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The Balance of Some Macro and Micro Fertilizers, The Effect on Nutrient Availability, Chlorophyll, Protein and Its Correlation with Yield Index of Rice in Inceptisols

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Abstract: Rice productivity is highly dependent on optimal soil nutrient availability. A balanced combination of macro and micro fertilizers is expected to improve soil nutrient balance and enhance plant nutrient uptake. This study aimed to determine the most effective combination balance of macro and micro nutrient, on nutrient availability, chlorophyll, protein, and its correlation with rice yield index of rice in Inceptisols. The experiment was conducted in Sroyo, Jaten, Karanganyar, using a randomized complete block design with nine treatments and three replications. The results showed that applying 75% of the recommended N,P,K dose combined with 100% microfertilizer (3 kg ha⁻¹) was the most effective treatment. This combination significantly increased soil nutrient availability, nutrient uptake, chlorophyll content, protein content, and yield index by 22.86% compared to the control group. The increase in yield index was mainly related to higher P, K, and Mn uptake, which improved photosynthetic efficiency and seed filling. Pearson's correlation analysis showed a positive relationship between nutrient uptake and yield index, while stepwise regression identified P, K, and Mn uptake as the main contributing factors. This treatment achieved high nutrient use efficiency by reducing N,P,K fertilizer input by 25% without reducing yield. Therefore, balanced macro- and micronutrient fertilization is an effective strategy for increasing rice productivity in Inceptisols.

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1. Introduction

Rice is a staple food for most of the world's population and the need for it continues to increase along with the increasing human population. Various activities have been made to fill this matter, such as improving irrigation, controlling pests and diseases, land intensification, and fertilization. Fertilization is necessary to meet the nutrient needs in soils with low and unbalanced nutrient levels (Zhang et al., 2021) such as Inceptisols, which are relatively undeveloped (Suryani et al., 2021), low nutrient availability (Romadhon and Hermiyanto, 2021), and including less fertile soil (Maimuna and Aminudin, 2021). Nutrients availability is an requirement for plant metabolism and yield quality (Krusk

et al., 2025). Nitrogen plays a role in photosynthesis because it is one of the components of chlorophyll (Sari, 2024), phosphorus is important for photosynthesis, energy transfer, and nucleic acid synthesis (Putriani et al., 2022), and potassium increases plant resistance to diseases and environmental stresses (Zhang et al., 2021).

Rice farmers in Indonesia often use NPK fertilizers, both single and compound fertilizers, because they are easy to obtain (Benauli, 2021). However, its intensive and continuous use can cause nutrient imbalances including micronutrients (Saidi, 2022). According to (Armita et al., 2022), intensive use of macro fertilizers is one of the factors causing micronutrient deficiencies. Which over the long term can lead to micronutrient deficiencies and reduced fertilization efficiency (Hasani and Zairani, 2021). Most farmers do not apply micro fertilization regularly, so that rice productivity is less than optimal (Sunar et al., 2021). Meanwhile, micronutrient have an important role as enzyme cofactors in physiological processes and plant metabolism (Syamsiyah et al., 2024).

Nitrogen is a macronutrient that serves as a structural component of chlorophyll and plays an essential role in photosynthesis, while zinc (Zn) functions in carbohydrate metabolism by converting sugars into starch, is involved in protein and auxin metabolism, and contributes to the maintenance of membrane integrity in plants (Utami et al., 2018; Sari, 2024). Phosphorus is an essential macronutrient that supports photosynthesis, root development, and the formation of flowers, fruits, and seeds, and functions as a component of metabolites and complex compounds as well as an activator and cofactor of enzymes (Ahmed et al., 2020), while potassium (K) contributes to root development, protein synthesis, disease resistance, and grain filling (Wang et al., 2021), and manganese (Mn) plays a crucial role as an enzyme activator, including enzymes involved in phosphate transfer and the Krebs cycle (Awa and Hambakodu, 2023). Chlorophyll-supported photosynthetic activity not only provides energy and carbon skeletons but also stimulates protein synthesis through enhanced translation initiation (Tcherkez et al., 2020), while a high harvest index indicates that plants efficiently allocate photosynthates to grains, resulting in greater grain yield relative to total biomass (Dinata et al., 2021).

Several studies have shown that the application of micronutrients in Alfisols India significantly increased plant height, leaves number, and leaf area of corn (Anitha and Kadalli, 2019), and improved the quality and protein content of wheat flour in Vertisols in China (Tao et al., 2018). Another researcher (Gomaa et al., 2015), reported that the application of micronutrients with macronutrients in Aridisols in Egypt increased grain yield and yield index, plant growth, physiological properties, crop yields, and value of wheat grains (Saquee et al., 2023), dry grain weight (GKP) (Purnomo, 2016), and rice yield index (Juanda, 2016). Several studies have shown positive results in the addition of micronutrients to balance the soil nutrient levels (Shcatula, 2021). This study aimed to determine the most effective combination balance of macro and micro nutrient, on nutrient availability, chlorophyll, protein, and its correlation with rice yield index of rice in Inceptisols.

2. Materials and Methods

2.1. Location and soil characteristics

The study was conducted in Sroyo, Jaten, Karanganyar, Central Java, Indonesia at (7°32'33" S; 110°52'32" E) from August 2024 - February 2025 with Inceptisols type which has low soil fertility (Suryani et al., 2021) (Table 1).

Table 1. Characteristics of inceptisols in Sroyo

| No. | Parameter | Value | Unit | Classification |
|-----|--------------------------|-------|------------------------|-------------------|
| 1. | pH H ₂ O | 7.67 | - | Slightly Alkaline |
| 2. | C-Organic | 2.14 | % | Currently |
| 3. | N Total | 0.12 | % | Low |
| 4. | P Available | 4.03 | ppm | Very Low |
| 5. | K Available | 0.15 | me 100 g ⁻¹ | Low |
| 6. | Zn Available | 0.32 | ppm | Low |
| 7. | Mn Available | 3.49 | ppm | Enough |
| 8. | Cation Exchange Capacity | 18.06 | me 100 g ⁻¹ | Currently |

Source : Primary Data.

Description : Award of BPSI Tanah dan Pupuk, 2023.

2.2. Experimental design and treatments

The study was a field experiment using a Randomized Completely Block Design (RCBD) with a single factor of 9 treatments repeated 3 times, namely:

Table 2. The dose of fertilizer

| Code | Treatment | Micro Fertilizer/MF kg ha ⁻¹ | NPK kg ha ⁻¹ | | |
|------|--|--|----------------------------|-------|-----|
| | | | Urea | SP-20 | KCl |
| A | Control | 0 | 0 | 0 | 0 |
| B | N,P,K Recommendation (rec) | 0 | 300 | 180 | 100 |
| C | Micro Fertilizer/MF (Zn 3.22%; Fe 4.97%; B 1.13%; Mn 9.04%; Mo 1.56%) | 3 | 0 | 0 | 0 |
| D | 50% N,P,K (rec) + 100% MF | 3 | 150 | 90 | 50 |
| E | 75% N,P,K (rec) + 100% MF | 3 | 225 | 135 | 70 |
| F | 100% N,P,K (rec) + 100% MF | 3 | 300 | 180 | 100 |
| G | 75% N,P,K (rec) + 25% MF | 0.75 | 225 | 135 | 75 |
| H | 75% N,P,K (rec) + 50% MF | 1.5 | 225 | 135 | 75 |
| I | 75% N,P,K (rec) + 75% MF | 2.25 | 225 | 135 | 75 |

Source: Permentan No. 1 2019.

Note: The nutrient contents of mineral fertilizers were as follows: urea (46% N), superphosphate (SP-20) (20% P₂O₅), and potassium chloride (KCl) (60% K₂O).

The land was cultivated according to local farmer's practices. Sunggal variety rice seedlings aged 28 days, were planted in a 3 m x 4 m plot with a planting distance of 25 x 25 cm. Urea fertilizer application is carried out 3 times, namely at planting time or 0 DAP (as much as 50% of the recommendation), at 14 DAP (as much as 25% of the recommendation), and at 28 DAP (as much as 25% of the recommendation), while SP-20 (P) and KCl (K) fertilizer application is carried out at planting time or day 0 DAP. Meanwhile, micro fertilizer applications were given twice at 14 and 28 DAP by mixing it with 100 grams of sand and then spreading it evenly. Soil sampling with a depth of 0-20 cm and plant samples were carried out during the maximum vegetative phase (56 DAP). Soil properties observed include soil pH (H₂O)(BPSI Tanah dan Pupuk, 2023). Total nitrogen using the Kjeldahl method (BPSI Tanah dan Pupuk, 2023). Available phosphorus using the Olsen method (BPSI Tanah dan Pupuk, 2023). Available potassium using the 25% HCl Extraction method (BPSI Tanah dan Pupuk, 2023). Available manganese and zinc using the Wet Digestion Method (BPSI Tanah dan Pupuk, 2023). Organic carbon using the Walkey and Black method (BPSI Tanah dan Pupuk, 2023). Cation Exchange Capacity (CEC) using the Ammonium Acetate Extraction method (BPSI Tanah dan Pupuk, 2023). Chlorophyll content using the equation from Lichtenthaler and Wellburn (1983) (Grzeszczuk et al., 2018). Nitrogen uptake using the Kjeldahl method (BPSI Tanah dan Pupuk, 2023). Phosphorus (P), Potassium (K), Manganese (Mn), and Zinc (Zn) uptake using the wet ashing method (BPSI Tanah dan Pupuk, 2023). Plant parameters include plant biomass weight, dry milled grain weight, rice protein content using the Kjeldahl method (Hernawan and Melyani, 2016) and Yield Index (YI) calculated using the formula by (Hambali and Lubis, 2015).

$$YI = \frac{\text{Dry Weight of Grain}}{\text{Total Dry Weight of Biomass}} \quad (1)$$

Data were analyzed using Analysis of Variance (ANOVA) at the 5% significance level (P < 0.05). Differences among treatment means were compared using Duncan's Multiple Range Test (DMRT) at P < 0.05. Pearson correlation analysis was performed to determine the relationships among observed parameters. In addition, stepwise regression analysis was conducted to identify the parameters that most strongly influenced the Yield Index.

3. Results

3.1. Soil nutrient status

The balanced application of macro and micro fertilizers significantly increased soil total N, available P, exchangeable K, and available Zn and Mn ($P < 0.05$) (Table 3). Among all treatments, the combination of 75% N,P,K (rec) + 3 kg ha⁻¹ micro fertilizer resulted in the highest increases in soil nutrient status, with total N increasing by 125%, available P by 31.85%, exchangeable K by 75%, available Zn by 126.31%, and available Mn by 96.18% compared to the control (Table 4). This higher and more balanced availability of macro and micro nutrients was directly reflected in plant physiological and yield-related responses. Increased total N availability, together with higher available Zn and Mn, supported greater chlorophyll content, which increased by 175.51% under this treatment, as these nutrients play essential roles in chlorophyll synthesis and photosynthetic enzyme activity. Furthermore, the increase in available P and exchangeable K enhanced photosynthate translocation and grain formation, resulting in a 22.86% increase in the yield index. Although several treatments were statistically similar, this combination consistently showed the highest values of soil nutrient availability, chlorophyll content, and yield index, indicating that it was the best treatment based on both statistical analysis and the highest biological performance.

Table 3. Soil nutrient status with various balances of macro and micro fertilizers

| Code | Treatment | Nutrient Availability | | | | |
|------|---|-----------------------|--------------------|------------------------------|--------------------|--------------------|
| | | Total N (%) | P (ppm) | K (me 100 mg ⁻¹) | Zn (ppm) | Mn (ppm) |
| A | Control | 0.16 ^a | 4.05 ^a | 0.16 ^a | 0.38 ^a | 3.93 ^a |
| B | N,P,K Recommendation (rec) | 0.37 ^{cd} | 5.42 ^c | 0.29 ^{de} | 0.42 ^a | 4.19 ^a |
| C | Micro Fertilizers (MF) | 0.19 ^a | 4.10 ^a | 0.18 ^a | 0.82 ^d | 7.16 ^c |
| D | 50% N,P,K (rec) + 3 kg ha ⁻¹ MF | 0.29 ^b | 5.03 ^b | 0.25 ^b | 0.83 ^d | 7.24 ^c |
| E | 75% N,P,K (rec) + 3 kg ha ⁻¹ MF | 0.36 ^{cd} | 5.34 ^{de} | 0.28 ^{cde} | 0.86 ^{de} | 7.71 ^d |
| F | 100% N,P,K (rec) + 3 kg ha ⁻¹ MF | 0.38 ^d | 5.45 ^e | 0.30 ^e | 0.90 ^e | 8.11 ^d |
| G | 75% N,P,K (rec) + 0.75 kg ha ⁻¹ MF | 0.31 ^b | 5.17 ^{bc} | 0.26 ^{bc} | 0.64 ^b | 6.61 ^b |
| H | 75% N,P,K (rec) + 1.5 kg ha ⁻¹ MF | 0.31 ^b | 5.20 ^{cd} | 0.27 ^{bc} | 0.68 ^b | 6.83 ^{bc} |
| I | 75% N,P,K (rec) + 2.25 kg ha ⁻¹ MF | 0.33 ^{bc} | 5.22 ^{cd} | 0.28 ^{bcd} | 0.75 ^c | 6.99 ^{bc} |

Source: Primary Data.

Note: Means followed by the same letter are not significantly different at the 5% significance level (DMRT, $P < 0.05$).

Table 4. Enhancement (%) of soil nutrient status under different macro and micro fertilizer balances relative to the control

| Code | Treatment | Enhancement (%) | | | | |
|------|---|-----------------|-------|-------|--------|--------|
| | | N | P | K | Zn | Mn |
| A | Control | 0 | 0 | 0 | 0 | 0 |
| B | N,P,K Recommendation (rec) | 131.25 | 33.83 | 81.25 | 10.53 | 6.62 |
| C | Micro Fertilizers (MF) | 18.75 | 1.23 | 12.5 | 115.79 | 82.19 |
| D | 50% N,P,K (rec) + 3 kg ha ⁻¹ MF | 81.25 | 23.95 | 56.25 | 118.42 | 84.22 |
| E | 75% N,P,K (rec) + 3 kg ha ⁻¹ MF | 125 | 31.85 | 75 | 126.32 | 96.18 |
| F | 100% N,P,K (rec) + 3 kg ha ⁻¹ MF | 137.5 | 34.57 | 87.5 | 136.84 | 106.36 |
| G | 75% N,P,K (rec) + 0.75 kg ha ⁻¹ MF | 93.75 | 27.65 | 62.5 | 68.42 | 68.19 |
| H | 75% N,P,K (rec) + 1.5 kg ha ⁻¹ MF | 93.75 | 28.40 | 68.75 | 78.95 | 73.79 |
| I | 75% N,P,K (rec) + 2.25 kg ha ⁻¹ MF | 106.25 | 28.89 | 75 | 97.37 | 77.86 |

Source: Primary Data.

3.2. Plant nutrient uptake

The balance of macro and micro fertilizers significantly increased the uptake of N, P, K, Mn, and Zn in rice plants ($P < 0.05$) (Table 5). Application of micro fertilizers alone (C) significantly increased Zn and Mn uptake compared to the control, but did not significantly affect N, P, and K uptake. Application of the recommended N,P,K dose (B) increased the uptake of N, P, and K, but did not

improve Mn and Zn uptake relative to the control. Among all treatments, the combination of 75% N,P,K (rec) + 3 kg ha⁻¹ micro fertilizer (E) resulted in the highest increases in nutrient uptake, reaching 99,94% for N, 80.36% for P, 146.33% for K, 75% for Zn, and 148.21% for Mn compared to the control (Table 6). Although this treatment was statistically similar to B and F, it consistently showed the highest nutrient uptake values, indicating superior nutrient acquisition efficiency and supporting higher growth performance. In contrast, reducing the micro fertilizer dose at the same N,P,K level (E, G, H, I) tended to decrease nutrient uptake. Therefore, the application of 75% N,P,K (rec) combined with 3 kg ha⁻¹ micro fertilizer was identified as the best practice based not only on statistical analysis but also on the highest nutrient uptake performance.

Table 5. Uptake of N, P, K, Mn and Zn with various balances of macro and micro fertilizers

| Code | Treatment | Nutrient Uptake (mg/plant) | | | | |
|------|---|----------------------------|---------------------|----------------------|-------------------|-------------------|
| | | N | P | K | Zn | Mn |
| A | Control | 78.97 ^a | 41.86 ^a | 81.58 ^a | 0.40 ^a | 0.56 ^a |
| B | N,P,K Recommendation (rec) | 160.65 ^e | 77.05 ^f | 210.57 ^{de} | 0.42 ^a | 0.60 ^a |
| C | Micro Fertilizers (MF) | 83.89 ^a | 42.77 ^a | 92.30 ^a | 0.62 ^d | 1.24 ^d |
| D | 50% N,P,K (rec) + 3 kg ha ⁻¹ MF | 128.19 ^b | 53.92 ^b | 143.07 ^b | 0.64 ^d | 1.28 ^d |
| E | 75% N,P,K (rec) + 3 kg ha ⁻¹ MF | 157.90 ^{de} | 75.50 ^e | 200.96 ^{cd} | 0.70 ^e | 1.39 ^e |
| F | 100% N,P,K (rec) + 3 kg ha ⁻¹ MF | 162.77 ^e | 77.72 ^e | 218.40 ^e | 0.73 ^e | 1.45 ^e |
| G | 75% N,P,K (rec) + 0.75 kg ha ⁻¹ MF | 148.02 ^c | 64.86 ^c | 182.76 ^c | 0.49 ^b | 1.06 ^b |
| H | 75% N,P,K (rec) + 1.5 kg ha ⁻¹ MF | 149.23 ^c | 66.90 ^{cd} | 186.17 ^{cd} | 0.54 ^c | 1.14 ^c |
| I | 75% N,P,K (rec) + 2.25 kg ha ⁻¹ MF | 153.15 ^{cd} | 69.31 ^d | 192.36 ^{cd} | 0.57 ^c | 1.23 ^d |

Source: Primary Data.

Note: Means followed by the same letter are not significantly different at the 5% significance level (DMRT, P < 0.05).

Table 6. Enhancement (%) of N, P, K, Mn, and Zn uptake with various balances of macro and micro fertilizers relative to the control

| Code | Treatment | Enhancement (%) | | | | |
|------|---|-----------------|-------|--------|-------|--------|
| | | N | P | K | Zn | Mn |
| A | Control | 0 | 0 | 0 | 0 | 0 |
| B | N,P,K Recommendation (rec) | 103.61 | 84.07 | 158.11 | 5 | 7.14 |
| C | Micro Fertilizers (MF) | 6.32 | 2.17 | 13.14 | 55 | 121.43 |
| D | 50% N,P,K (rec) + 3 kg ha ⁻¹ MF | 62.47 | 28.81 | 75.37 | 60 | 128.57 |
| E | 75% N,P,K (rec) + 3 kg ha ⁻¹ MF | 99.94 | 80.36 | 146.33 | 75 | 148.21 |
| F | 100% N,P,K (rec) + 3 kg ha ⁻¹ MF | 106.30 | 85.67 | 167.71 | 82.50 | 158.93 |
| G | 75% N,P,K (rec) + 0.75 kg ha ⁻¹ MF | 87.60 | 54.95 | 124.03 | 22.50 | 89.29 |
| H | 75% N,P,K (rec) + 1.5 kg ha ⁻¹ MF | 89.14 | 59.82 | 128.21 | 35 | 103.57 |
| I | 75% N,P,K (rec) + 2.25 kg ha ⁻¹ MF | 94.11 | 65.58 | 135.79 | 75 | 119.64 |

Source: Primary Data.

3.3. Chlorophyll, protein and yield index

The combination of macro and micro fertilizers significantly affected chlorophyll content, rice protein content, and yield index (P < 0.05) (Table 7). Application of micro fertilizers alone (C) significantly increased chlorophyll content and protein content compared to the control, however, it did not result in a significant increase in grain weight or yield index. Among all treatments, the application of 75% N,P,K (rec) + 3 kg ha⁻¹ micro fertilizer (E) resulted in the highest increases in chlorophyll content (175.51%), protein content (99.31%), and yield index (22.86%) relative to the control (Table 8). Although this treatment was statistically similar to treatments B and F, it consistently showed the highest values for these physiological and yield related parameters. This indicates that the application of 3 kg ha⁻¹ micro fertilizer enabled a 25% reduction in N,P,K fertilizer without compromising yield performance. Furthermore, at the 75% N,P,K (rec) level, chlorophyll content, protein content, and yield index tended to increase with increasing micro-fertilizer rates (E, G, H, I).

Table 7. Effect of macro and micro fertilizer balance on chlorophyll, protein and yield index

| Code | Treatment | Chlorophyll (g leaf ⁻¹) | Protein (%) | Biomass Weight (kg plot ⁻¹) | Grain Weight (kg plot ⁻¹) | Yield Index (%) |
|------|---|--|--------------------|---|---|-----------------------|
| A | Control | 0.49 ^a | 4.37 ^a | 7.67 ^a | 4.15 ^a | 0.35 ^a |
| B | N,P,K Recommendation (rec) | 1.36 ^f | 8.81 ^f | 10.32 ^c | 7.95 ^f | 0.44 ^c |
| C | Micro Fertilizers (MF) | 0.83 ^b | 5.93 ^b | 9.33 ^b | 5.71 ^b | 0.38 ^b |
| D | 50% N,P,K (rec) + 3 kg ha ⁻¹ MF | 1.24 ^c | 7.35 ^c | 9.60 ^c | 6.49 ^c | 0.40 ^c |
| E | 75% N,P,K (rec) + 3 kg ha ⁻¹ MF | 1.35 ^f | 8.71 ^{ef} | 10.29 ^c | 7.90 ^f | 0.43 ^c |
| F | 100% N,P,K (rec) + 3 kg ha ⁻¹ MF | 1.37 ^f | 9.81 ^f | 10.42 ^c | 8.06 ^f | 0.44 ^c |
| G | 75% N,P,K (rec) + 0.75 kg ha ⁻¹ MF | 1.28 ^d | 7.97 ^d | 9.99 ^d | 7.08 ^d | 0.41 ^d |
| H | 75% N,P,K (rec) + 1.5 kg ha ⁻¹ MF | 1.29 ^{de} | 8.23 ^d | 10 ^d | 7.18 ^{de} | 0.42 ^d |
| I | 75% N,P,K (rec) + 2.25 kg ha ⁻¹ MF | 1.30 ^e | 8.31 ^{de} | 10.06 ^d | 7.33 ^e | 0.42 ^d |

Source: Primary Data.

Note: Means followed by the same letter are not significantly different at the 5% significance level (DMRT, P < 0.05).

Table 8. Enhancement (%) of chlorophyll, protein and yield index with various balances of macro and micro fertilizers relative to the control

| Code | Treatment | Enhancement (%) | | | | |
|------|---|--|----------------|---|---|-----------------------|
| | | Chlorophyll (g leaf ⁻¹) | Protein (%) | Biomass Weight (kg plot ⁻¹) | Grain Weight (kg plot ⁻¹) | Yield Index (%) |
| A | Control | 0 | 0 | 0 | 0 | 0 |
| B | N,P,K Recommendation (rec) | 177.55 | 101.60 | 34.55 | 91.57 | 25.71 |
| C | Micro Fertilizers (MF) | 69.39 | 35.70 | 21.64 | 37.59 | 8.57 |
| D | 50% N,P,K (rec) + 3 kg ha ⁻¹ MF | 153.06 | 68.19 | 25.16 | 56.39 | 14.29 |
| E | 75% N,P,K (rec) + 3 kg ha ⁻¹ MF | 175.51 | 99.31 | 34.16 | 90.36 | 22.86 |
| F | 100% N,P,K (rec) + 3 kg ha ⁻¹ MF | 179.59 | 124.49 | 35.85 | 94.22 | 25.71 |
| G | 75% N,P,K (rec) + 0.75 kg ha ⁻¹ MF | 161.22 | 82.38 | 30.25 | 70.60 | 17.14 |
| H | 75% N,P,K (rec) + 1.5 kg ha ⁻¹ MF | 163.27 | 88.33 | 30.38 | 73.01 | 20 |
| I | 75% N,P,K (rec) + 2.25 kg ha ⁻¹ MF | 165.31 | 90.16 | 31.16 | 76.63 | 20 |

Source: Primary Data.

4. Discussion

4.1. Nutrient status

The application of macro fertilizers combined with micro fertilizers significantly increased soil nutrient availability compared to the initial conditions, as indicated by significant increases in total N, available P, exchangeable K, available Zn, and Mn (P < 0.05). The soil before treatment was classified as having low to very low nutrient status, thus requiring external nutrient inputs to support rice production. Similar improvements in soil nutrient availability following balanced macro and micronutrient fertilization have also been reported in rice cultivation systems (Syamsiyah et al., 2025). Among all treatments, the combination of 75% N,P,K (recommendation) with 3 kg ha⁻¹ of microfertilizer showed the highest and most balanced increase in nutrient availability, although some treatments were not statistically different. Therefore, this treatment was determined to be the best practice based on statistical significance and the magnitude of the increase in nutrient availability. The increase in macro and micro nutrient availability in the combination treatment is in line with the nutrient requirements of rice plants, which reach 17.5 kg N, 3 kg P, and 17 kg K per ton of grain (Badan Penelitian dan Pengembangan Pertanian, 2021), and is supported by the findings of Saquee et al. (2023) who reported that combination fertilization is more effective than single fertilization. Higher nutrient availability also correlates with increased nutrient uptake, as nutrient availability is a major factor determining uptake efficiency by plants (Peniwiratri et al., 2023). Additionally, a 25% reduction in N,P,K dosage balanced with microfertilizer did not significantly reduce nutrient uptake, indicating that a balance of macro and microfertilization can improve fertilization efficiency (Wang et al., 2022).

4.2. Chlorophyll and protein

The balance of nutrients in the combination of macro and micro fertilizers (D, E, F, G, H, and I) significantly increased the absorption of N, P, K, Zn, and Mn, which synergistically supported the formation of chlorophyll and rice protein (Figure 1). This is reinforced by the significant positive correlation between plant nutrient content and chlorophyll and rice protein levels. Among all treatments, the combination of macro and micro fertilizers showed the highest nutrient uptake values, although some treatments were not statistically different, so it can be determined as the best practice based on statistical test results and the magnitude of the physiological response produced. Nitrogen plays an important role in the formation of amino acids such as glutamate and glutamine and as a major component of chlorophyll (Taiz et al., 2015; Pramitasari et al., 2016; Fathi, 2022). Phosphorus plays a role in photosynthesis and protein synthesis through energy transfer (Ahmed et al., 2020), while potassium functions in enzyme activation, protein synthesis, and seed filling (Wang et al., 2021). The microelement Zn acts as an activator of various enzymes involved in protein synthesis, including glutamate dehydrogenase, ATPase, and ribonuclease, and supports auxin synthesis and the activation of chlorophyll forming enzymes (Alloway, 2004; Hassan et al. 2020). Meanwhile, Mn functions as an enzyme cofactor in phosphate transfer reactions, the Krebs cycle, oxidation-reduction reactions, and chlorophyll and carbohydrate formation (Awa and Hambakodu, 2023). The increase in rice protein content due to the application of macro and micro fertilizers is thought to be closely related to the increase in chlorophyll content (Figure 1). Rice varieties with higher chlorophyll content showed higher productivity and protein content (Wei et al., 2022). In addition, chlorophyll plays a role in the absorption of light energy for photosynthesis, which produces carbohydrates that are then converted into amino acids as the basic material for protein synthesis in rice grains (Zhang et al., 2021). These findings are in line with Saquee et al. (2023), who stated that a combination of macro and micro fertilizers can increase nutrient absorption efficiency and crop quality.

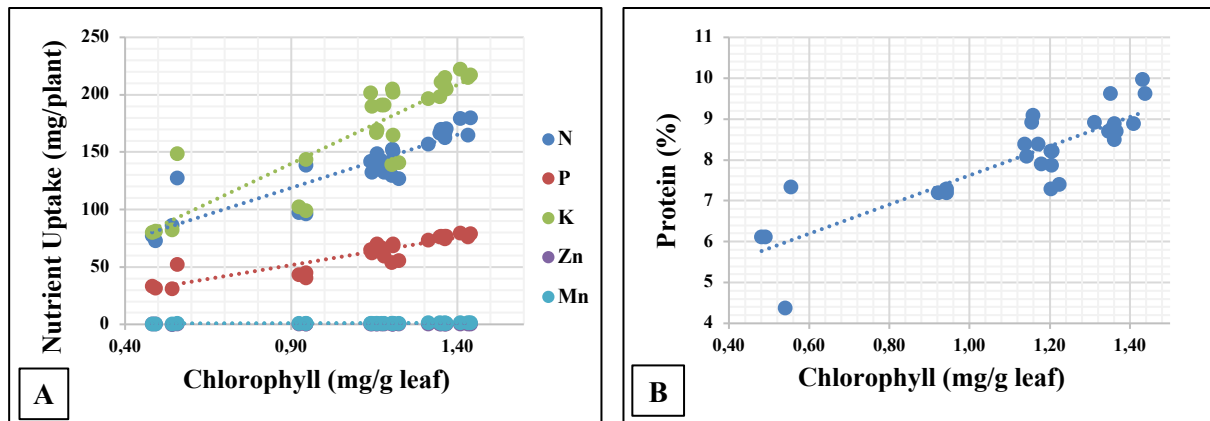


Figure 1. Relationship between N, P, K, Zn, and Mn uptake with chlorophyll (A) and protein (B).

4.3. Yield index

The application of macro and micro fertilizers significantly increased grain weight, plant biomass, and yield index compared to the control ($P < 0.05$) (Table 7). This increase is closely related to higher plant nutrient uptake (Figure 2A). Among all treatments, the combination of 75% N,P,K (recommendation) with microfertilizer (treatment E) showed the highest grain weight, biomass, and yield index values, although statistically not different from some higher N,P,K dose treatments. Therefore, this treatment can be established as a best practice because it can increase yields with more efficient N,P,K use (25% reduction in dosage). Similar yield improvements under integrated macro and micronutrient fertilization have been reported, emphasizing enhanced physiological performance and yield-related traits in rice (Agustina et al., 2024). Increased crop yields are in line with increased macro and micro nutrient uptake. Hartatik et al. (2023) reported that increased crop yields correlate with increased nutrient uptake by plants. Nitrogen, phosphorus, and potassium play an important role in supporting photosynthesis and photosynthate formation (Bojtor et al., 2022). Potassium functions in the translocation and storage of carbohydrates and seed filling, resulting in higher grain yield (Manik, 2020).

The microelements Mn and Zn also contribute through increased photosynthetic efficiency, enzyme activation, and increased leaf chlorophyll content (Seran, 2017).

The results of the Pearson correlation test show that the yield index correlates positively and significantly with N uptake ($r = 0.952^{**}$), P ($r = 0.955^{**}$), K ($r = 0.952^{**}$), Zn ($r = 0.387^*$), and Mn ($r = 0.394^*$), indicating that an increase in the yield index follows an increase in nutrient uptake. However, stepwise regression analysis identified P, Mn, and K uptake as the main factors contributing to the yield index, as shown by the regression equation:

$$Y_1 = 0.2825 + 0.0012X_1 + 0.0123X_2 + 0.0002X_3 \quad (2)$$

The increase in yield index was mainly influenced by P uptake, which plays a role in seed filling and grain formation (Simorangkir and Annisah, 2022), as well as K uptake, which supports panicle formation, grain number, and grain weight (Kurnia et al., 2021). In addition, the increase in yield index is also related to increased chlorophyll content (Figure 2B), which improves photosynthetic efficiency. Gadallah et al. (2022) stated that Mn^{2+} uptake increases photosynthetic efficiency, thereby increasing the accumulation of photosynthates in the yield organs. This finding is in line with Meliawati et al. (2023) who reported that grain weight and total biomass are the main determinants of rice yield index.

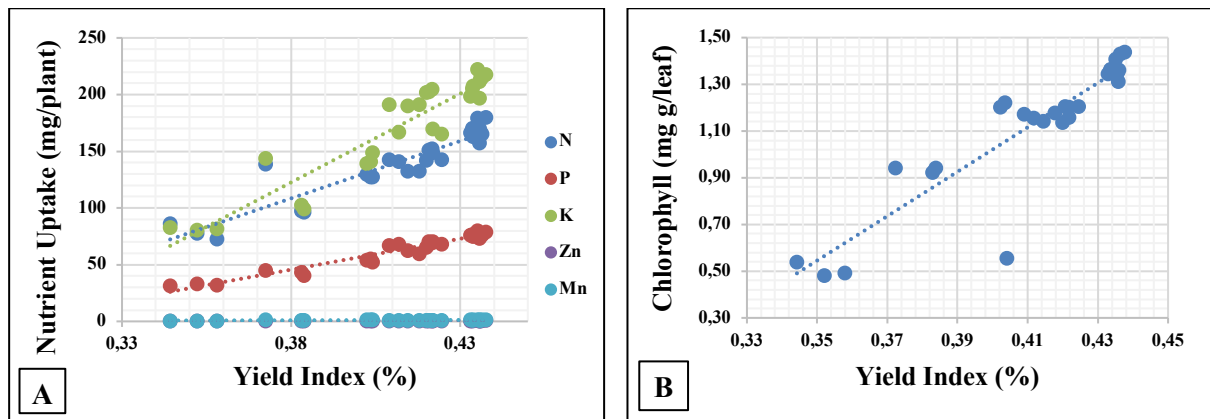


Figure 2. Relationship between yield index with N, P, K, Zn, and Mn uptake (A) and chlorophyll (B).

Conclusion

The balanced application of macro and micro fertilizers has been proven to increase soil nutrient availability (total N, available P, exchangeable K, available Zn, and Mn), plant nutrient uptake, chlorophyll content, rice protein content, and rice yield index compared to the control. Among all treatments, the combination of 75% N,P,K (recommended) with 3 kg ha⁻¹ of micro fertilizer was determined to be the best practice, as it produced high nutrient uptake and yield index and was statistically equivalent to treatments using higher N,P,K doses. These findings indicate that N,P,K fertilizer doses can be reduced by up to 25% without decreasing rice productivity if balanced with adequate microfertilizer application. Thus, nutrient balance between macro and microelements is a key factor in improving fertilizer efficiency and maintaining sustainable rice yields.

Ethical Statement

Ethical approval is not required for this study.

Conflict of Interest

The authors declare that there are no conflicts of interest.

Artificial Intelligence Declaration

The authors acknowledge the limited use of artificial intelligence–assisted tools (e.g., Scopus AI) for literature retrieval, language editing, and minor drafting support. No artificial intelligence tools were used for data analysis, result interpretation, or scientific decision-making. The authors take full responsibility for the originality, accuracy, and integrity of the manuscript.

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Author Contributions

A1 conceived and designed the study and served as the principal investigator. A2 and A3 contributed to the supervision of the research methodology and provided academic guidance throughout the study. A4 and A5 conducted the field investigation, collected and analyzed the data, and prepared the original draft of the manuscript. A6 and A7 contributed to critical review, evaluation, and revision of the manuscript. All authors have read and approved the final version of the manuscript.

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