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Methanol Application and Soil Water Content: Increased Production and Cultivation of Soybean Plants

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Abstract: Soybean cultivation can increase its production by providing methanol and groundwater. This research aims to analyze the application of methanol, soil water content, and the interaction between methanol and soil water content to increase soybean production. The research was conducted from June to October 2022 in Pangkajene District, Pangkep Regency, South Sulawesi, Indonesia. The research used a split-plot design, and each treatment was repeated three times. The first plot is the level of water content, which consists of three levels: A1: 25–50%, A2: 50–75%, and A3: 75–100%, while the subplot is the methanol concentration, which consists of four levels: Mo: no methanol, M1: 15% methanol, M2: 30% methanol, and M3: 45% methanol. The research results show that soil water levels of 75-100% and methanol 30% have a good influence on growth and production components. Providing methanol can increase water use efficiency.

Keywords: methanol application; groundwater use; production; soybean cultivation

甲醇施用和土壤含水量：提高大豆产量和种植面积

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摘要:

大豆种植可以通过提供甲醇和地下水来提高产量。本研究旨在分析甲醇的应用、土壤含水量以及甲醇与土壤含水量之间的相互作用以提高大豆产量。该研究于2022年6月至10月在印度尼西亚南苏拉威西省邦格县庞卡杰内区进行。该研究采用裂区设计，每个处理重复3次。第一个图是含水量水平，由三个水平组成：A1：25-50%、A2：50-75%、A3：75-100%，而次图是甲醇浓度，由四个水平组成含量：Mo：无甲醇，M1：15%甲醇，M2：30%甲醇，以及M3：45%甲醇。研究结果表明，土壤含水量75-100%、甲醇30%对生长和生产成分有良好的影响。提供甲醇可以提高水的利用效率。

关键词：甲醇应用；地下水的利用；生产; 大豆种植

1 Introduction

Soybean (Glycine max (L) Merrill) is an annual crop that has long been cultivated and known in Indonesia. The soybean plant is classified as a traditional secondary crop, which has changed from a side crop to a strategic crop in the economy because the demand for soybeans as a raw material for industry is increasing[1–3].

Soy is a food that has a high nutritional value; therefore, it can be used to improve people's nutrition because it is cheap and affordable for all levels of society[4,5]. In addition, soybeans are also easy to find in everyday life. This is because soybeans are a type of plant that can grow and develop well in tropical areas, including Indonesia. This makes soybeans very popular in Indonesia[6,7].

Soybean seeds contain 30%–50% protein and 25%–30% fat, so they are an important food ingredient; thus, efforts to increase soybean production need attention[8]. Furthermore, soybeans are used as food, and the slightly dried leaves and stems can also be used as animal feed and green manure[9-11].

The rate of increase in soybean production from year to year programed by the government has shown encouraging results but is still unable to keep up with the rate of increase in domestic soybean demand[12]. Furthermore, the government continues to prioritize soybeans in agricultural development toward food self-sufficiency to reduce the rate of increase in imports, which have so far absorbed quite large amounts of foreign exchange so that the prospects for increasing production per hectare and expanding the planting area still need to be improved[13]. In 1997, Indonesian soybean production only reached 1,356,891 tons with an average productivity of 1.2 tons per hectare[39]. However, the average annual production achieved is still below the productivity of soybean-producing countries such as the United States (17.0 quintals per hectare) and Canada (19.8 quintals per hectare). In addition, productivity is still far below the potential yield of national superior varieties, namely 16.0-20.0 quintals per hectare[14].

Indonesia's soybean production is still low because it is influenced by the fact that sufficient water is not always available during the plant growth, and the land fertility lowers day by day[15]. This occurs because water availability is very dependent on rainfall. In addition, water problems can be influenced by climatic conditions and the number of rainy periods or wet periods that are relatively narrow or small, especially if the soybean development area is emphasized in areas that do not have irrigation[16–18].

Soybean planting in such land conditions often fails due to drought or excess water when planted at the beginning or end of the rainy season. In a study, it was found that, from the results of experiments carried out, soybeans planted in the dry season with sufficient irrigation could produce an average of 1.97 tons per hectare, whereas in the rainy season, they produced only 0.61 tons per hectare at the same planting location.

Low soybean production is related to low photosynthesis rates[19,20], because high radiation intensity in tropical areas causes high photorespiration rates, especially for C_3 group plants such as soybeans; therefore, low production and productivity cannot be separated from the carbon dioxide (CO_2) cycle in photosynthesis[21,22]. In general, the C_3 plant group has a lower net photosynthesis rate than the C_3 plant group. The low level of net photosynthesis in the C_3 plant group is due to intense competition between CO_2 and O_2 in using ribulose bisphosphate (RuBP). Use of RuBP by oxygen with the help of the rubisco enzyme results in the release of CO_2 back into the atmosphere, and the rate of CO_2 fixation in photosynthesis decreases and indirectly reduces plant production and productivity.

Many efforts have been made to increase the production, but the results obtained have not been as desired. Research on the use of methanol in C_3 plants in recent years has received considerable attention among plant physiologists[23] who reported that the use of methanol in C_3 plants can increase the production by up to 100% when grown in areas receiving high-intensity sunlight[24,25]. Methanol is a short-chain carbon compound that has a lower polarity than water, is easily absorbed by plants, and easily decomposes into CO_2 in plant leaves. Thus, it is likely that CO_2 levels in leaf chlorophyll will increase, resulting in increased plant photosynthesis rates, which in turn can increase production.

The urgency of this research is to build a breakthrough innovation in soybean production technology by conducting trials combining the use of methanol concentration for soybean growth and production at various levels of soil water content, which has not been widely used in Indonesia. Furthermore, this research can reduce Indonesia’s dependence on importing soybeans from abroad. Indonesia is currently still importing 2.6 million tons of soybeans from abroad. Seeing this, Indonesia should increase domestic soybean production to meet the public’s need for soybeans. Increasing soybean production in Indonesia will help the economic sector, especially farmers.

2 Data and Methods

2.1 Research Design

This research uses a quantitative experimental design approach carried out in Pangkajene District, Pangkep Regency, South Sulawesi. This study was conducted from June to October 2022. The research used a split-plot design, and each treatment was repeated three times[26]. The first plot is the water content level, which consists of three levels: A1: 25-50%, A2: 50-75%, and A3: 75-100%, while the subplot is the methanol concentration, which consists of four levels: Mo: without methanol, M1: 15% methanol, M2: 30% methanol, and M3: 45% methanol (basic considerations for using methanol).

The following is a flow diagram for determining the sample for this research:

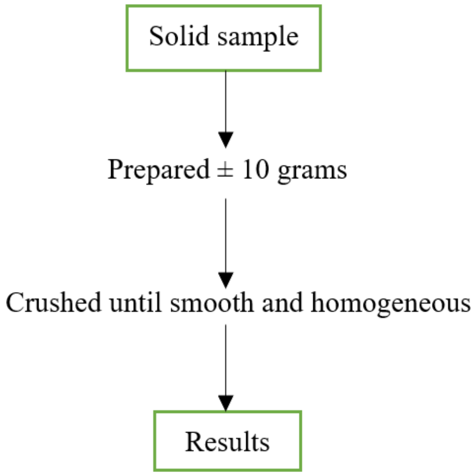

Fig. 1 Flowchart of the revision method

2.2 Land Preparation, Methanol Application, and Soil Water Content

The research land was cultivated using a tractor, combed to clean up plant debris and destroy chunks of soil, and the determined plot area was measured. The distance between main plots is 50 cm, between subplots 30 cm, and between replications/groups is 100 cm. Between the plots, a channel was made 20 cm deep and plastic was placed on the edge of the channel vertically 30 cm deep to avoid water seepage between one plot and another when the treatment was administered.

The planting was performed in a hole approximately 5 cm deep with a spacing of 40 cm x 20 cm. Each hole was filled with 3-4 soybean seeds. Next, the fertilizer given was urea 50 kg/ha, TSP 100 kg/ha, and KCI 50 kg/ha given twice each; the first fertilizer was given as basic simultaneously with planting: urea 25 kg/ha, TSP 50 kg/ha, and KCI 25 kg/ha. The second fertilizer was given 30 days after planting: urea 25 kg/ha, TSP 50 kg/ha, and KCI 25 kg/ha.

The embroidery is carried out 7 days after planting, while thinning is carried out 30 days after planting, leaving 2 plants per hole. The weeding is performed every week, and pest control is performed by administering Dursban 20 EC when filling the pods.

Methanol treatment is applied when the plants are 30 days after planting and then carried out every two weeks until a week before harvest. The concentration of methanol applied is adjusted to the treatment, namely without methanol, 15% methanol, 30% methanol, and 45% methanol, with a spray volume such that the entire plant canopy is wet.

Water is given on the basis of the field capacity (KP) and permanent wilting point (TLP). Their determination was performed in the soil science laboratory at Hasanuddin University. Based on the soil water content, the amount of water given for each treatment is determined using the following formula:

$$\mathrm{KAT} = 0{,}1\ x\ h\frac{S}{w}\ x\ AT$$

where KAT - soil water content expressed in water storage units, h - soil layer thickness (cm), S - density or dry weight of the soil volume (g cm³), w - water density (g. cm), and AT - available water.

2.3 Observation and Measurement

2.3.1 Growth Components

(1) Plant height is determined at harvest (cm) by measuring from the cotyledon scar to the top growing point;

(2) The number of leaves is calculated at harvest;

(3) The number of branches per plant is calculated at harvest;

(4) Leaf area is calculated using formula: L x W x k, where P - length, L - width, K – constant;

(5) Dry weight per plant (per plot and per hectare);

(6) Age of harvest (days) (add what components and how to measure them and what lab equipment to use).

2.3.2 Production Components

(1) Number of filled pods per plant;

(2) Weight of 100 dry seeds (grams);

(3) Weight of dry seeds per plot (kg) and per hectare (tons).

2.3.3 Climate Components

(1) The average water used per day is calculated by measuring it and then dividing it by the age of the plant at harvest;

(2) The efficiency of plant water use is calculated as follows:

$$EPA = \frac{Dry\ Ingredients\ (g)}{Water\ Required\ During\ Growth\ \ (l)}$$

2.3.4 Analysis Method

The data from the experiment were statistically analyzed using SPSS version 16 software[27]. The treatments were compared using analysis of variance (ANOVA) with the least significant difference (LSD) post-hoc test at a 5% probability level.

3 Results

3.1 Vegetative Growth Components

Analysis of variance showed that the level of soil water content and the application of methanol had a significant effect on plant height, number of leaves, number of branches, and harvest age (Tab. 1), but had no effect on the interaction between the soil water content and methanol. Tab. 1 shows that the soil water content of 75-100% (A3) provides the highest plant height, number of leaves, and harvest age and is significantly different from the other treatments because the soil water content of 75-100% (A3) is quite available to fulfill plant growth, while the 30% methanol (M3) treatment provided the highest plant height and number of branches and was significantly different from other treatments due to the contribution of CO_2 from methanol, which caused an increase in the net photosynthesis rate of the plants.

Tab. 1 Average plant height, number of leaves, number of branches, and harvest age at various methanol concentrations and soil water contents

Treatment	Plant height (cm)	Number of leaves	Number of branches	Harvest age
Main Plot				
A1	51.18 ^{b}	20.05 ^{b}	2.92 ^{a}	83.00 ^{c}
A2	55.32 ^{b}	21.14 ^{b}	3.47 ^{a}	86.00 ^{b}
A3	56.48 ^{a}	24.14 ^{a}	3.59 ^{a}	89.00 ^{a}
Subplot				
M0	51.94 ^{b}	20.81 ^{a}	2.80 ^{b}	86.00 ^{a}
M1	53.34 ^{ab}	21.64 ^{a}	3.38 ^{a}	85.66 ^{a}
M2	56.88 ^{a}	23.04 ^{a}	3.39 ^{a}	85.33 ^{a}
M3	54.55 ^{ab}	21.87 ^{a}	3.39 ^{a}	86.00 ^{a}

Note: Average numbers followed by the different letters are significantly different according to the Duncan test at $\alpha = 0.05$.

Tab. 2 and 3 show that methanol treatment and soil water content showed an interaction effect on the leaf area and plant dry matter weight. The treatment with 30% methanol (M2) and the soil water content of 75-100% (A3) provided the largest leaf area and the highest plant dry matter weight and was significantly different from the other treatments.

Tab. 2 Average leaf area at various methanol concentrations and soil water contents

Methanol	Soil water content		
	A1	A2	A3
M0	1294.83^{b}_{y}	1414.99^{b}_{z} 1599.46^{b}_{y}	1862.48^{a}_{y}
M1	1345.93^{c}_{y}	1526.10^{b}_{y}	1843.03^{a}_{y}
M2	1593.76^{b}_{x} 1493.96^{b}_{x}	1739.06^{a}_{x}	2222.12^{a}_{x}
M3			1874.28^{a}_{y}

Note: Average numbers followed by the different letters indicate that they are different in rows (a, b, c) and columns (x, y, z), which means they are significantly different according to the Duncan test level = 0.05.

Tab. 3 Average plant dry matter weight per plot at various methanol concentrations and soil water contents

Methanol	Soil water content		
	A1	A2	A3
M0	$7,57^{b}_{y}$	$9,25^{a}_{y}$	$9,59^{a}_{y}$
M1	$8,49^{c}_{y}$	$12,45^{a}_{x}$	$9,95^{b}_{y}$
M2	$8,07^{c}_{y}$	$10,73^{b}_{y}$	$12,34^{a}_{x}$
M3	$11,78^{a}_{x}$	$9,69^{b}_{y}$	$11,84^{a}_{y}$

Note: Average numbers followed by the different letters indicate that they are different in rows (a, b, c) and columns (x, y, z), which means they are significantly different according to the Duncan test at α = 0.05.

3.2 Result Components

Analysis of variance showed that the soil water content and application of methanol had a significant effect on the number of pods, weight of 100 seeds, and weight of dry seeds per plot (Tab. 4), but had no effect on the interaction between the soil water content and methanol. Tab. 4 shows that the soil water content of 75-100% (A3) provided the highest number of pods, weight of 100 seeds, and dry seed weight per plot and was significantly different from the other treatments, while the treatment with 30% methanol (M3) provided the highest number of pods, weight of 100 seeds, and dry seed weight per plot and was significantly different from the other treatments.

Tab. 4 Average number of pods, weight of 100 seeds, and dry seed weight per plot at various methanol concentrations and soil water contents

Treatment	Number of pods (fruits)	Weight of 100 seeds (grams)	Dry seed weight per plot (kg)
Main Plot			
A1	$47,80^{c}$	$12,14^{b}$	$1,52^{c}$
A2	$62,70^{b}$	$12,16^{b}$	$1,84^{b}$
A3	$89,50^{a}$	$13,32^{a}$	$2,62^{a}$
Subplot			
M0	$59,90^{c}$	$11,99^{c}$	$1,72^{b}$
M1	$62,70^{bc}$	$12,62^{b}$	$2,05^{a}$
M2	$70,80^{a}$	$12,84^{a}$	$2,25^{a}$
M3	$70,50^{ab}$	$12,71^{b}$	$2,04^{a}$

Note: Average numbers followed by the different letters are significantly different according to Duncan's test at α = 0.05.

3.3 Climate Components

Analysis of variance showed that the soil water content had a significant effect on the amount of water used per day (Tab. 5), but did not have a significant effect on the application of methanol. Tab. 5 shows that the soil water content of 75-100% (A3) shows the highest amount of water used per day and is significantly different from other treatments, while the application of methanol tends to be better at a concentration of 30% (M2).

Tab. 5 Average amount of water used per day (liters) at various methanol concentrations and soil water contents

Methanol	Soil water content			
	A1	A2	A3	Average
M0	9,47	16,58	29,41	19,49
M1	9,48	16,63	29,52	18,54
M2	9,56	16,71	29,73	18,67
M3	9,48	16,61	29,39	18,49
Average	$9,49^{c}$	$16,03^{b}$	$29,51^{a}$	

Note: Average numbers followed by the different letters are significantly different according to the Duncan test at α = 0.05.

Analysis of variance showed that the interaction between the soil water content and methanol application had a significant effect on the average water use efficiency (Tab. 6). Tab. 6 shows that soil water content of 25-50% (A1) and 45% methanol (M3) show water use efficiency and are significantly different from the other treatments; soil water content of 50-75% (A2) and 15% methanol (M1) show water use efficiency and are significantly different from the other treatments, while soil water content of 75-100% and methanol do not show a real effect.

Tab. 6 Water use efficiency at various methanol concentrations and soil water contents

Methanol	Soil water content		
	A1	A2	A3
M0	$9,63^{a}_{y}$	$6,52^{b}_{y}$	$3,70^{c}_{x}$
M1	$11,05^{a}_{y}$	$8,83^{b}_{x}$	$3,84^{c}_{x}$
M2	$10,40^{a}_{y}$	$7,60^{b}_{y}$	$4,75^{c}_{x}$
M3	$14,81^{a}_{x}$	$6,97^{b}_{y}$	$4,53^{c}_{x}$

Note: Average numbers followed by the different letters indicate that they are different in rows (a, b, c) and columns (x, y, z), which means they are significantly different according to the Duncan test at α = 0.05.

4 Discussion

The productivity of cultivated plants in principle depends on the results of the net accumulation of CO_2 assimilated during photosynthesis throughout the growing season. Photosynthesis is influenced by many factors, including CO_2 concentration and water availability.

Duncan's test results (Tab. 1) show that soil water content of 75-100% produces the highest plant height, number of leaves, and number of branches compared with soil water contents of 25-50% and 50-75%. This is thought to be because a soil water level of 75-100% can provide the water needed by plants in sufficient quantities to support their growth, especially in increasing cell turgor by absorbing water and nutrients from below (in the soil) to above (plant crown). Plants need water during the growth, and water plays an important role in plant growth, from vegetative growth to the reproductive phase[28].

Furthermore, the more water is given in accordance with the plant's needs, the more turgor it provides to the plant cells so that it can promote the cell enlargement and improve plant structure. Meanwhile, the harvest age shows that increasing soil water levels cause the harvest to last longer. This proves that water plays an important role in the rate of development of plant morphology. This situation means that the level of water supply affects the rate of fruit ripening. High soil moisture slows down the growth in the vegetative and flower phases and the fruit ripening, whereas low soil moisture results in faster growth and development. This means that the lengthening of plant growth in the vegetative phase will affect the generative phase of the plant so that the lifespan of the harvested plants will be longer[29].

Duncan's test results (Tab. 1) show that plant height, number of leaves, and number of branches increased with increasing methanol concentration up to 30% and then decreased at a concentration of 45%. This is because methanol is a short-chain carbon compound that is easily broken down into CO_2 in the leaf mesophyll so that the CO_2 concentration in the leaf mesophyll increases. Increasing CO_2 concentration causes the ratio of CO_2 to O_2 to increase. This increase causes the photorespiration rate to be depressed, whereas the photosynthesis rate increases. Photorespiration occurs because CO_2 concentrations decrease and O_2 concentrations increase in the leaf mesophyll[30].

An increase in internal CO_2 concentration (leaf mesophyll) is then thought to increase the activity of nibisco (ribulose bisphosphate carboxylase/oxidase) toward carboxylase so that the rate of photosynthesis increases, causing the amount of assimilate to be translocated to actively growing tissue to support the growth, including the plant height, number of leaves, and number of branches. Providing methanol up to 22.5% increased soybean plant height and was still linear at this concentration[31].

Duncan's test results (Tab. 2) show that methanol concentration and soil water content increased the leaf area. The area increase is caused by an increase in methanol concentration, which increases the internal CO_2 concentration and is supported by the availability of sufficient water, thereby increasing the rate of photosynthesis and causing an increase in assimilation. Part of this assimilate is directed toward leaf formation, further increasing the number of leaves and leaf area. Increasing the methanol concentration to 22.5% increases the number of Arabica coffee plant leaves and is still linear[32].

There is an interaction between the soil water content and methanol concentration in the leaf area. This interaction occurs because of a dependent relationship between soil water content and methanol concentration. The availability of CO_2, followed by the availability of sufficient water, can stimulate photosynthetic activity. Increasing the rate of photosynthesis, the production of photosynthesis (assimilate) increases, thus allowing the formation of all larger plant organs, including the leaf area.

There is an interaction between methanol concentration and soil water content on plant dry matter weight (Tab. 3), which is caused by the formation of larger plant organs. An increase in methanol concentration of up to 30% at soil water content of 75-100% indicates an increasingly high level of photosynthetic efficiency. Increasing the efficiency of photosynthesis causes the amount of assimilate formed to increase so that the plant fruit weight also increases[33].

The results of Duncan's test on the number of pods (Tab. 4) showed an increase in rhythm with increasing methanol concentration and soil water content. This is due to the increasing level of net photosynthetic efficiency of the plants, especially in the generative phase, so that more assimilate is directed toward the formation of a higher number of filled pods per plant. The formation and filling of pods is largely determined by the provision of photosynthesis occurring at that time[34].

Likewise, providing higher levels of water (75-100%) before the reproductive phase causes increased plant photosynthesis, resulting in increased assimilation directed toward pod formation and filling. Furthermore, the weight of 100 seeds and the weight of dry seeds per plot (Tab. 4) showed an increase in rhythm with increasing methanol concentration up to 30% and soil water content up to 75-100%. Soil water content of 75-100% showed the best response compared to soil water contents of 25–50% and 50–75%. Methanol concentration of 30% also showed the best response compared with other concentrations (without methanol, 15% methanol, and 45% methanol).

The influence of soil water content and methanol concentration on the production components cannot be separated from the vegetative growth phase of soybean plants. The vegetative growth phase determines the generative growth phase of the plant. In this experiment, it was seen that the vegetative growth of the plants (plant height, number of leaves, number of branches, leaf area, and plant dry matter weight) was better at the treatment with 30% methanol and the soil water content of 75-100%.

The results of Duncan's test on the average water used per day (Tab. 5) show that increasing soil water content increases the amount of water used per day. Water absorption by the plant roots runs normally, causing smooth transport of water vapor from the stomata to the atmosphere (transpiration). An increase in water potential in the plant area will be followed by an increase in ground water potential, which is then followed by an increase in leaf water potential. An increase in water potential followed by an increase in turgor pressure in leaf cell tissue results in the development of protective (guard) cells in the stomata and causes the stomata to open. This causes transpiration to increase so that the need for water used by plants increases.

The results of the Duncan test on water use efficiency (Tab. 6) show that increasing methanol concentration increases water use efficiency. Water use efficiency is a general description of the amount CO_2 which is used (dry matter produced) with only the water used by plants in the respiration process. Treatment with methanol concentration of 45% at the soil water content of 25-50% produces high water use efficiency. The highest water use efficiency results from increasing internal CO_2 levels and decreasing transpiration rates so that the effectiveness of photosynthesis increases[35,36]. Thus, increasing atmospheric CO_2 can double water use efficiency in C_3 plant groups such as soybeans.

There is an interaction between methanol concentration and soil water content. Methanol concentration of 45% at the soil water content of 25-50% produces the highest water use efficiency. This is because water influences the rate of transpiration and CO_2 diffusion due to the regulation of stomata. This is also related to the fact that methanol can increase the efficiency of water use at any level of water availability. This is because methanol has an effect on increasing the internal CO_2 concentration, which will cause the stomata to partially close, which occurs quickly so that transpiration decreases. Partial closure of stomata at high internal CO_2 concentrations is a plant reaction that maintains the internal CO_2 concentration at a favorable level[37]. Thus, partial closure of the stomata will reduce the rate of transpiration, as stated by Takahashi[38] that high CO_2 levels in leaves cause the stomata to narrow.

5 Conclusions

Based on the research results, it can be concluded that soil water content and the application of methanol have a good influence on the growth and production components. Soil water content of 75-100% provides the highest dry seed production of 2.24 tons/ha. 30% methanol provides the highest dry seed production of 1.87 tons/ha or increases the production by 30% and can increase water use efficiency.

In the treatment at the field water content of 80%, the highest N nutrient uptake was inoculated with Acaulospora sp. 1, namely K_2M_1 treatment, compared with other treatment combinations. Meanwhile, inoculation of Glomus sp. mycorrhizae under water stress conditions (60% field capacity (K_3M_3)) increased plant N nutrient uptake compared with other types of mycorrhizae. In treatments at the field water content of 80%, P nutrient uptake was the highest in the K_2M_1 treatment compared with other treatment combinations. Meanwhile, Glomus sp. mycorrhizal inoculation under conditions of 60% field capacity (K_3M_3) increased plant P nutrient uptake compared with other types of mycorrhizae. The lower the soil water content, the more significantly the plant uptake of N and P nutrients will decrease. Inoculation of various types of mycorrhizae also showed decreased results despite P uptake by the Aeciospore sp inoculant. 1 (M_1) has the highest P uptake. However, the highest combination of N and P uptake treatments was found in the K_2M_1 treatment, namely 950,959 N mg/plant and 66,338 P mg/plant. Under water stress conditions (60% field capacity), inoculation of mycorrhizal Glomus sp. (K_3M_3) increased the plant N and P nutrient uptake, namely 755,932 mg/plant and 45,543 mg/plant, compared with other treatment combinations.

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