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Yield and Zinc Concentration of Several Rice Plant Varieties (*Oryza Sativa* L.) with Zinc Sulfate Heptahydrate ($ZnSO_4$) Fertilization

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Abstract. Zinc is an essential micronutrient for the survival of plants. Rice cultivation in Indonesia is primarily conducted on submerged soil, resulting in low zinc availability in rice plants. Applying zinc fertilizer is a method to augment plant nutrients, consequently enhancing rice yield. This study aimed to analyze and determine the best ZnSO4 fertilization dose for yield and zinc concentration in Ciherang, Rojolele and Mentik Wangi rice varieties. This study was conducted in the experimental garden of Sebelas Maret University from August 2023 to January 2024. The study was conducted using a 3x5 factorial Randomized Complete Block Design. The first factor was rice varieties consisting of Ciherang, Rojolele, and Mentik Wangi. The second factor was ZnSO4 fertilization consisting of doses of 0 $kg.ha^{-1}$, 12 $kg.ha^{-1}$, 16 $kg.ha^{-1}$, 20 $kg.ha^{-1}$, and 24 $kg.ha^{-1}$. Statistical analysis in this study used ANOVA with a significance level of 5% and DMRT with a significance level of 5%. Zinc sulfate heptahydrate (ZnSO4) foliar application significantly differs in rice's zinc concentration. Mentik wangi rice variety (V3) was given dose 4 (D4) (24 $kg.ha^{-1}$) is the best combination to increase the zinc concentration in rice by 40.01 ppm compared to no zinc fertilization treatment.

Keywords: foliar; rice; yield; zinc sulfate heptahydrate (ZnSO4)

INTRODUCTION

Zinc is an essential micronutrient for the survival of humans and plants. The presence of zinc is crucial in the human body for protein synthesis, cell growth, and cell differentiation (Farzana et al., 2021). Plants only need a small amount of zinc, but it plays a vital role in several key physiological functions, including structural membrane formation, photosynthesis, phytohormone activity, lipid, and nucleic acid metabolism, gene expression and regulation, protein synthesis, defense against drought and disease (Anggraeni et al., 2021).

Zinc deficiency in rice plants poses a major threat to food security (Salawati et al., 2021). This is a concern in several countries, including Indonesia, as rice is the primary food commodity (Zhang et al., 2021). The zinc content of rice is significantly lower than that of animal-based foods. An analysis of zinc levels in rice revealed a range of 16.2 to 35 ppm, with ciherang rice variety has zinc concentration of 16.5 ppm, while the rojolele rice variety has zinc concentration of 31 ppm (Indrasari, 2006). This range is still below the recommended daily intake for humans (Yang et al., 2021).

Rice, derived from the rice plant (*Oryza sativa L.*), is a staple food for most Indonesians and should provide sufficient zinc content to support growth. However, the majority of rice cultivation in Indonesia utilizes submerged soil, resulting in low zinc availability in rice plants (Prom-u-thai et al., 2020). Lowland rice is at high risk of zinc deficiency due to the decline in zinc levels in submerged soil. Submergence leads to an increase in soil pH, causing the formation of zinc sulfide (ZnS), and an increase in the concentrations of divalent ferrous (Fe^{2+}) and manganese (Mn^{2+}) ions. These ions, in turn, significantly hinder zinc uptake by plant roots (Sunar et al., 2021).

One of the ways farmers to get maximum rice yields is using inorganic fertilizer (Marpaung et al., 2024)and the most effective strategies for enhancing zinc absorption in rice plants is zinc fertilization (Islam et al., 2021). The foliar spray method or the method of spraying zinc (Zn) onto leaves is the most effective fertilization method compared to spraying onto the soil in improving crop production quality. Zinc deficiency in plants is most commonly addressed using zinc sulfate heptahydrate (ZnSO4) due to its high

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solubility and affordability. Foliar application of zinc is considered an effective method as the nutrients can easily penetrate the leaf cuticle, leading to better absorption (Saikh et al., 2022).

Studies emphasizing plant cultivation techniques useful for increasing yield and zinc content in rice are needed to meet human nutritional needs. This study aimed to analyze and determine the best dose of Zinc Sulfate Heptahydrate (ZnSO4) fertilization to increase growth, yield, and zinc content in the Ciherang, Rojolele, and Mentik Wangi rice varieties.

METHODS

This study was conducted at the experimental garden of the Faculty of Agriculture, Sebelas Maret University (UNS) in Jumantono sub-district, Karanganyar Regency, with an altitude of 345 meters above sea level (geographic location 7°37'48.82" South Latitude and 110°56'52.17" East Longitude), and the analysis of zinc content (soil, plants, and rice) was carried out at the Chemistry and Soil Laboratory of Sebelas Maret University (UNS). The study was conducted over a period of 5 months, from August 2023 to January 2024. The study was conducted using a 3x5 factorial Randomized Complete Block Design. The first factor was rice varieties consisting of Ciherang, Rojolele, and Mentik Wangi. The second factor was foliar fertilization of Zinc Sulfate Heptahydrate (ZnSO4) consisting of doses of 0 kg.ha^{-1} (control), 12 kg.ha^{-1}, 16 kg.ha^{-1}, 20 kg.ha^{-1}, and 24 kg.ha^{-1}. The zinc content of the soil samples was analyzed before any treatment was applied.

Rice seeds (Ciherang, Rojolele, and Mentik Wangi) were dried under sunlight for 2 hours, then rinsed to remove dirt and empty seeds. Next, they were soaked for 24 hours. The seeds were then incubated for 36 hours until the radicle emerged, and then sown in seedling trays. Seedling care included irrigation, fertilization, and pest and disease control. Seedlings were transplanted into plastic gallons with one rice seedling per planting hole 15 days after sowing. Each gallon was filled with a growing medium composed of soil with added manure/compost. A total of 45 rice seedlings (45 gallons) were transplanted based on the formula first factor x second factor x 3 replications. ZnSO4 fertilization was applied foliarly or by spraying to rice plants at 21 days after transplanting (DAT) (25% of each dose), 42 DAT (35% of each dose), and 63 DAT (40% of each dose).

Observed variables were plant zinc content, rice zinc content, panicle length, number of filled grains, number of empty grains, 1000-grain weight, harvest index, dry grain weight per clump and per hectare. Statistical analysis in this study used ANOVA (analysis of variance) with a significance level of 5% and DMRT (Duncan Multiple Range Test) with a significance level of 5%.

RESULTS AND DISCUSSION

Soil Analysis Results from the Experimental garden

Soil is an essential component in agriculture, providing plants with the essential nutrients they need to thrive (Sumarniasih et al., 2023). To determine the nutrient content in the soil, soil analysis needs to be conducted before planting crops in agricultural fields (Kalambe, 2021).

Soil analysis is a series of chemical processes that determine the amount of available plant nutrients in the soil (Kalambe, 2021). Based on the results of soil analysis in **Table 1**, the total nitrogen content is moderate (0.38%), phosphorus content is low (6.64 ppm), potassium content is very low (0.34%), organic matter content is very high (5.89%), soil pH is slightly acidic (6.09), cation exchange capacity is high (25.40 cmol/kg) (Satiti et al., 2020), (Eviati et al., 2023) and the available zinc content in the soil is low at 1.55 ppm (Primary laboratory analysis data, 2023), low soil zinc content can occur due to increased iron, nitrogen and phosphorus content. The average zinc content in

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agricultural land ranges from 50 ppm (Brian J. Alloway, 2008).

Research by (Coffin & Slaton, 2020), who tested zinc in rice through soils with low and medium zinc levels, showed that the increased concentration of zinc in rice was not detected significantly. Low zinc content in the soil results in low zinc (Zn) content in plants (Amin et al., 2022). The availability of Zn for plants is highly dependent on site-specific deposits, diffusion conditions, weeds, and the availability of other nutrients such as N, P, K, Ca, and Fe (Salawati et al., 2021).

Table 1. Results of nutrient analysis in the soil prior to the study

Nutrients	Soil Chemistry Concentration	Valuation
N-Total	0.38 %	Moderate
P	6.64 ppm	Low
K	0.34%	Very low
C-Organik	5.89%	Very high
pH	6,09	Slightly acidic
Zn	1.55 ppm	Low
Cation exchange capacity	25.40 cmol/kg	Hight

Source: Primary Data Laboratory Analysis (2023) &; Laboratory Analysis, based on research (Satiti et al., 2020) and guidelines for chemical analysis of soil, plants, water and fertilizer (Eviati et al., 2023).

Panicle Length

Panicle length plays a crucial role in rice growth. The longer the panicle size, the more grains it can produce (Yagya et al., 2023). Based on the observation data of panicle length (**Table 2**), there was no interaction in rice varieties (ciherang (V1), rojolele (V2) and Mentik Wangi (V3)) with zinc fertilization doses (dose 1 (D1) (12 kg.ha^{-1}), dose 2 (D2) (16 kg.ha^{-1}), dose 3 (D3) (20 kg.ha^{-1}) and dose 4 (D4) (24 kg.ha^{-1})). While the single factor of rice varieties showed significantly different results and the dose of zinc fertilization showed no significant difference in the length of panicles. The rice variety factor showed the highest panicle length in the rojolele rice variety (V2) of 25.40 cm compared to the Ciherang (V1) and Mentik Wangi (V3) rice varieties.

Zinc plays a crucial role in plant biochemical activities, including in the formation of the auxin hormone, essential for plant growth, particularly in stem and panicle development (S K Rautaray & Swati Sucharita, 2023). This study found no significant differences among the zinc sulfate heptahydrate fertilization treatments applied. However, some treatment doses had a positive effect on increasing panicle length in several rice varieties. This is in line with another research by (Yagya et al., 2023) which states that applying zinc fertilizer through foliar spraying to the leaves is less effective in increasing panicle length. The fertilization that increases panicle length is the fertilization of zinc through the soil followed by foliar spray. These results are also in line with the research conducted by (Kandil et al., 2022) which states that the application of zinc fertilizer through foliar produces a significant difference in all plant attributes including panicles length, viewed from the side of single factor, that is the variety factor.

Number of Filled Grains Panicle

The number of filled grains per panicle in several rice varieties indicated no significant difference among all treatments (**Table 2**). However, the application of zinc sulfate heptahydrate (ZnSO4) fertilizer at several treatment doses indicated better results compared to the 0-dose treatment. This is in line with the study by Sunar et al., (2021).

Based on the data presented in **Table 2**, showed no interaction in rice varieties

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(ciherang (V1), rojolele (V2) and mentik wangi (V3)) with zinc fertilization doses (dose 1 (D1) (12 $kg.ha^{-1}$), dose 2 (D2) (16 $kg.ha^{-1}$), dose 3 (D3) (20 $kg.ha^{-1}$) and dose 4 (D4) (24 $kg.ha^{-1}$)). As for the single factor, the rice variety showed significantly different results, while for the zinc fertilization dose, it showed no significant difference in the amount of number of filled grains per panicle. The rice variety factor showed that the number of grains filled with beans was the highest in the Ciherang rice variety (V1) of 125.04 grains compared to the rojolele (V2) and mentik wangi (V3) rice varieties.

Table 2. The effect of zinc sulfate heptahydrate fertilizer application on the yield components of several rice varieties

Treatment	Penicle Length (cm)	Grain Filled Penicle	Hollow Grain Filled Penicle	Weight 1000 Seeds (g)
Varietas				
V1	24.46a	125.04b	20.62a	25.71a
V2	25.40b	97.96a	24.58a	27.53b
V3	25.05ab	93.78a	22.65a	28.46b
Dosis				
D0	24.33a	102.58a	27.00a	25.98a
D1	25.03a	103.61a	24.57a	27.06a
D2	25.39a	106.46a	20.71a	27.59a
D3	25.05a	104.42a	20.70a	27.64a
D4	25.07a	110.88a	20.11a	27.90a
Interaction	0.962 (ns)	0.669 (ns)	0.136 (ns)	0.527 (ns)

Remarks: The values in the same column followed by the same letter show no significant difference based on the DMRT test at the 5% level. Description n = significantly different, ns = not significantly different.

Several factors affect the number of filled grains per panicle. One crucial factor is the minimal infestation of brown planthopper, which typically suck the sap out of rice grains, rendering them empty. According to Maulana et al., (2023), the appropriate application of ZnSO4 fertilizer can enhance plant resistance to pests and diseases, leading to a higher yield of filled grains. The length of the panicle also plays a role in determining the number of filled grains per panicle, as it depends on the plant's photosynthesis process. The longer the panicle, the more filled grains it can potentially support, and the more efficient the plant's photosynthesis, the better the quality of the grains produced (Nena Nurhasanah et al., 2020).

Hollow Grains Panicle

Based on the observation data of the number of hollow grains penicle (**Table 2**), there was no interaction in rice varieties (ciherang (V1), rojolele (V2) and mentik wangi (V3)) with zinc fertilization doses (dose 1 (D1) (12 $kg.ha^{-1}$), dose 2 (D2) (16 $kg.ha^{-1}$), dose 3 (D3) (20 $kg.ha^{-1}$) and dose 4 (D4) (24 $kg.ha^{-1}$)). This is also in line with the results of the single factor of rice varieties and the dose of zinc fertilization which showed no significant difference in the number of vacuum grains. Empty grains per panicle are inversely proportional to the number of filled grains per panicle. The higher the dose of zinc sulfate heptahydrate fertilizer on several rice varieties, the better the results compared to the 0 dose group.

The application of ZnSO4 fertilizer can help reduce the number of empty grains per panicle because ZnSO4 plays a role in plant metabolism that synergistically supports growth, resulting in a decrease in the number of empty grains (Salawati et al., 2021).

Weight of 1000 Grain

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The results of the observation of the weight of 1000 seeds showed that there was no interaction in rice varieties (ciherang (V1), rojolele (V2) and Mentik Wangi (V3)) with zinc fertilization doses (dose 1 (D1) (12 kg.ha^{-1}), dose 2 (D2) (16 kg.ha^{-1}), dose 3 (D3) (20 kg.ha^{-1}) and dose 4 (D4) (24 kg.ha^{-1})). While the single factor of rice varieties showed significantly different results, but for the dose of zinc fertilization showed no real difference in the weight of 1000 seeds. The rice variety factor showed that the weight of 1000 seeds was highest in the mentik wangi rice variety (V3) of 28.46 g compared to the Ciherang (V1) and Rojolele (V2) rice varieties. This can happen because different varieties carry genetic influences, so the rice seeds produced are different in each variety, which affects the weight of each rice seed. Well-filled rice grains produce heavier grain weight. This indicates that the biomass content is increasing due to the adequate availability of nutrients (S K Rautaray & Swati Sucharita, 2023). Planting rice using plastic gallons around each clump ensures that the applied ZnSO4 is evenly distributed to each clump, providing each one with maximum nutrients without competing for fertilizer absorption (Mehmood et al., 2022).

Although there was no real interaction in the statistical test regarding the effect of zinc fertilization on weight with rice varieties, the zinc fertilization performed showed an increase in the weight yield of 1000 grain weight at each treatment dose when compared to dose 0. The results also align with the research by (Khampuang et al., 2023), which states that zinc fertilization has a good effect because it can increase 1000-grain weight by 12%. The better 1000-grain weight compared to the control group aligns with the findings of Choukri et al., (2022) stating that applying zinc sulfate heptahydrate (ZnSO4) fertilizer has a positive effect on rice grain yield.

Dry Grain Weight at Harvest

Dry grain weight at harvest can be seen in **Table 3**. Foliar application of zinc sulfate heptahydrate (ZnSO4) has a positive effect on increasing the dry grain weight at harvest (Bhavik et al., 2019).

Foliar application of zinc sulfate heptahydrate (ZnSO4) can increase the dry grain weight at harvest for several rice varieties. However, variables of dry grain weight harvest per clump and dry grain weight of harvest per ton showed no interaction in rice varieties (ciherang (V1), rojolele (V2) and fragrant picking (V3)) with zinc fertilization doses (dose 1 (D1) (12 kg.ha^{-1}), dose 2 (D2) (16 kg.ha^{-1}), dose 3 (D3) (20 kg.ha^{-1}) and dose 4 (D4) (24 kg.ha^{-1})). Likewise, the single factor of rice varieties showed no significant difference in the weight of dry grain harvested by per clump and the weight of dry grain harvested by per ton. Meanwhile, the single factor of rice zinc fertilization dose showed significantly different results on the weight of dry grain weight of per clump harvest and dry grain weight of per ton harvest.

The total weight of dry grain harvest per clump for the zinc fertilization dose factor shows that the weight of dry grain harvest per clump is the most at dose 3 (D3) (20 kg.ha^{-1}) of 139.22 g compared to no zinc fertilization treatment. In the weight of dry grain harvest per ton, for the zinc fertilization dose factor, the weight of dry grain harvested per ton was the largest at dose 3 (D3) (20 kg.ha^{-1}) of 22.27 tons/ha^{-1} compared to no zinc fertilization treatment. These results were better than the control group (dose 0).

Zinc functions as cofactor in carbonate anhydrase enzyme in CO_2 fixation, respiration, ion exchange, pH regulation and fixation photosynthesis. Zinc application in leaves at the reproductive stage efficiently increases the weight of dry grain yield at harvest weight occurs due to photosynthesis activity stimulated by the application of zinc fertilizer so that the yield is directly translocated through xylem and phloem in the formation of panicles and grains(Khampuang et al., 2022).

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Table 3. Effect of zinc sulfate heptahydrate (ZnSO4) fertilizer application on Dry Grain Weight at Harvest for several rice varieties

Treatment	Dry Grain Weight at Harvest	
	Clump (g)	**Hectare (ton)**
Varieties		
V1	124.73a	19.95a
V2	129.53a	20.75a
V3	116.80a	18.68a
Dosage		
D0	99.55a	15.92a
D1	119.55b	19.12b
D2	121.00b	19.36b
D3	139.22b	22.27b
D4	139.11b	22.25b
Interaction	0.704 (ns)	0.704 (ns)

Remarks: The values in the same column followed by the same letter show no significant difference based on the DMRT test at the 5% level; * = significant (α= 5%). Description n = significantly different, ns = not significantly different.

The increase in dry grain yield at harvest was partly due to the increase in number of filled grains per panicle, number of productive tillers, 1000-grain weight, and dry grain weight per hectare (Sunar et al., 2021), followed by a decrease in the number of empty grains per panicle. Foliar application of zinc sulfate heptahydrate (ZnSO4) at an appropriate dosage can increase the weight of rice grains. This is because the right dosage of zinc sulfate heptahydrate (ZnSO4) can efficiently participate in various metabolic processes, such as metal enzyme reactions, auxin regulation, and production, thus maximizing the seed formation process and ultimately increasing rice yield (Islam et al., 2021).

Harvest Index

The harvest index of rice treated with foliar zinc fertilizer application can increase optimally if applied at the right dose (Amanullah et al., 2020).

Table 4. Effect of zinc sulfate heptahydrate (ZnSO4) fertilizer application on Harvest Index for several rice varieties

Treatment	Harvest Index
Varieties	
V1	0.13a
V2	0.14a
V3	0.12a
Dosage	
D0	0.10a
D1	0.12ab
D2	0.13b
D3	0.13b
D4	0.17c
Interaction	0.801 (tn)

Remarks: The values in the same column followed by the same letter show no significant difference based on the DMRT test at the 5% level; * = significant (α= 5%). Description n = significantly different, tn = not significantly different.

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In **Table 4**. It can be seen that there was no interaction in rice varieties (ciherang (V1), rojolele (V2) and mentik wangi (V3)) with zinc fertilization doses (dose 1 (D1) (12 kg.ha^{-1}), dose 2 (D2) (16 kg.ha^{-1}), dose 3 (D3) (20 kg.ha^{-1}) and dose 4 (D4) (24 kg.ha^{-1})). Likewise, the single factor of rice varieties showed no significant difference in results, but for the zinc fertilization dose showed a significantly different on the harvest index.

Zinc fertilizer application also significantly affected grain and straw yield, as well as the harvest index. Foliar application of ZnSO4 increased rice yield compared to soil-based zinc application (Zinzala & Narwade, 2019).

Foliar zinc application resulted in the highest grain and straw yields, along with a significant increase in rice harvest index, with 1% Zn EDTA applied during the tillering and grain filling stages (El-Sobky et al., 2022).

Zinc Concentration in Rice

Zinc concentration in rice, as shown in **Table 5**, significantly increased with the application of zinc sulfate heptahydrate (ZnSO4) fertilizer on several rice varieties. Based on **Table 5**, There was an interaction in rice varieties (ciherang (V1), rojolele (V2) and mentik wangi (V3)) with zinc fertilization doses (dose 1 (D1) (12 kg.ha^{-1}), dose 2 (D2) (16 kg.ha^{-1}), dose 3 (D3) (20 kg.ha^{-1}) and dose 4 (D4) (24 kg.ha^{-1})). Mentik wangi rice variety (V3) given a dose of 4 (D4) (24 kg.ha^{-1})) was the best combination to increase the zinc content in rice by 40.01 ppm compared to no zinc fertilization treatment.

The combination of rojolele (V2) and mentik wangi (V3) varieties with zinc fertilization doses (dose 1 (D1) (12 kg.ha^{-1}), dose 2 (D2) (16 kg.ha^{-1}), dose 3 (D3) (20 kg.ha^{-1}) and dose 4 (D4) (24 kg.ha^{-1})) showed significantly different results of zinc levels in rice. Meanwhile, the combination of the ciherang variety (V1) with the dose of zinc fertilization showed that the results of zinc levels in rice were not significantly different. The graph of the increase in zinc content in rice is presented in **Figure 1**.

ZnSO4 fertilizer was applied using the foliar technique to increase the zinc content in the resulting rice. Foliar fertilization is an alternative to avoid zinc fixation in the soil, causing suboptimal zinc uptake by plant tissues (Mandal & Ghosh, 2021). In principle, applying ZnSO4 fertilizer can increase the zinc content in plants, which can also increase the zinc content in grain yield (Wahid et al., 2022). A study by Zhang et al. (2021) revealed that zinc fertilizer application significantly affected zinc concentration in plants and rice. This study is also in line with a study by Islam et al., (2021) and Tuiwong et al., (2022), stating that the application of ZnSO4 fertilizer can increase the zinc content in rice for several rice varieties. Another study also mentioned that ZnSO4 fertilization can result in higher zinc content in rice compared to the 0 dose group and increase rice's zinc content by 29% (Yogi et al., 2023). Also in line with research by (Khampuang et al., 2023) Foliar zinc application is one of ways to improve zinc concentration and to decrease the phytate: zinc ratio in rice grain.

Table 5. Effect of zinc sulfate heptahydrate (ZnSO4) fertilizer application on Zinc Concentration in Rice of several rice varieties

Varieties	**Zinc sulfate heptahydrate (ZnSO4) fertilizer**					
	D0	D1	D2	D3	D4	Rataan
V1	15.92a	17.48a	18.38a	18.90a	18.90a	17.92a
V2	28.52bc	29.57bc	32.30cd	33.25cd	39.02e	32.53b
V3	25.20b	26.08b	28.87bc	35.70de	40.01e	31.17b
Average	23.21a	24.37ab	26.51ab	29.28bc	32.64c	

Remarks: The values in the same column followed by the same letter show no significant difference based on the DMRT test at the 5% level.

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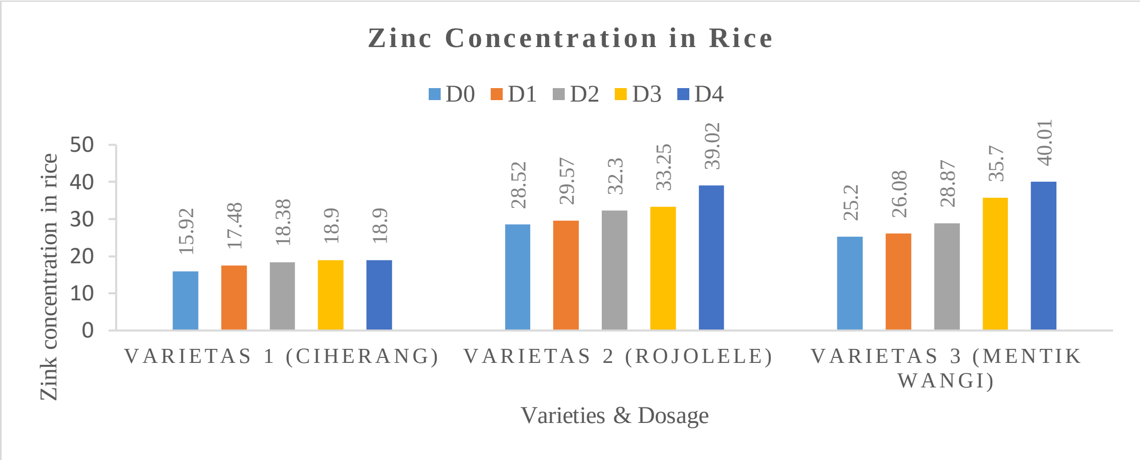

Figure 1. Distribution of the effect on increasing Zinc Content in Rice for several rice varieties treated with ZnSO4 fertilizer

In this study, it can be seen there is a very significant difference zinc concentration in rice between each variety. This can occur due to the influence of metabolism and genetic inheritance of each different variety (S K Rautaray & Swati Sucharita, 2023). Genetic factors cause the absorption of zinc fertilizers in each variety to be different. Basically, according to previous research, the Ciherang rice variety has a lower zinc content in rice grains. In the previous study, the zinc concentration in Ciherang rice variety around 16 ppm (Sunar et al., 2021). However, foliar fertilization of zinc at each doses given still provides results to increasing zinc concentration in Ciherang, Rojolele and Mentik Wangi rice varieties.

CONCLUSION

Foliar application of zinc sulfate heptahydrate (ZnSO4) with rice varieties did not show interaction, however foliar application in single factor of rice varieties showed significant increase in length, number of filled grains penicle, hollow grains penicle, and 1000-grain weight. There was interaction in rice varieties (ciherang (V1), rojolele (V2) and mentik wangi (V3)) with zinc fertilization doses (dose 1 (D1) (12 $kg.ha^{-1}$), dose 2 (D2) (16 $kg.ha^{-1}$), dose 3 (D3) (20 $kg.ha^{-1}$) and dose 4 (D4) (24 $kg.ha^{-1}$)). Mentik wangi rice variety (V3) was given dose 4 (D4) (24 $kg.ha^{-1}$) is the best combination to increase the zinc concentration in rice by 40.01 ppm compared to no zinc fertilization treatment.

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