Agronomic performance and zinc concentration in plant tissues of several rice fields varieties with zinc sulfate heptahydrate ($ZnSO_4$) fertilization

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Abstract. An essential micronutrient for rice growth is zinc. Rice grown in flooded fields is at risk of zinc deficiency. Applying zinc fertilizer through foliar spray is an effective method to meet the zinc nutrient requirement for rice plant growth. This study aimed to determine the proper ZnSO4 fertilizer dosage to provide optimal rice growth in several rice varieties. The research was conducted at the UNS experimental Garden. The first factor was the foliar ZnSO4 fertilization dosage consisting of 5 levels: without ZnSO4 fertilizer (D0), 12 kg ha^{-1} ZnSO4 fertilizer (D1), 16 kg ha^{-1} ZnSO4 fertilizer (D2), 20 kg ha^{-1} ZnSO4 fertilizer (D3) and 24 kg ha^{-1} ZnSO4 fertilizer (D4). The second factor consisted of Ciherang (V1), Mentik Wangi (V2) and Rojolele (V3) rice varieties. Statistical analysis in this study used analysis of variance 5% and DMRT test at 5%. The results found there was no interaction in rice varieties (Ciherang, Rojolele and Mentik Wangi) with zinc fertilization doses 12, 16, 20 and 24 kg ha^{-1}. However, this study showed significant difference at single factor of rice varieties and zinc fertilization doses and significant increase in plant height, number of tillers, number of productive tillers, number of leaves per hill, fresh biomass weight and zinc concentration in plant tissues compared to the dose 0.

1 Introduction

The majority of the population in Indonesia is a staple food on rice (Oryza sativa L.) and also to relies their calorie and carbohydrate intake. For Indonesians, rice is a strategic crop commodity because, being a food source, and then rice farming is also a means of earning a decent income for farmers. Rice as a food crop has an increase demand every year in line with the growing population. Thus, rice production must be able to meet the needs of the community. Therefore, increasing rice production is consistently carried out by the government as the main supporter of farmers in optimal rice cultivation [1].

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One way to achieve optimal rice cultivation is by meeting its nutritional needs (fertilization). Adequate plant nutrient supply is a crucial part of the rice farming system to increase biomass production and meet food needs, particularly rice. Fertilization is a crucial agronomic activity in modern agriculture, because the availability of the amount of nutrients, particularly micronutrients such as zinc available in the soil, is typically less than the amount needed for optimal plant growth [2].

Zinc is an essential micronutrient required by rice plants because it functions as a key role in growth and development. Zinc is useful in the process of protein formation, as an enzyme cofactor, and useful as a cofactor for several enzymes involved in various processes such as protein synthesis, DNA replication and metabolism of lipid. In addition, zinc useful a major role in various plant metabolic processes, such as activity of enzyme, chlorophyll formation, photosynthesis and other biochemical functions [3]. The second element required most abundant for all organisms living is zinc. Under physiological conditions, it can It occurs as not redox-active and divalent cation (Zn^{2+}). This clarify in various biological activities, zinc has different performance [4].

Foliar zinc fertilization is more effective than root fertilization due to the larger surface area of leaves, allowing for more efficient nutrient uptake. Zinc fertilization through the soil is at risk of not being available in sufficient and optimal quantities due to leaching, so that the availability of zinc in the soil is reduced. Therefore, the problem of meeting the zinc requirements of rice plants can only be solved by foliar spraying [5]. Micronutrients useful a direct role in rice production, plant growth, and yield attributes. Foliar zinc fertilization is also considered more practical and economical compared to soil zinc fertilization, as it tends to be more expensive and impractical [6]. The objectives of this study to investigate the physiology of rice and find the right dose to ensure optimal rice growth and maximize rice yield.

2 Materials and Methods

This study was occured from August 2023 to January 2024 at the experimental farm of Sebelas Maret University (UNS) in Jumantono Sub-District, Karanganyar Regency, with an altitude of 177,7 meters above sea level. The research design used was a Complete Randomized Design with two factors, namely ZnSO4 fertilizer doses and rice varieties.

The first factor, ZnSO4 fertilizer dose, consisted of 5 levels: no ZnSO4 fertilizer treatment (D0), ZnSO4 fertilizer dose of 12 kg ha^{-1} (0.192 g/pot/L) (D1), ZnSO4 fertilizer dose of 16 kg ha^{-1} (0.256 g/pot/L) (D3), ZnSO4 fertilizer dose of 20 kg ha^{-1} (0.320 g/pot/L) (D3), and ZnSO4 fertilizer dose of 24 kg ha^{-1} (0.380 g/pot/L) (D4) [7]. The second factor, varieties, were Ciherang rice (V1), Mentik Wangi rice (V2), and Rojolele rice (V3). Zinc Sulfate Heptahydrate (ZnSO4) fertilizer was applied using the foliar application method with doses of no ZnSO4 fertilizer, ZnSO4 fertilizer treatment (D0), ZnSO4 dose of 12 kg ha^{-1} (0.192 g/pot/L) (D1), ZnSO4 dose of 16 kg ha^{-1} (0.256 g/pot/L) (D3), ZnSO4 dose of 20 kg ha^{-1} (0.320 g/pot/L) (D3), and ZnSO4 dose of 24 kg ha^{-1} (0.380 g/pot/L) (D4). ZnSO4 fertilization was applied by foliar or 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).

The observed variables in the study were plant height, number of tillers, number of productive tillers, panicle length, and fresh biomass weight. Zinc content in plant tissues test was carried out in chemistry and soil Laboratory UNS, using the Atomic Absorption Spectrophotometer (AAS) test (instrument to measure the level of metals present in samples). The statistical analysis in this study used ANOVA and DMRT at 5%.

3 Results

3.1 Plant Height

As shown in Table. 1, the observation of plant height growth in several rice varieties demonstrated an increase difference in the treatment doses and rice varieties tested.

Table 1. Effect of Zinc Sulfate Heptahydrate Fertilizer Application on Plant Height of Several Rice Varieties

Treatment	Plant Height (cm)				
	3 MST	5 MST	7 MST	9 MST	11 MST
Varieties					
V1 (Ciherang)	22,33[a]	44,10[a]	66,74[a]	80,28[a]	84,23[a]
V2 (Rojolele)	23,10[a]	46,83[a]	68,10[a]	83,06[a]	91,73[b]
V3 (Mentik Wangi)	23,16[a]	44,60[a]	66,70[a]	85,36[a]	88,33[ab]
Dosage					
D0 (0 kg/ha)	21,12[a]	42,66[a]	63,23[a]	79,23[a]	82,77[a]
D1 (12 kg/ha)	21,66[ab]	43,88[a]	66,32[ab]	81,83[ab]	85,19[a]
D2 (16kg/ha)	24,00[b]	46,00[a]	67,15[ab]	84,00[ab]	86,28[a]
D3 (20kg/ha)	23,12[ab]	46,55[a]	69,33[b]	82,12[ab]	88,26[a]
D4 (24kg/ha)	24,41[b]	46,77[a]	69,85[b]	87,32[b]	97,97[b]
Interaction	0,587 (tn)	0,573 (tn)	0,995 (tn)	0,996 (tn)	0,889 (tn)

Note: Values in the same column followed by the same letter indicate no significant difference based on the DMRT test at the 5% level. Description n = significantly different, tn = not significantly different.

Based on table 1, it can be seen there was no interaction in rice varieties (Ciherang (V1), Rojolele (V2) and Mentik Wangi (V3)) with zinc fertilization (dose 1 (D1) (12 kg/ha), dose 2 (D2) (16 kg/ha), dose 3 (D3) (20 kg/ha) and dose 4 (D4) (24 kg/ha)) on plant height. Meanwhile, the single factor of rice variety and zinc fertilization dose showed significantly different results on plant height. The factor of rice varieties aged 11 MST showed the highest plant height in the Rojolele rice variety (V2) at 91.73 cm compared to the Ciherang (V1) and Mentik Wangi (V3) rice varieties. Zinc fertilization dose showed that the highest plant height aged 11 MST at dose 4 (D4, 24kg/ha) was 97.97 cm compared with no zinc fertilization treatment.

3.2 Number of Tillers

Observations from table 2 reveal that the application of zinc sulfate heptahydrate fertilizer led to an increase in the number of tillers in several rice varieties. However, the effect was not statistically significant for all the tested rice varieties.

Table 2. Effect of Zinc Sulfate Heptahydrate Fertilizer Application on the Number of Tillers of Several Rice Plant Varieties

Treatment	Number of Tillers				
	3 MST	5 MST	7 MST	9 MST	11 MST
Varieties					
V1 (Ciherang)	2,33[a]	15,60[a]	27,33[a]	38,60[a]	43,80[a]
V2 (Rojolele)	2,60[a]	16,93[a]	30,66[b]	43,73[b]	50,86[b]
V3 (Mentik Wangi)	2,73[a]	17,46[a]	31,00[b]	44,80[b]	55,40[c]
Dosage					
D0 (0 kg/ha)	1,66[a]	13,22[a]	24,11[a]	33,77[a]	43,88[a]
D1 (12 kg/ha)	2,22[ab]	15,55[ab]	29,00[b]	41,77[b]	47,88[ab]

D2 (16kg/ha)	2,66[ab]	16,44[b]	30,22[b]	44,44[b]	52,33[bc]
D3 (20kg/ha)	3,00[b]	18,00[bc]	31,00[bc]	43,44[b]	50,33[bc]
D4 (24kg/ha)	3,22[b]	20,11[c]	34,00[c]	48,44[c]	55,66[c]
Interaction	0,997 (tn)	,000 (tn)	0,941 (tn)	0,508 (tn)	0,644 (tn)

Note: Values in the same column followed by the same letter indicate no significant difference based on the DMRT test at the 5% level. Description n = significantly different, tn = not significantly different.

Based on teble 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), dose 2 (D2) (16 kg/ha), dose 3 (D3) (20 kg/ha) and dose 4 (D4) (24 kg/ha)) on the number of tillers. Meanwhile, the single factor of rice variety and zinc fertilization dose showed significantly different results on the number of tillers. The single factor of Mentik Wangi rice varieties (V3) at 11 MST showed the highest number of tillers of 55.40 compared to Ciherang (V1) and Rojolele (V2) rice varieties. The zinc fertilization dose factor showed the highest number of tillers in 11 MST at dose 4 (D4) (24kg/ha) of 55.66 compared to no zinc fertilization treatment.

3.3 Number of Productive Tillers and Fresh Biomass Weight

Aplication of zinc sulphate heptahydrate fertilizer led to an increase in the number of productive tillers and fresh biomass weight of several rice varieties presented in table 3. However, no significant effect was observed on the tested rice varieties with zinc sulphate heptahydrate fertilizer.

Table 3. Effect of Zinc Sulfate Heptahydrate Fertilizer Application on the Number of Productive Tillers and Fresh Biomass Weight of Several Rice Plant Varieties

Treatment	Number of Productive Tillers	Fresh Biomass Weight (kg)
Varieties		
V1 (Ciherang)	38,40[a]	1,108[a]
V2 (Rojolele)	45,00[b]	1,32[b]
V3 (Mentik Wangi)	48,93[c]	1,22[b]
Dosage		
D0 (0 kg/ha)	42,11[a]	1,11[a]
D1 (12 kg/ha)	42,22[a]	1,16[ab]
D2 (16kg/ha)	44,77[a]	1,29[b]
D3 (20kg/ha)	45,77[a]	1,22[ab]
D4 (24kg/ha)	45,66[a]	1,30[b]
Interaction	0,085 (tn)	0,802 (tn)

Note: Values in the same column followed by the same letter indicate no significant difference based on the DMRT test at the 5% level. Description n = significantly different, tn = not significantly different.

The observational data on the number of productive tillers and fresh biomass weight indicates the number of productive tillers and fresh biomass weight showed no interaction in rice varieties (Ciherang (V1), Rojolele (V2) and Mentik Wangi (V3)) with zinc fertilization doses (dose 1 (D1) (12 kg/ha), dose 2 (D2) (16 kg/ha), dose 3 (D3) (20 kg/ha) and dose 4 (D4) (24 kg/ha)). Meanwhile, the single factor of rice varieties showed significantly different results on the variables number of productive tillers and fresh biomass weight. The single factor of zinc fertilization dose showed significantly different results for fresh biomass weight.

In rice variety factor showed the highest number of productive tillers at Mentik Wangi rice variety (V3) of 48.93 compared to Ciherang (V1) and Rojolele (V2) rice varieties. The

zinc fertilization dose factor showed no significant difference in the number of productive tillers. And then, in rice variety factor showed the highest fresh biomass weight in the Rojolele rice variety (V2) of 1.32 kg compared to the Ciherang rice variety (V1) and Mentik Wangi (V3) rice varieties. The single factor of zinc fertilization dose showed the highest fresh biomass weight at dose 4 (D4) (24 kg/ha) of 1.30 kg compared to no zinc fertilization treatment.

3.4 Number of Leaves per Hill

The observation data in Table 4 on the effect of zinc sulfate heptahydrate fertilizer application on the number of leaves per hill of several rice varieties indicates an increase effect on the number of leaves per hill.

Table 4. Effect of Zinc Sulfate Heptahydrate Fertilizer Application on the Number of Leaves per Hill of Several Rice Varieties

Treatment	Number of Leaves per Hill				
	3 MST	5 MST	7 MST	9 MST	11 MST
Varieties					
V1 (Ciherang)	7,00^{a}	46,80^{a}	82,00^{a}	115,80^{a}	133,93^{a}
V2 (Rojolele)	7,80^{a}	50,80^{a}	92,00^{a}	134,40^{b}	163,86^{b}
V3 (Mentik Wangi)	8,20^{a}	52,40^{a}	93,00^{a}	131,20^{b}	152,60^{b}
Dosage					
D0 (0 kg/ha)	5,00^{a}	39,66^{a}	72,33^{a}	101,33^{a}	131,66^{a}
D1 (12 kg/ha)	6,66^{ab}	46,66^{ab}	87,00^{b}	125,33^{b}	143,66^{ab}
D2 (16kg/ha)	8,00^{ab}	49,33^{b}	90,66^{b}	133,33^{b}	157,88^{bc}
D3 (20kg/ha)	9,00^{b}	54,00^{bc}	93,00^{bc}	130,33^{b}	152,11 ^{bc}
D4 (24kg/ha)	9,66^{b}	60,33^{c}	102,00^{c}	145,33^{c}	165,33 ^{c}
Interaction	0,997 (tn)	1,000 (tn)	0,941 (tn)	0,508 (tn)	0,541 (tn)

Note: Values in the same column followed by the same letter indicate no significant difference based on the DMRT test at the 5% level. Description n = significantly different, tn = not significantly different.

Based on the observational data, the number of leaves per hill showed no interaction in rice varieties (Ciherang (V1), Rojolele (V2) and Mentik Wangi (V3)) with zinc fertilization doses (dose 1 (D1) (12 kg/ha), dose 2 (D2) (16 kg/ha), dose 3 (D3) (20 kg/ha) and dose 4 (D4) (24 kg/ha)). Meanwhile, the single factor of rice variety and zinc fertilization dose showed significantly different results on the number of leaves per Hill. The factor of rice varieties aged 11 MST showed the highest number of leaves per Hill in Rojolele rice variety of 163.86 leaves, compared to the Ciherang rice varieties (V1) and Mentik Wangi(V3). The zinc fertilization dose factor showed that the number of leaves per hill in 11 MST at dose 4 (D4) (24kg/ha) of 165.33 compared to no zinc fertilization treatment.

3.5 Zinc Content in Plant Tissues

Figure 1 presents the data on zinc content in the plant tissues of several dosage and rice varieties. Application of zinc sulfate heptahydrate fertilizer showed 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), dose 2 (D2) (16 kg/ha), dose 3 (D3) (20 kg/ha) and dose 4 (D4) (24 kg/ha)). Single factor rice varieties showed no significant difference, while zinc fertilization doses showed significantly different results on zinc content in plant tissues. Zinc fertilization dose factor showed the highest zinc content in plant tissues at dose 4 (D4) (24kg/ha) of 51.21 ppm compared to no zinc fertilization treatment.

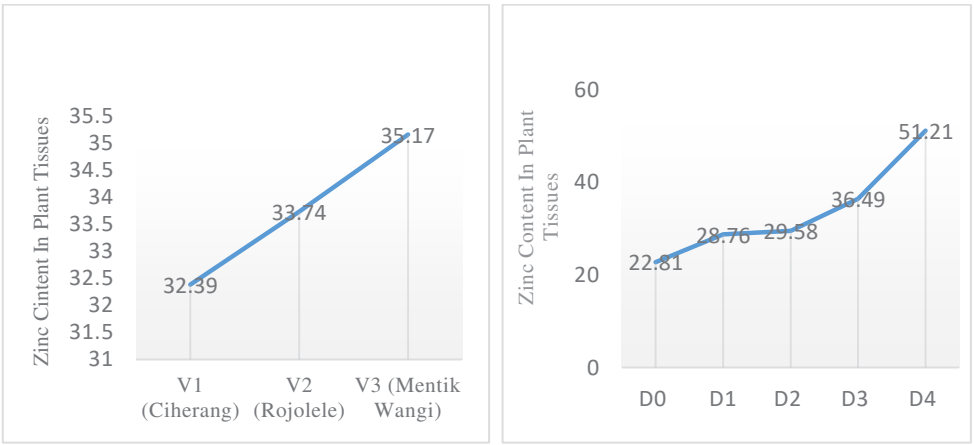

Fig. 1. Graph of zinc sulfate heptahydrate fertilizer application on increasing zinc content in plant tissues of several rice varieties. (D1 = Dosis 12 kg/ha; D2 = Dosis 16 kg/ha; D3 = Dosis 20 kg/ha; D4 = Dosis 24 kg/ha).

Base on observational data, single factor of rice varieties has no significant differences. However, Figure 1 showed the highest zinc content in plant tissues at Rojolele variety of 33.74 ppm, the scond at Mentik Wangi variety of 35.17 ppm and the third varieties Ciherang 32.39 ppm. In general, the application of $ZnSO^4$ fertilizer can increase the zinc content in plant tissues, which in turn can also increase the zinc content in the grain yield [3]. Previous research showed, Rojolele rice variety has zinc content in rice of 31 ppm [21], and than Ciherang as the most popular rice variety has zinc content in rice of 24,7 ppm [22].

4 Discussion

This study aimed to investigate the physiology of rice and find the right dose to ensure optimal rice growth and maximize rice yield. Based on the results, the criteria for rice plant height based on the Rice Standard Evaluation System are divided into several categories: short (<90 cm), medium (90-125 cm), and tall (>125 cm) (Fahmi & Sunarya, 2022). Based on the data about single factor of rice variety, the highest rice plant heights data were recorded in 11 MST for Ciherang at 84,23 cm, Rojolele at 91,73 cm, and Mentik Wangi at 88,33 cm, placing them in the medium height category. Meanwhile, single factor of zinc fertilization dose, the highest plant height were recorded in 11 MST contained in D4 at 97,97 cm.Variations in plant height, including rice plants, can be attributed to differences in genetic makeup and nutrient availability [8].

The data obtained from zinc fertilization experiments at different dosage levels revealed an increase in plant height due to zinc application. Zinc useful a crucial role in various essential in metabolic of plant, such as activities of enzyme, photosynthesis, chlorophyll formation, and other biochemical functions [9]. Photosynthesis is the primary process that enables plants to grow, develop, and produce yield. An adequate supply of zinc enhances the availability and optimizes the utilization of other essential nutrients, leading to increased plant height growth [10].

The results shows about number of tillers of several rice varieties. Rojolele Sinuk rice variety has a harvest age of 120 days, however rojolele local rice variety has age of 150 days. resistance to lodging, approximately 22 vegetative tillers, medium grain shape, straw yellow color, easy shattering, medium grain shape, sticky texture, 15.9% amylose content, and moderate resistance to tungro/blast [11]. Ciherang rice has a harvest age of 125 days,

moderate lodging, 14-17 vegetative tillers, long slender grain shape, clean yellow color, moderate shattering, sticky texture, 23% amylose content, and resistance to brown planthopper biotypes 1, 2, and 3 [12]. Mentik Wangi rice has a harvest age of 135 days, moderate lodging, 15 vegetative tillers, oblong round grain shape, brownish yellow color, fragrant rice aroma, moderate shattering, and sticky texture [13]. Based on the description of the number of vegetative tillers of each variety compared to the results of zinc fertilization treatments, the number of vegetative tillers is higher with zinc treatments. Based on the observational data obtained, zinc fertilization at several doses resulted in a higher number of rice tillers compared to dose 0. Number of tillers is a crucial component of rice in determining grain yield. The number of tillers was increased can be attributed to foliar application of zinc due to its role in various zinc-induced enzymatic activities and auxin metabolism, which regulate plant growth and development [14]. Foliar application of fertilizers, particularly those containing nitrogen and zinc during the vegetative stage, can enhance the number of tillers per plant. Meanwhile, foliar application during the reproductive stage leads to an increase in the tillers productive number [15].

Rojolele rice variety, particularly Srinuk, has advantages in terms of cultivation and economic value. Rojolele Srinuk rice variety has a significantly higher number of productive tillers, reaching 30-40 productive tillers. In contrast, the original Rojolele rice variety has only a few productive tillers, ranging from 10 to 15 [18]. The Ciherang rice variety has approximately 15 productive tillers, while the Mentik Wangi rice variety has approximately 14 productive tillers [19]. According to the data obtained, the number of productive tillers in the three varieties, Ciherang, Rojolele, and Mentik Wangi, treated with zinc fertilization indicated a higher increase compared to the description of productive tillers and without treatment for each variety.

The proper application of foliar zinc fertilization causes an increase in vegetative growth, such as in the growth of productive tillers that regenerate/multiply. This is attributed to the stimulation of optimal photosynthesis, chlorophyll synthesis, and nitrogen fixation as a result of the applied fertilization treatment [20]. The application of foliar zinc fertilizer provides sufficient zinc availability, facilitating optimal plant growth. This is due to the involvement of zinc in enzyme systems, auxin regulation and production, enhanced carbohydrate synthesis, and transport to plant tissues, leading to increased rice straw weight [15]. The application of zinc fertilization treatment resulted in significantly higher fresh biomass weight compared to dose 0 in all tested rice varieties.

Physiologi factors that need to be considered, in addition in plant height and the number of tillers, number of productive tillers and fresh iomass weight, the factor of the number of leaves per hill is one of the physiologi components that must be considered. Application of zinc sulphate heptahydrate fertilizer on leaves cause adequate zinc levels can accelerate enzymatic activity and auxin metabolism in plants [16]. Zinc fertilization plays a role in increasing photosynthesis and chlorophyll structure formation, causing an increase in plant metabolism, thereby increasing growth parameters, including the number of leaves compared to dose 0 [17]. This is in accordance with the research findings that the zinc fertilizer treatment at several doses (1, 2, 3, and 4) indicated higher results compared to dose 0.

A study indicated that the application of zinc fertilizer to seeds successfully increased the zinc content in rice tissues at low concentrations. The application of zinc fertilizer during the nursery stage anticipated zinc deficiency in plant tissues during the rice irrigation period. However, this could not increase the zinc content in plant tissues until the rice harvest phase [23] . The application of zinc fertilizer to the soil indicated a smaller increase in zinc content in rice grains and plant tissues compared to foliar zinc fertilization application [4].

Application of zinc fertilizer with foliar can increase the zinc concentration until the end of the rice harvest period. This, in turn, can lead to increased rice yield and higher zinc content in the grains [24]. Zinc concentration in straw can be increased more effectively through foliar application compared to soil application due to ion uptake mechanisms. Foliar zinc fertilization penetrates the cuticle and cellulose walls through limited or free diffusion, and ions are also absorbed through stomata on the leaves [25].

5 Conclusions

The conclusions of this study are as follows:

There was no interaction in rice varieties (Ciherang, Rojolele and Mentik Wangi) with zinc fertilization doses 12, 16, 20 and 24 kg/ha. However, this study showed significant difference at single factor of rice varieties and zinc fertilization doses. Foliar application of Zinc Sulfate Heptahydrate (ZnSO4) indicated a significant increase in plant height, number of tillers, number of productive tillers, number of leaves per hill, fresh biomass weight and zinc content in plant tissues compared to the dose 0. The highest results on each observation variable were plant height at ZnSO4 dose 4 (24 kg/ha) of 97,97 cm, number of tillers at ZnSO4 dose 4 (24 kg/ha) of 55,66 tillers, number of leaves per hill at ZnSO4 dose 4 (24 kg/ha) of 165,33 leaves, number of productive tillers at ZnSO4 dose 3 (20 kg/ha) of 45,77 tillers, and the fresh biomass weight at ZnSO4 dose 4 (24 kg/ha) of 1.30 kg, and the highest zinc content in plant tissues at dose 4 (D4) (24kg/ha) of 51.21 ppm.

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