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Increasing Fe Content in Rice Plants with the Application Liquid Fertilizer of *Moringa oleifera* and Golden Snail

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***Abstract*—Increasing iron (Fe) content in rice is needed because Fe is a critical mineral that plays a crucial role in body metabolisms, such as a form of hemoglobin, antibody production, and a catalyst for several compounds. Meanwhile, the Fe absorption by rice plants is relatively low. *Moringa oleifera* and golden snail contain high Fe, potentially liquid fertilizer ingredients. This research aims to increase Fe uptake and Fe content of rice plants by application of liquid fertilizer of *Moringa oleifera* and golden snail. The study used a completely randomized design with two factors. The first factor is the composition of liquid organic fertilizer (P), six levels (P0: without fertilizer, P1: fresh extract of Moringa leaf, P2: fermented Moringa leaf, P3: fresh extract of Golden snail, P4: fermented Golden snail, P5: Mixed 1:1 by volume fermented of Moringa leaf and golden snail). The second factor is liquid fertilizer concentrations (K), which are four levels (K1: 2%, K2: 4%, K3: 6%, and K4: 8% concentration). Repetition of treatment three times. The results showed that the treatment affected increasing fresh weight of the plant, dry weight of the plant, 100 grain weight, number of leaves, total chlorophyll, and Fe content. The fermented mixture of *Moringa* and golden snail at 4% concentration increased the Fe available in the soil by 6,677% or 4,788% higher than the control. The fermented Moringa leaf with an 8% concentration increased Fe in rice, which was 8,165% or 30.50% higher than the control.**

***Keywords*— Iron (Fe); paddy; liquid organic fertilizer.**

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I. Introduction

Fe deficiency is the most common deficiency case in the world [1]. The element Fe is part of the hemoglobin molecule; with reduced Fe, hemoglobin synthesis will decrease, decreasing hemoglobin levels [2]. Hemoglobin is vital for the human body because low hemoglobin levels affect the ability to deliver O_2, which all body tissues need [3]. Fe is needed every day to replace Fe lost from the body, and this growth varies depending on age and gender. Needs increase in infants, adolescents, pregnant, lactating women, and menstruating women [4]. Fe deficiency is the leading cause of anemia [5]. This anemia affects more than a third of the world's population which has very detrimental health and severe social impacts. Therefore, efforts are needed to overcome these problems by enriching staple foods with micro-nutrients through plant breeding strategies or agronomy, commonly called biofortification [6]. Micronutrient requirements for Fe is 9-27 mg/person/day, respectively [7]. To meet these needs, the minimum consumed rice contains Fe 26.1-78.2 ppm. The micronutrient content of Fe in rice was only around 9.4-16.2 (average 11.68) ppm. Rice will be combined with liquid organic fertilizer from Moringa leaf extract to increase the Fe content. Moringa leaf extract is known to have a crude protein content of 30.3% and 19 amino acids. In addition, it also contains the following minerals: calcium (3.65%), phosphorus (0.3%), magnesium (0.5%), potassium (1.5%), sodium (0.164%), sulfur (0. 63%), zinc (13.03 mg/kg), copper (8.25%), manganese (86.8 mg/kg), iron (490 mg/kg) and selenium (363 mg/kg) [8]. Conversion in 100 g of research material obtained in young Moringa leaves contains 497.8 mg/100g of calcium and 6.24 mg/100g of iron [9]. Biofortification of rice with Moringa leaf extract and golden snail is expected to increase the availability of Fe in rice. Increasing the Fe content in rice will increase Fe intake for consumers, improving their health status. The purpose of this study was to determine which treatment can increase the availability of Fe for rice plants.

II. MATERIALS AND METHOD

The research was conducted at the Greenhouse of the Disease Pest Control Laboratory Mojolaban, Soil Chemistry Laboratory of Universitas Sebelas Maret, Plant Physiology Laboratory of Universitas Sebelas Maret, and Laboratory of Universitas Gadjah Mada. The research period runs from March 2021 to May 2022.

A. Materials and equipment

The material used in this study was menthik wangi rice seeds obtained from organic rice farmers in Karanganyar, Central Java. The fresh Moringa leaves are harvested from the bottom third branch of the plant. The soil was obtained from Gentungan Village, Mojogedang, Karanganyar, with Inceptisols soil type, taken on a topsoil layer (30 cm) using organic cultivation since 2009. The tools used in this study were planter bags, rulers, sprayers, blenders, boilers, furnaces, plain filter paper, 300 mesh linen sieves, and fermentation devices.

B. Experimental design

The experimental design was factorial with Completely Randomized Design as the basic design, consisting of 2 factors: liquid organic fertilizer (P0: no liquid organic fertilizer, P1: fresh extract of moringa leaf, P2: fermented moringa leaf, P3: fresh extract of golden snail, P4: fermented golden snail, P5: Mixed 1:1 by volume fermented of Moringa leaf and golden snail) and concentrations (K1: 2% concentration, K2: 4% concentration, K3: 6% concentration, K4: 8% concentration) obtained 20 treatment combinations, each repeated 3 times, so there are 60 experimental units.

C. Procedure

Liquid fertilizer from Moringa leaf is made by grinding 1 kg of moringa leaves and mixing them with 2 liters of water, 2 liters of rice washing water, and 50 ml of molasses. Cover the container with the lid. Stir the ingredients again every day. Liquid fertilizer can be used if it has passed 10-14 days. The soil used for planting media was dried and then filtered using a multilevel sieve. It then weighed as much as 31 kg/planterbag. Rice planting is carried out by planting 1 rice seed in each planting hole. The seeds that have been planted are then covered with husk ash to maintain a balance of temperature and soil moisture. Embroidery is done if there are seeds that do not grow. Harvesting is done when the plant enters the vegetative phase; the maximum is 120 DAP.

D. Parameters of laboratory observation and analysis

Parameters observed were plant height, number of leaves, fresh weight of the plant, dry weight of the plant, 100-grain weight, Fe available in the soil, Fe plant, and Fe rice.

E. Data analysis

Data were statistically analyzed using ANOVA (95% significance level) followed by the Duncan Multiple Range Test (DMRT) (95% significance level). A correlation test is carried out to determine the relationship between the observed parameters.

III. RESULTS AND DISCUSSION

A. Growth Parameters

1) Plant height: Based on the results of ANOVA, the application of liquid organic fertilizer, the difference in the concentration of liquid organic fertilizer, and the interaction of the two did not significantly affect plant height ($p>0.05$). The results of the data analysis of rice plant height, as listed in Table 1. It shows that the value of F Count < F Table. Duncan's 5% analysis showed that applying liquid organic fertilizer with concentrations of 2%, 4%, 6%, and 8% gave no significant difference. This means that applying liquid organic fertilizer from each treatment concentration does not affect the height of the rice plant. The increase in plant height was initially slow, then gradually became faster until a maximum stem elongation rate was reached, namely at the eighth week, and finally, the stem elongation rate was constant until the eleventh week. The study's results, which ranged from 65,333 to 86,000 cm, showed a value that was not significantly different from the height of the rice plant. This is reinforced by the Duncan test, which shows a significant difference in each application of liquid fertilizer. Applying liquid organic fertilizer containing elements of N, P, K, Mg, and Ca will stimulate the synthesis and division of anticlinal cell walls to accelerate the increase in plant height [11].

TABLE I

DATA ON AVERAGE PLANT HEIGHT, NUMBER OF LEAVES, WEIGHT OF 100 SEEDS, FRESH WEIGHT OF PLANT, DRY WEIGHT OF PLANT AND TOTAL CHLOROPHYLL IN RICE PLANTS

Sample	Plant Height (cm)	Number of Leaves (strands)	Weight of 100 Grain (g)	Fresh Weight of Plant (g)	Dry Weight of Plant (g)	Total Chlorophyll
Control	76.750	15.333 abcdefg	22.375abcde	17.000abcd	8.204abc	0.511abc
P1K1	73.667	21.333 bcdefg	21.667a	19.333abcd	12.309abc	0.571abc
P1K2	72.333	12.667 abcd	22.533abcdef	10.667ab	5.376a	0.547abc
P1K3	77.667	24.000 efg	22.933bcdefg	21.667abcd	10.715abc	0.527abc
P1K4	67.000	27.000 g	23.900g	23.333abcd	11.594abc	0.440ab
P2K1	81.333	20.333 bcdefg	23.633fg	26.667bcd	11.819abc	0.427a
P2K2	76.333	22.333 cdefg	22.400abcde	20.333abcd	9.744abc	0.596bc
P2K3	72.333	15.000 abcdef	25.900h	17.000abcd	8.420abc	0.530abc
P2K4	80.333	17.333 abcdefg	23.200cdefg	12.000ab	5.579a	0.542abc
P3K1	79.667	21.667bcdefg	21.700a	19.000abcd	8.921abc	0.516abc
P3K2	85.667	22.000 bcdefg	23.333defg	27.667bcd	12.115abc	0.522abc
P3K3	71.000	19.333 bcdefg	22.100abc	14.667abcd	7.496abc	0.429a
P3K4	72.000	8.000 a	22.100abc	7.000a	4.330a	0.501abc
P4K1	71.000	11.333 ab	22.767abcdefg	12.000ab	5.908ab	0.503abc

Sample	Plant Height (cm)	Number of Leaves (strands)	Weight of 100 Grain (g)	Fresh Weight of Plant (g)	Dry Weight of Plant (g)	Total Chlorophyll
P4K2	72.333	21.333 bcdefg	22.300abcde	14.667abcd	7.617abc	0.541abc
P4K3	86.000	25.333 fg	22.333abcde	30.000cd	14.250c	0.554abc
P4K4	78.333	23.000 defg	23.467efg	21.000abcd	11.287abc	0.475abc
P5K1	75.333	22.000 bcdefg	21.667a	25.000abcd	11.574abc	0.465abc
P5K2	65.333	21.000 bcdefg	21.767a	15.333abcd	7.890abc	0.427a
P5K3	76.667	22.333 cdefg	22.333abcde	21.333abcd	9.776abc	0.531abc
P5K4	70.000	16.333 abcdefg	22.867abcdefg	13.333abc	6.063ab	0.600c

Notes: P1: fresh extract of moringa leaf, P2: fermented moringa leaf, P3: fresh extract of golden snail, P4: fermented golden snail, P5: mixed 1:1 by volume fermented of moringa leaf and golden snail; K1: 2% concentration, K2: 4% concentration, K3: 6% concentration, K4: 8% concentration.

2) Number of leaves: Based on ANOVA, liquid organic fertilizer and fertilizer concentration did not significantly affect the number of leaves ($p>0.05$), but the interaction between liquid organic fertilizer and fertilizer concentration significantly affected the number of leaves ($p<0.05$). Based on Table 1, the administration of Moringa extract + 8% concentration (P1K4) increased the number of leaves by 27 strands or 44,44% higher than the control. This increase in the number of rice leaves is thought to suggest that Moringa extract can cause the cells at the tip of the stem to be stimulated or accelerated to conduct cell division and enlargement immediately, especially in the meristematic area. Periclinal division and enlargement of meristematic cells at the tip of the stem, although the rates were not the same.

3) 100 grain Weight: Based on ANOVA, liquid organic fertilizer, fertilizer concentration, and their interaction also significantly affected the weight of 100 seeds ($p<0.05$). Based on Table 1, moringa fermentation + 6% concentration (P2K3) increased the weight of 100 seeds by 25, 90 g or 13,62% higher than the control. This is because the given moringa fermentation is able to stimulate metabolism in rice plants. Nitrogen contained in Moringa fermentation is a protein constituent [12]. The content of phosphorus and calcium in *Moringa* plays a role in stimulating meristem tissue division root growth and leaf development [13]. This causes the level of absorption of nutrients and water by plants to its optimum limit, which will be used for cell division, elongation, and differentiation. Potassium regulates the activity of opening and closing stomata [14]. The optimal arrangement of stomata will control plant transpiration and increase the reduction of carbon dioxide converted into carbohydrates. Nutrients nitrogen, phosphorus, potassium, and microelements contained in moringa fermentation will increase plants' photosynthetic activity, thereby increasing the carbohydrates produced as food reserves [15].

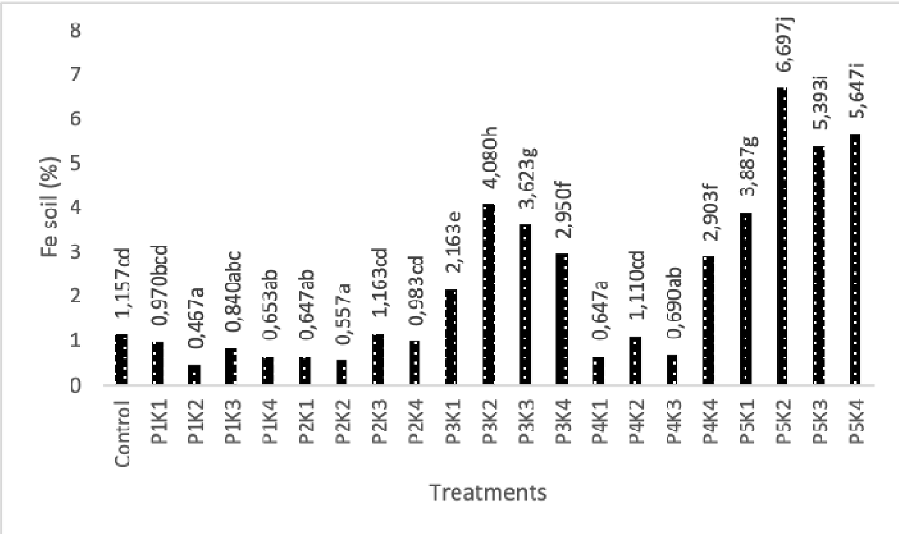
4) Fresh weight of plant: Based on the ANOVA, liquid organic fertilizer and fertilizer concentration had no significant effect on wet plant weight ($p>0.05$), but the interaction between liquid organic fertilizer and fertilizer concentration significantly affected wet plant weight ($p<0.05$). Based on Table 1, golden snail fermentation + 6% concentration (P4K3) increased the wet plant weight by 30.00 g or 43,33% higher than the control. The wet weight of the plant is the weight of the plant when the plant is still alive and is weighed directly after harvest before the plant wilts due to water loss [16]. The response of rice plants to the giving of golden snail fermentation gave an increased yield at a concentration of 6% (30 g). The response of rice plants in terms of wet plant weight to liquid organic fertilizer fertilization is in line with the conditions of growth and development of the number of leaves. The number of leaves accompanied by green leaves indicates the presence of chlorophyll content which can produce photosynthate for plant growth and development, ultimately affecting the wet weight of plant rice.

5) Dry weight of plant: Based on ANOVA, liquid organic fertilizer and fertilizer concentration had no significant effect on dry plant weight ($p>0.05$), but the interaction between liquid organic fertilizer and fertilizer concentration had a significant effect on dry plant weight ($p<0.05$). The dry weight of the plant rice results from three processes: the accumulation of assimilation through photosynthesis, the decrease of assimilate due to respiration, and the accumulation of the food reserves. Based on Table 1, golden snail fermentation + 6% concentration (P4K3) increased the wet plant weight by 14.25 g or 42.45% higher than the control. It indicates that the dry weight of plant rice is the balance between CO_2 uptake (photosynthesis) and CO_2 expenditure (respiration) [17]. If respiration is greater than photosynthesis, the plant will decrease its dry plant weight [18]. Liquid organic fertilizer contains potassium and calcium nutrients, which will increase the growth and development of lateral roots, thus affecting the ability of rice plants to absorb water. This causes rice plants with different treatments to absorb different amounts of water, evaporating during drying.

*6) Total chlorophyll: 4f*Based on the ANOVA, liquid organic fertilizer, fertilizer concentration, and their interaction also significantly affected total chlorophyll ($p<0.05$). Based on Table 1, applying a mixture of *Moringa* and golden snail fermentation + 8% concentration (P5K4) increased the total chlorophyll by 6.00 or 17.41 % higher than the control. The difference in the rate of growth and activity of the meristematic tissue that is not the same will cause differences in the rate of formation that are not the same in the formed organs [19]. In addition, the application of liquid organic fertilizer with complete nutrient content will cause a different synthetic growth rate. Liquid organic fertilizers, besides containing nitrogen, are composed of all proteins, nucleic acids and chlorophyll, they also contain micro-nutrients, including Mn, Zn, Fe, S, B, Ca and Mg elements [20]. These micronutrients act as catalysts in the process of protein synthesis and the formation of chlorophyll. Protein is the main constituent of protoplasm which functions as the center of metabolic processes in plants which in turn will stimulate cell division and elongation. Nitrogen nutrients and micro nutrients act as constituents of chlorophyll so that increasing photosynthetic activity will produce photosynthate, which results in the development of leaf meristematic tissue [21].

B. *Effect of Treatment on Fe Content*

1) Fe soil: Based on the ANOVA, liquid organic fertilizer, fertilizer concentration, and their interaction significantly affected the available Fe in the soil ($p<0.05$). The following is a graph of the available Fe in the soil (Fig. 1).



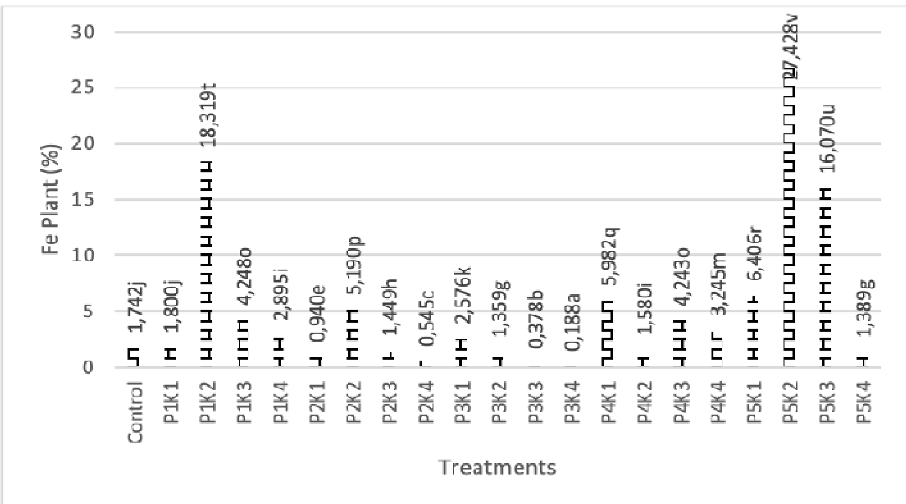
Notes:

P1: fresh extract of moringa leaf, P2: fermented moringa leaf, P3: fresh extract of golden snail, P4: fermented golden snail, P5: mixed 1:1 by volume fermented of moringa leaf and golden snail; K1: 2% concentration, K2: 4% concentration, K3: 6% concentration, K4: 8% concentration.

Fig. 1 Fe soil

Fe available in the soil in the P5K2 treatment (a mixture of fermented *Moringa* and golden snail + 4%) had the highest mean of 6.677% or 4.788% higher than the control (Fig. 1). This shows that the addition of moringa fermentation can increase the available Fe in the soil so that it can increase the Fe absorbed by plants. The amount of Fe given will affect Fe levels in plants. In the fermentation process, many important elements in the form of complex and simple compounds will be released into the fermentation solution [22]. This is because fermented Moringa leaves and golden snails are rich in zeatin, cytokinin, ascorbate, phenolics, and minerals such as Ca, K, and Fe, which can trigger plant growth. Cytokinin are plant hormones that induce cell division and growth, promote new cell growth, and delay cell aging [23]. Zeatin is a strong antioxidant with anti-aging properties. Given its nutritional content, Moringa leaf extract is the best organic fertilizer for all types of plants. So, *Moringa* leaves can be used as liquid fertilizer [24], an effect of giving Moringa leaf liquid organic fertilizer on the growth of pakchoy (*Brassica rapa* L.). The results showed that applying 4% concentration of liquid organic fertilizer Moringa leaf extract positively affected each growth parameter.

2) Fe plant: Based on the results of ANOVA, liquid organic fertilizer, fertilizer concentration, and their interaction significantly affected the Fe plant ($p<0.05$). The following is a graph of Fe plant (Fig. 2).

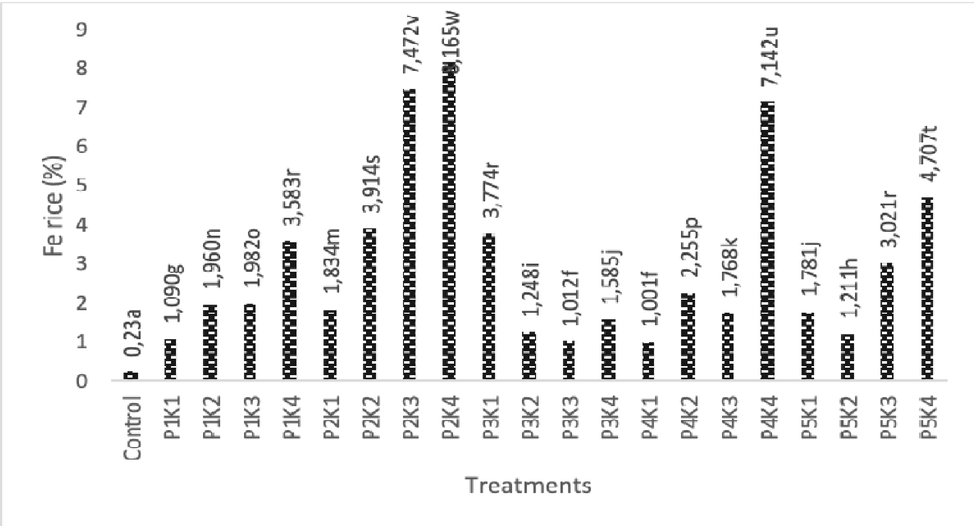


Notes:
P1: fresh extract of moringa leaf, P2: fermented moringa leaf, P3: fresh extract of golden snail, P4: fermented golden snail, P5: mixed 1:1 by volume fermented of moringa leaf and golden snail; K1: 2% concentration, K2: 4% concentration, K3: 6% concentration, K4: 8% concentration.

Fig. 2 Fe plant

Based on Fig.2, the highest Fe plant was in the P5K2 treatment with a mixture of fermented *Moringa* and golden snail + 4%), which was 27.42% or 90.25% higher than the control. A mixture of fermented moringa leaves and golden snails + 4% can be used to accelerate plant growth by spraying on the leaves. As a weathering process, fermented organic matter will undergo physical and chemical changes due to microbial activity [25]. Physical changes are indicated by the destruction of tissue and cell materials, and this will be followed by chemical changes which are characterized by an increase in the elemental content in the fermented solution. *Moringa* contains many vitamins such as C, B, and A, riboflavin, pyridoxine, folic acid, beta-carotene, ascorbic acid,, nicotinic acid, alpha-tocopherol, with high mineral content for iron and calcium in order to a major source of essential amino acids [26]. Moringa leaves were used as liquid fertilizer which was tested on various plants, namely peanuts, soybeans, and corn [27] [28]. The results were very significant in the yield of plants that were given moringa leaf liquid fertilizer, which was 20-35% greater than those without liquid fertilizer. The results of Moringa leaf extract (MLE) used on safflower plants show that it can increase the antioxidant content [29].

3) Fe rice: Based on the results of ANOVA, liquid organic fertilizer, fertilizer concentration, and their interaction significantly affected Fe rice ($p<0.05$). The following is a graph of Fe rice (Fig. 3).



Notes:
P1: fresh extract of moringa leaf, P2: fermented moringa leaf, P3: fresh extract of golden snail, P4: fermented golden snail, P5: mixed 1:1 by volume fermented of moringa leaf and golden snail; K1: 2% concentration, K2: 4% concentration, K3: 6% concentration, K4: 8% concentration.

Fig. 3 Fe Rice

Based on Fig. 3, the highest Fe rice in the P2K4 treatment (fermented moringa leaf + 8% concentration) was 8.165% or 30.50% higher than the control. This is presumably because adding fermented moringa leaf with 8% concentration increased the Fe available in the soil, so the Fe plant also increased. Moringa leaves contain 12.5% carbohydrates, 7% protein, vitamins A, B1, B2, B6 19%, C 12%, calcium, potassium, and various other minerals. While in dry conditions, moringa leaves have a protein content of up to 27%. Moringa leaf extract contains hormones that can increase plant growth, namely the cytokinin hormone. The mixture of moringa leaf extract uses variations in concentration differences [30]. The best treatment for giving liquid organic fertilizer to plants is in a 4% concentration moringa leaf extract. Utilization of Moringa leaf extract is an organic liquid fertilizer on the performance of apple plants [31]. This treatment gave the best results for each observation parameter: the number of leaves, plant height, fresh weight of the plant, and dry weight.

IV. Conclusions

The application of liquid organic fertilizer and the difference in liquid organic fertilizer concentration significantly increased the number of leaves, wet plant weight, dry plant weight, 100 seeds, and total chlorophyll. The P5K2 treatment (a mixture of fermented *Moringa* and golden snail + 4%) increased the available Fe in the soil by 6.677% or 4.788% higher than the control. P2K4 treatment (fermented moringa leaf + 8% concentration) was able to increase Fe in rice, 8,165% or 30.50% higher than control.

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