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Effect of zinc application on growth, yield, and zinc and protein content in Ciherang rice (*Oryza sativa* L.)

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Abstract: Ciherang rice is a popular variety in Indonesia. However, the zinc (Zn) content in Ciherang rice is relatively low, barely reaching 40.1% of the agronomic and nutritional standard. The purpose of this study was to determine the best effect of Zn application on growth, yield, protein and Zn content in Ciherang rice. The study involved seven treatments, including seed priming and/or spraying with combinations of $ZnSO_4$ (0.07% or 0.5%) and urea (1%), as well as a control. Seed priming was carried out for 24 hours and spraying was applied four times: at tillering (day 35), booting (day 45), flowering (day 75), and seven days after flowering (day 82). The results showed that the application of priming and spraying with Zn and urea increased the production of Ciherang variety rice by increasing the number of productive tillers and the production of filled grains. Spraying 1% urea + 0.5% $ZnSO_4$ and priming 1% urea + 0.5% $ZnSO_4$ + spraying 1% urea + 0.5% $ZnSO_4$ increased growth by increasing the number of tillers, dry weight of shoots, and green leaves. Priming 1% urea + 0.5% $ZnSO_4$ + spraying 1% urea + 0.5% $ZnSO_4$ was also the best treatment in increasing the Zinc content to 33.5 ppm compared to the control of 11.41 ppm and protein of Ciherang rice. The combination of priming and spraying with 1% urea + 0.5% $ZnSO_4$ is the best treatment to overcome the low zinc content in Ciherang rice, while increasing growth, yield, and protein content.

Keywords: Biofortification, rice yield, zinc content.

Introduction

Rice is the staple food of the Indonesian people. In general, the nutritional content of rice per 100 g is 74.9 – 79.95 g of carbohydrates, 6 – 14 g of protein, 0.5 – 1.08 g of total fat, 0.07 – 0.58 mg of vitamin B1 (thiamine), 0.04 – 0.26 mg of B2 (riboflavin), and 1.6 – 6.7 mg of B3 (niacin) (Fitriyah et al. 2020). However, the Zinc (Zn) and protein content in rice is still low compared to other plants, especially cereals. Zn is known as an anti-stunting nutrient in preventing growth disorders. In humans, Zn functions in protein synthesis and cellular immunity, contains antioxidants and anti-inflammatory agents (Dhaliwal et al. 2021; Tuiwong et al. 2022). The Zn content in the Ciherang variety of rice, which is widely planted in Indonesia, may usually reach 24.06 ppm (Balai Besar Pengujian Standar Instrumen Padi 2019), far from 60 ppm to be beneficial agronomically and health-wise (Boonchuay et al. 2013). This shows the need for efforts to increase the Zn content in rice in the Ciherang variety.

Several strategies have been suggested to increase Zn concentration in rice, such as conventional breeding, fertilizer management, seed preparation and agronomic biofortification (Talsma et al., 2017; Nissar et al., 2019; Choukri et al., 2022). Agronomically, Zn in plants plays a role in the carbohydrate metabolism process during photosynthesis and the conversion of sugar to starch, in protein metabolism, auxin, and helps maintain membrane integrity in plants (Alloway, 2008). Other functions of Zn include influencing several physiological processes, including enzyme activation in N metabolism, protein synthesis, detoxification of reactive oxygen species, gene expression and regulation, and reproductive development of pollen formation (Phuphong et al., 2018). In addition to the micro element Zn, plants also need nitrogen as a source of nutrition. Nitrogen (N) plays an important role in rice plants, such as increasing plant growth, improving yield levels and grain quality by increasing the number of tillers, expanding the leaf area, the number of panicles, flowering, grain formation, grain filling, and protein synthesis. According to Boonchuay et al. (2013), foliar application of Zn can effectively increase Zn in rice grain.

Combined application of Zn and N increases Zn availability by enhancing the transformation of exchangeable Zn, loose organics, and carbonate-bound from other fractions, N uptake facilitates Zn translocation into grain (Zhao et al., 2016). Ji et al. (2022) reported that there was a clear synergistic effect between Zn and N in root-to-shoot transport in rice plants through increased gene expression. Zn supply also increased the N assimilation rate in rice leaves, further increasing biomass production and yield. In addition, N supply

increased the distribution rate of Zn in brown rice. Application of N and Zn fertilization in rice increased seed germination, seed vigor, grain production, and increased Zn concentration (Hussain et al., 2018; Tuiwong et al., 2022).
Research conducted by Tuiwong et al. (2022), seed priming and fertilization with Zn and urea showed significant results in increasing growth, production, and Zn content in SPT1 rice varieties. This study was also strengthened by Moradi & Siosemardeh (2023), showing that application of priming and foliar spraying increased the Zn content in rice. This provides an idea to evaluate these treatments in finding the best combination of Zn and urea applications in other rice varieties. Therefore, it is necessary to find the best combination of seed priming and spraying with Zn and urea applications to improve the agronomic profile of Ciherang rice which are favored and popular in Indonesia.

Results and Discussion

Plant growth
Based on the plant growth parameters, the priming and spraying treatments of Ciherang rice plants with Zn and urea were not significant on the plant height variable (Table 1). This is supported by the research of Tuiwong et al. (2022), reporting that application of Zn did not significantly affect the height of SPT1 variety of rice. However, in this study, all priming and spraying treatments with Zn and urea significantly affected the number of productive tillers and dry weight of the stover with the best treatment resulting from the combination of priming with a solution of 1% urea + 0.5% $ZnSO_4$ + spraying with 1% urea + 0.5% $ZnSO_4$ with the number of productive tillers of 35 compared to the control with only 27 tillers. The total number of tillers was also significantly influenced by the spraying of 1% urea + 0.5% $ZnSO_4$ and priming with a solution of 1% urea + 0.5% $ZnSO_4$ + spraying with 1% urea + 0.5% $ZnSO_4$. This also reported by Wahid et al. (2022), which stated that the addition of nitrogen regulates the physiological and morphological processes in plants, so that the right dose is needed to produce optimal growth. The effect of giving nitrogen in the form of urea combined with Zn applied by priming or priming + spraying on the total number of tillers and productive tillers and dry weight of the stover was thought to occur because giving Zn to the leaves in the early stages will ensure better plant nutrition at the tiller stage (Tuiwong et al. 2022). Zn played a role in the formation of tryptophan which was a precursor enzyme for auxin, where auxin itself plays a role in cell elongation (Choukri et al., 2022).
The green leaves were increased through spraying with $ZnSO_4$ 75 days after planting (DAP) (Table ***2***). The best treatment in increasing the greenness of Ciherang rice leaves was obtained from spraying treatment with a combination of priming solution of 1% urea + 0.5% $ZnSO_4$ + spraying of 1% urea + 0.5% $ZnSO_4$ with a value of 27.2 cci, compared to control 21.2 cci. The enhanced green coloration of rice leaves likely reflects an increase in chlorophyll content, which may result from improved nitrogen availability and enhanced stress tolerance mechanisms. This finding is consistent with the previous studies reporting that leaf greenness is positively correlated with chlorophyll concentration, photosynthetic efficiency, nutritional status, and ultimately, yield potential in rice and other plant species (Yoshida et al., 1976; Sepaskhah and Karimi-Goghari 2005; Zhao et al., 2016).
An optimal photosynthesis process may cause the increase in leaf greenness. An increase in photosynthesis will increase the amount of glucose converted into energy for plants to form the development of leaves, stems, and roots of plants. The application of Zn and urea has its respective roles which influence the plant growth including leaf greenness. Zn in plants plays an important role in metabolic processes such as protein metabolism and during the synthesis of carbohydrates into starch, while nitrogen for plants has a role in stimulating overall growth and the formation of green leaves (Asmuliani et al., 2021).

Yield
We found that the treatment of Zn and a combination of urea + Zn by priming and spraying had a significant effect, compared to the control, on the total number of grains, filled grains, and the percentage of filled grains per hill of Ciherang rice (Table 3). The best treatment on the variables of the total number of grains and filled grains was the priming treatment of 1% urea + 0.5% $ZnSO_4$ solution + spraying of 1% urea + 0.5% $ZnSO_4$ with a total number of grains per hill of 4,664 and filled grains per hill of 3,710 significantly higher compared to other treatments. It is much higher than the control which only reached 3,711 total grains per hill and 2,766 filled grains per hill. However, the percentage comparison between the number of filled grains to the total number of grains per hill was not better than the spraying treatment of 1% urea + 0.5% $ZnSO_4$ solution, which were 79.5% and 81.6%, respectively. However, to obtain maximum rice production quantity, the priming treatment of 1% urea + 0.5% $ZnSO_4$ solution + spraying of 1% urea + 0.5% $ZnSO_4$ was still better with a difference of 157 grains per hill compared to the spraying treatment of 1% urea + 0.5% $ZnSO_4$ solution.
All treatments did not affect the total number of grains per panicle (Table 4). However, all treatments affected the percentage of filled grains compared to the control. In addition, the treatment of spraying with $ZnSO_4$ and the combination of priming and spraying with $ZnSO_4$ significantly affected the variable of the number of filled grains per panicle. This directly affected the quantity of Ciherang rice production. The treatment with a combination of priming with a solution of 1% urea + 0.5% $ZnSO_4$ + spraying with 1% urea + 0.5% $ZnSO_4$ was the best in increasing the number of filled grains per panicle of Ciherang rice compared to other treatments.
The best treatment for the variable of the number of filled grains was priming with a solution of 1% urea + 0.5% $ZnSO_4$ + spraying with 1% urea + 0.5% $ZnSO_4$ with a value of the number of filled grains per panicle of 108.8, which was significantly different from the control treatment with a value of 103.6 filled grains per panicle. The effect of administering nitrogen in the form of urea combined with Zn applied to rice plants had a significant effect on the production of Ciherang rice. The priming treatment with a combination of 1% urea + 0.5% $ZnSO_4$ + spraying with 1% urea + 0.5% $ZnSO_4$ had a significant effect on the variables of total grains and filled grains per hill and panicle. This is in line with the results of research by Tuiwong et al. (2022), which stated that the effect of administering N and Zn to rice plant seeds and leaves increased the yield of straw and grains. The results of research by Wahid et al. (2022) stated that the application of urea with Zn was most responsive to grain and maximum filling. The application of urea with Zn reduced the presence of unfilled or empty grains in rice plants. This is in line with the research of (Sarwar et al., 2020), which reported that nitrogen fertilizer reduced the number of unfilled grains.
Zn application to leaves at the early stage of maximum tillering is only transported by the xylem to the leaves, where Zn remains until leaf senescence begins, and a small amount of Zn that had been applied was expected to flow into the developing grains to become entire seeds (Saha et al., 2022). In addition, the interaction between Zn and N applications provides the basis for higher yields due to increased N uptake, which increases chlorophyll content (Tuiwong et al., 2022). According to research by Shah et al. (2023), it states that the application of Zn and urea increases grain yields in wheat and rice caused by Zn, which supports pollination through improvements in photosynthesis, sugar transformation, pollen tube development, pollen viability, flowering and grain formation thereby increasing grain content of rice plants. Ghoneim (2016), showed that spraying Zn on rice leaves can increase the yield of grain per hill and per panicle. It will affect the production of rice plants, and the weight of 1000 grains. The increase in grain production was also in line with

Table 1. Effect of seed priming and spraying with Zn and urea on height, number of tillers, number of productive tillers and stalks of Ciherang rice.

Treatment	Plant Height (cm)	Total Number of Tillers	Total Number of Productive Tillers	Dry Weight of Slabs (g)
Priming with distilled water (Control)	65.08	34b	27d	65.2c
Priming with 0.07% $ZnSO_4$ solution	66.54	37ab	32b	86.0b
Priming with 1% urea + 0.5% $ZnSO_4$ solution	66.25	36ab	31bc	86.5ab
Spraying with 0.07% $ZnSO_4$ solution	66.63	35b	31c	77.1bc
Spraying with 1% urea + 0.5% $ZnSO_4$ solution	63.36	39a	32b	89.8ab
Priming with 0.07% $ZnSO_4$ solution + spraying with 0.07% $ZnSO_4$	64.45	36ab	32b	80.3b
Priming with 1% urea + 0.5% $ZnSO_4$ solution + spraying with 1% urea + 0.5% $ZnSO_4$	65.56	39a	35a	100.8a
LSD 0,05	ns	3.25	1.45	11.70

[1]Numbers followed by the same letter are not significantly different based on the LSD 0.05 and ns is not significant.

Table 2. Effect of seed priming and spraying with Zn and urea on the greenness of Ciherang rice leaves.

Treatment	Greenish leaves 75 DAP	
	Before (cci)	After (cci)
Priming with distilled water (Control)	19.3	21.2e
Priming with 0.07% $ZnSO_4$ solution	20.9	21.9de
Priming with 1% urea + 0.5% $ZnSO_4$ solution	19.9	23.6cd
Spraying with 0.07% $ZnSO_4$ solution	22.2	24.3bc
Spraying with 1% urea + 0.5% $ZnSO_4$ solution	23.0	25.8ab
Priming with 0.07% $ZnSO_4$ solution + spraying with 0.07% $ZnSO_4$	22.3	25.3abc
Priming with 1% urea + 0.5% $ZnSO_4$ solution + spraying with 1% urea + 0.5% $ZnSO_4$	23.0	27.2a
LSD 0,05	ns	1.95

[2]Numbers followed by the same letter are not significantly different based on the LSD 0.05 and ns is not significant.

Table 3. Effect of seed priming and spraying with Zn and urea on the total number of grains, filled grains, and percentage of filled grains per hill of Ciherang rice.

Treatment	Total number of grains	Number of filled grains	Percentage of filled grains (%)
Priming with distilled water (Control)	3,711e	2,766d	74.5e
Priming with 0.07% $ZnSO_4$ solution	3,955d	3,125c	79.0bc
Priming with 1% urea + 0.5% $ZnSO_4$ solution	4,201bc	3,246c	77.2d
Spraying with 0.07% $ZnSO_4$ solution	4,119cd	3,199c	77.6cd
Spraying with 1% urea + 0.5% $ZnSO_4$ solution	4,354b	3,553b	81.6a
Priming with 0.07% $ZnSO_4$ solution + spraying with 0.07% $ZnSO_4$	4,160c	3,276c	78.7bcd
Priming with 1% urea + 0.5% $ZnSO_4$ solution + spraying with 1% urea + 0.5% $ZnSO_4$	4,664a	3,710a	79.5b
LSD 0,05	169.9	155.8	1.63

[3]Numbers followed by the same letter are not significantly different based on the LSD 0.05.

Table 4. Effect of seed priming and spraying with Zn and urea on the total number of grains, filled grains, and percentage of filled grains per panicle of Ciherang rice.

Treatment	Total number of grains	Number of filled grains	Percentage of filled grains (%)
Priming with distilled water (Control)	138.9a	103.6bc	74.6e
Priming with 0.07% $ZnSO_4$ solution	127.2d	100.7c	79.1abc
Priming with 1% urea + 0.5% $ZnSO_4$ solution	134.4abc	103.9bc	77.3d
Spraying with 0.07% $ZnSO_4$ solution	136.1ab	106.7ab	78.3cd
Spraying with 1% urea + 0.5% $ZnSO_4$ solution	131.0cd	105.5ab	80.5a
Priming with 0.07% $ZnSO_4$ solution + spraying with 0.07% $ZnSO_4$	133.2bc	104.8b	78.6bcd
Priming with 1% urea + 0.5% $ZnSO_4$ solution + spraying with 1% urea + 0.5% $ZnSO_4$	135.9abc	108.8a	80.0ab
LSD 0,05	4.98	3.29	1.60

[4]Numbers followed by the same letter are not significantly different based on the LSD 0.05.

Table 5. Effect of seed priming and spraying with Zn and urea on the zinc content of Ciherang rice.

Treatment	Zinc content (ppm)
Priming with distilled water (Control)	11.41e
Priming with 0.07% $ZnSO_4$ solution	14.05de
Priming with 1% urea + 0.5% $ZnSO_4$ solution	14.05de
Spraying with 0.07% $ZnSO_4$ solution	16.74d
Spraying with 1% urea + 0.5% $ZnSO_4$ solution	21.40bc
Priming with 0.07% $ZnSO_4$ solution + spraying with 0.07% $ZnSO_4$	23.95b
Priming with 1% urea + 0.5% $ZnSO_4$ solution + spraying with 1% urea + 0.5% $ZnSO_4$	33.50a
LSD 0,05	3.96

[5]Numbers followed by the same letter are not significantly different based on the LSD 0.05.

Table 6. Effect of seed priming and spraying with Zn and urea on the protein content of Ciherang rice.

Treatment	Protein content (%)
Priming with distilled water (Control)	7.82c
Priming with 1% urea + 0.5% $ZnSO_4$ solution	8.48bc
Spraying with 1% urea + 0.5% $ZnSO_4$ solution	10.05a
Priming with 1% urea + 0.5% $ZnSO_4$ solution + spraying with 1% urea + 0.5% $ZnSO_4$	9.50ab
LSD 0,05	1.56

[6]Numbers followed by the same letter are not significantly different based on the LSD 0.05.

Arifiyatun et al. (2016), which stated that fertilization with an optimum dose of NPK 300 kg/ha + 1.75% Zn increases the growth, production, and nutrient absorption of lowland rice.

Zinc content

Spraying of 0.07% $ZnSO_4$ solution, spraying of 1% urea solution + 0.5% $ZnSO_4$, priming of 0.07% $ZnSO_4$ solution + spraying of 0.07% $ZnSO_4$ and priming of 1% urea solution + 0.5% $ZnSO_4$ + spraying of 1% urea + 0.5% $ZnSO_4$ were higher compared to the control, while other treatments were not significantly different (

Table ***5***). The best treatment for the Zn content variable was priming of 1% urea solution + 0.5% $ZnSO_4$ + spraying of 1% urea + 0.5% $ZnSO_4$ with a value of 33.50 ppm which was significantly different from the control treatment with a value of 11.41 ppm. The increase in Zn content was already reported by Shivay et al. (2016), which stated that the provision of Zn can increase the Zn content in rice grain, husks, and straw. The results of the study by Shah et al. (2023) also stated that administering Zn to rice plants increased the concentration and absorption of Zn in rice compared to the control treatment.

Zn treatment was only by priming or spraying application increased Zn content but did not provide the best results as with priming and foliar spraying. This is in line with Tuiwong et al. (2022), which states that Zn application through leaves on plants was an effective way to increase Zn concentration in grains. In addition, spraying a combination of N and Zn carried out at the flowering and seed development stages can increase productivity and Zn accumulation in seeds. This is also in line with Yuan et al. (2013), who stated that Zn accumulation in rice seeds increased significantly with the application of Zn through leaves. Zn application through leaves during flowering also increased Zn levels in rice plants.

The results of research by Shivay et al. (2016) stated that coating N fertilizer with Zn through foliar fertilization increased nutrient availability in basmati rice. This is also in line with the results of research by Shivay et al. (2016), which stated that Zn concentrations in rice, wheat, barley, and corn seeds can be easily increased through adequate Zn fertilization or with agronomic biofortification.

Protein content

The combination of N and Zn spraying can not only increase the Zn content in Ciherang rice, but also increase the protein content. The protein content in Ciherang rice treated with a combination of N and Zn spraying was significantly higher than the control (Table 6). One of the functions of Zn was to influence N metabolism and protein synthesis (Phuphong et al., 2018). The spraying treatment of 1% Urea + 0.5% $ZnSO_4$ solution produced the highest protein content in rice (10.05%) which was not significantly different from the priming treatment of 1% Urea + 0.5% $ZnSO_4$ solution + spraying 1% Urea + 0.5% $ZnSO_4$. Spraying application of N and Zn can be an alternative in an effort to increase the nutritional content of rice. The results of the study showed that the content of Zinc and protein increased after being treated with N and Zn spraying. The increase in Zinc and protein content in Ciherang rice after being treated with N and Zn spraying is very beneficial in efforts to reduce stunting rates and improve nutrition in Indonesia.

The exogenous application of nitrogen and zinc has been demonstrated to significantly enhance the protein content of rice grains through complementary physiological pathways. Nitrogen plays a direct role in the biosynthesis of amino acids and storage proteins, particularly when applied during critical developmental stages (Fageria et al., 2008). Zinc, on the other hand, is essential for the activation of key enzymes involved in nitrogen assimilation and protein metabolism; thereby, improving nitrogen use efficiency and promoting protein synthesis (Cakmak et al., 2004; Hafeez, 2013). When applied in combination, zinc and nitrogen exhibit a synergistic effect that not only enhances grain yield but also improves grain quality, particularly through increased protein accumulation (Ali et al., 2014; Baral et al., 2023).

Materials and Methods

Plant material and experimental design

The experiment was conducted from April to September 2024 in Tegineneng District, Pesawaran Regency, Indonesia, the Seed and Plant Breeding Laboratory, Faculty of Agriculture and Integrated Laboratory and Technology Center of the University of Lampung, University of Lampung. This study used a randomized block design with 7 treatments, namely control with seed soaking using distilled water, priming with 0.07% $ZnSO_4$ solution, priming with a combination solution of 1% urea + 0.5% $ZnSO_4$, spraying with 0.07% $ZnSO_4$, spraying with 1% urea + 0.5% $ZnSO_4$, priming with 0.07% $ZnSO_4$ + spraying with 0.07% $ZnSO_4$, priming with a combination of 1% urea + 0.5% $ZnSO_4$ + spraying with 1% urea + 0.5% $ZnSO_4$. Each treatment was repeated four times as a group and each group consisted of two pot units, so that there was a total of 56 plant units.

Treatments

Making a combination solution of 1% urea + 0.5% Zn, namely by weighing 10 g of urea dissolved in 500 mL of distilled water and making a solution of 0.5% Zn, namely by weighing 5 g of Zn dissolved in 500 mL of distilled water. Then, the two solutions were mixed evenly until they become a homogeneous 1,000 mL solution, with the making of other treatment solutions.

The priming application was carried out by soaking 100 seeds in a control solution (aquades), a solution of 0.07% $ZnSO_4.7H_2O$, and a combination solution of 1% urea + 0.5% $ZnSO_4.7H_2O$. Seed priming was carried out for 24 hours (Tuiwong et al., 2022). Ciherang variety rice seeds with spraying treatment were carried out 4 times, namely in the tillering phase (tillers) applied on the 35th day, the booting stage phase applied on the 45th day (Tuiwong et al., 2022), the flowering phase applied on the 75th day, and one week after application on the 75th day (82 days) (Boonchuay et al., 2013).

Traits measured

Plant growth observations were conducted on plant height (cm) at the ages of 7, 14, 21, 28, and 35 days. The number of tillers per hill was calculated from each sample plant hill by counting the total number of plants in the hill. The results obtained were then reduced by the number of parent plants, which was two plants, then divided by two to obtain the average total number of tillers for one plant. The total number of tillers was calculated when the plants were 11-week-old after planting. The number of productive tillers per hill was calculated based on the number of tillers that produced panicles when the plants were 13-week-old after planting. Leaf greenness (cci) was carried out using the SPDA tool, by taking samples of each plant as many as 3 leaves and each leaf was observed at 3 points on the leaf.

Yield observations were made on the total number of grains per plant by counting all the filled grains and empty grains per harvested rice plant. The number of filled grains per hill was calculated based on the number of grains that felt hard when pressed. The percentage of filled grains was the ratio between the number of filled grains and the total number of grains. The calculation of the number of grains was carried out on each plant per hill and panicle. The weight of 1,000 grains was carried out by weighing the grain at a water content of 14% using a countermatic type seed counter with 10 weighings of 100 grains each.

Zinc content was tested in the Laboratory by bringing samples of Ciherang rice production results. The samples tested were taken from each experimental unit. The seed samples that had been ground with a mass of 1 g were then destructed in a destruction flask. The samples that had been put into the destruction flask were then added with 5 mL of HNO_3 (1:1) and 1.5 mL of HCI (1:1). The samples were destructed using a heavy metal digester at a temperature of 95 for 30 minutes. After the samples were cooled down, they were diluted by adding aquapure until the volume became 50 mL. The sample solution was then filtered using filter paper. Furthermore, the samples were analyzed using the Inductively Coupled Plasma Optical Emission Spectroscopy instrument.

Protein content measurement was carried out by inserting 2 g of homogenate sample into a digestion flask along with 2 catalyst tablets and several boiling stones, then 15 mL of concentrated H_2SO_4 (95% -97%) and 3 mL of H_2O_2 were added slowly and left for 10 minutes in the acid chamber. Furthermore, destruction at a temperature of 4100 °C for ± 2 hours or until the solution was clear, let it stand until it reached room temperature and add 50-75 mL of distilled water. Then the results of the sample destruction were installed in a steam distillation apparatus connected to an Erlenmeyer flask containing 25 mL of 4% H_3BO_3 solution containing an indicator as a distillate container. Then 50-75 mL of sodium hydroxide-thiosulfate solution was added. Distillation and tamping of the distillate in the Erlenmeyer flask until the volume reaches a minimum of 150 mL (the distillate will turn yellow). The distillation results and blanks were titrated using standardized 0.2 N HCl until the color changed from green to neutral gray (natural gray) in duplicate (twice). Calculation of protein content using the formula:

$$PC(\%) = \frac{[(Va - Vb)HCl]\ (NHCl\ x\ 14.007\ x\ 5.95)}{W\ x\ 1000}\ x\ 100\%$$

Where PC is protein content expressed in % units, Va is mL HCl for sample titration, Vb is mL HCl for blank titration, N is normality of standard HCl used, 14.007 is atomic weight of nitrogen, 5.95 is protein conversion factor for rice, and W is sample weight (g).

Statistical analysis

The data were analyzed using variance analysis, then to calculate the differences between treatments, the Least Significant Difference (LSD) test was continued at the α = 0.05 level using RStudio statistics.

Conclusions

The application of a combination of priming urea 1% and $ZnSO_4$ 0.5% followed by spraying urea 1% and $ZnSO_4$ 0.5% proved to be the most effective method in increasing the zinc content of Ciherang rice. This treatment also had a positive impact on increasing plant growth, harvest yields, and protein levels in grain.

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