consistent with the results of this research. The application of silicon in rice leads to an increase in the Si concentration in the shoots, especially in the grains (Cuong et al., 2017). Kheyri et al. (2019b) reported that the Si application, especially nano-Si foliar application significantly increased the Si concentration in rice plant tissue.

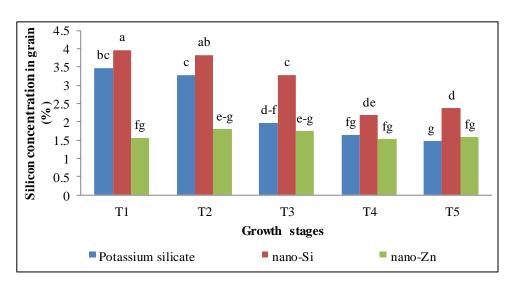


Figure 2. Interaction between fertilizers foliar application and growth stages on silicon concentration in grain of rice (T1: early tillering + middle tillering + late tillering + full heading, T2: middle tillering + late tillering + full heading, T3: late tillering + full heading, T4: late tillering and T5: full heading)

Zinc concentration in grain

The maximum zinc concentration in the grain was obtained by foliar application of nano-Zn in middle tillering + late tillering + full heading stage $(21.67 \text{ mg kg}^{-1})$ (Figure 3). In general, with decreasing the number of foliar application stages, the zinc concentration in the grain decreased. Kheyri et al. (2019b) found that foliar application of nano-Zn resulted in a 33.2% increase in zinc concentration in rice grain compared to the control. Foliar application of micronutrient such as zinc at different times can help to eliminate the deficiency of this element (Whitty and Chambliss, 2005). Wu et al. (2010) demonstrated that foliar application of zinc during booting and pollination stages caused more transfer of zinc from flag leaves to seeds. Shivay et al. (2016) stated that foliar application of zinc during three stages of plant growth causes more uptake of zinc in the grain than one stage of foliar application, which is consistent with the results of this study.

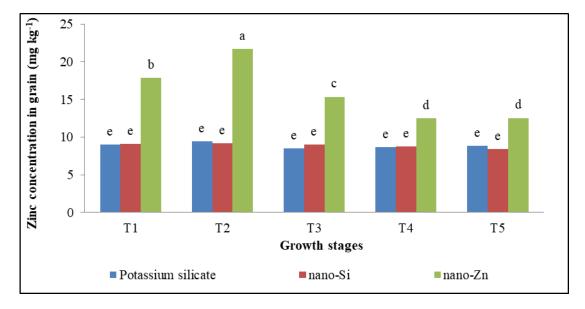


Figure 3. Interaction between fertilizers foliar application and growth stages on zinc concentration in grain of rice (T1: early tillering + middle tillering + late tillering + full heading; T2: middle tillering + late tillering + full heading; T3: late tillering + full heading; T4: late tillering; T5: full heading)

CONCLUSIONS

The results of this study showed the effectiveness of nanofertilizers, as well as the importance of each growth stages to improve the quantitative and qualitative yield of rice grains. The silicon and zinc nanoparticles were able to significantly increase yield components, vield and nutrients concentration in grain. Foliar application of nutrients during different growth stages, especially at the middle tillering + late tillering + full heading stage led to improved grain quantity and quality. Therefore, foliar application of nanoparticles at the middle tillering + late tillering + full heading stage is essential for increase yield and rice grain enrichment.

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