BUKTI KORESPONDENSI
SYARAT KHUSUS ARTIKEL JURNAL BEREPUTASI

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Jurnal : Journal of Ecological Engineering, Vol 24, No. 8, Tahun 2023, Hal. 128-136
Penulis : Zulkifli Maulana, Andi Muhibuddin, Muhammad Arief Nasution, Abri, Amiruddin Amirudin, Baharuddin, Andi Tenri Fitriyah, Sitti Nurani Sirajuddin, Abdel Razzaq M. Al Tawaha

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Utilization of Dry Land using Molybdenum, Lime, and Rhizobium Strains to Increase Soybean Yield

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I am pleased to inform you that your manuscript, entitled: Utilization of Dry Land using Molybdenum, Lime, and Rhizobium Strains to Increase Soybean Yield, might be accepted for publication in our journal, pending some minor changes suggested by reviewers.

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A. MANUSCRIPT

Journal	Journal Of Ecological Engineering
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B. REVIEWER'S SPECIFIC COMMENTS PER SECTION OF MANUSCRIPT

Title	The title is mostly appropriate
Abstract	The authors should correct all grammatical, spellings and typo
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Introduction	The authors should briefly discuss the relevance of the research methods to the research. Authors should correct all grammatical, spellings, italic and typo.
Materials and Methods	The authors should improve the table to make it easy to read. Authors should clarified using what statistical analysis. All grammatical, spellings and typo should be corrected.
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INTRODUCTION

Indonesia's soybean demand continues to in-crease along with population growth. This is due to the need for soybeans in the community for consumption, sothe increase in soybean productionis one indicator of the success of the agricultural sector. The high demand for soybeans is becauseit is one of the basic needs and the main source ofvegetable protein for most Indonesians. In addi- tion, soybeans are a group of leguminous plants that can fix nitrogen from the air that occurs in root nodules (Al-Tawaha et al., 2022a; Al-Tawa-ha et al., 2022b; Saranraj et al., 2021; Shiri Janagard and Ebadi Segherloo, 2015) with an average protein content of 40% based on dry matter (Al- Tawaha and Al-Tawaha, 2017; Cruz-Suárez et al.,2009; Banaszkiewicz, 2011), soybean consumption in Indonesia is 2.2 million tons per year. Thisfigure is far from the average production level ofonly 819,442 tons per year. As a result, the gov-ernment must import soybeans as much as 2–2.6 million tons per year. Domestic soybean production is currently only able to meet about 35% of the consumption (Ningrum et al., 2018). The governments have made several attempts to increase domestic soybean production, such as by clearing new farming land. Ultisol soil is one of the alternative lands for developing soybean areas inIndonesia, whose distribution reaches 45,794,000 ha or about 25% of the total land area of Indone-sia (Syahputra et al., 2015), with great potential to be developed into productive land. However, ultisol soils are poor in organic matter (<5%), lowpH, high Al saturation, low base saturation, and organic C, causing nutrient deficiencies, especially molybdenum (Mo) (Fitriatin et al., 2014). On the other hand, the element Mo is needed in the

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Reply

Table 1. Chemical and physical properties of ultisol soils before and after treatments

Parameters	Content before treatment (R_0)	Content after treatment (R_1)	Content after treatment (R_2)	Content after treatment (R_3)
pH (1:2,5) H_2O	4,9	5,50	5,65	5,70
C- Organik (%)	0,82	0,83	0,83	0,84
N-Total (%)	0,18	0,28	0,36	0,38
Available-P (ppm)	10,00	10,20	10,37	10,48
K-dd (me/100 g)	0,32	0,43	0,47	0,51
CEC (me/100 g)	15, 07	15,10	16,10	16,20
Base Saturation (%)	15,15	16,10	16,12	16,40
Al-dd (me/100 g)	1,92	10,20	10,20	10,20
Mo (ppm)	0,35	1,51	1,69	2,10
Al Saturation	22,64	22,12	21,78	21,60
Fraktion: Sand (%)	55,23	55,21	55,21	55,10
Dust (%)	24,10	24,12	24,13	24,32
Clay (%)	20,67	20,67	20,66	20,58
Tekstur	(Sandy clay loam)	(Sandy clay loam)	(Sandy clay loam)	Sandy clay loam)

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Observation and measurement

Vegetative growth observation included plantheight, age of flower appearance, number of root nodules, percentage of effective root nodules, andnitrogen (N) and potassium(K) uptake. Mean-while, observations for the generative phase werethe weight of 100 seeds, the number of pods per plant, and dry seed weight per plant.

Data analysis

The data from the experiment were statistically analyzed. Thedifferences among treatments were compared using analysis of variance (ANOVA) with the leastsignificant difference (LSD) posthoc test at a 5% probability level.

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Table 2. Average plant height, age of flower appearance, number of root nodules, and percentage of effective root nodules on soybean cultivars and the combinations of *Rhizobium* strain *Nod⁺Fix⁺*, $CaCO_3$ and NH_4 -molybdate

Treatment	Plant height (cm)	Age of flower appearance (days)	Number of root nodules (seeds)	Percentage of effective root nodules (%)
Cultivars				
V1	83.96 ^{x}	32.78 ^{x}	40.22 ^{x}	62.21 ^{x}
V2	75.62 ^{y}	38.96 ^{y}	34.64 ^{y}	58.28 ^{y}
Sub-plots				
R0	66.04 ^{a}	35.38 ^{a}	09.80 ^{a}	27.23 ^{a}
R1	82.59 ^{b}	36.05 ^{a}	26.14 ^{b}	67.76 ^{b}
R2	90.16 ^{c}	35.66 ^{a}	57.22 ^{cd}	79.76 ^{cd}
R3	80.37 ^{b}	36.10 ^{a}	48.47 ^{c}	73.74 ^{c}

Note: Numbers followed by the same letter in each column are not significantly different at Duncan's test level =0.05

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The results of Duncan's test in Table 2 onthe observation of the number of root nodules showed that different treatments gave different results on the number of root nodules. Treatment R_2 showed the highest number of root nodules. Thiswas due to the suitability of the needs for bacte- rial strain *Nod⁺ Fix⁺*, Lime ($CaCO_3$), Molybdenum($(NH_4)6Mo_7O_{24}.4H_2O$), and Cobalt (CoC_{12}). Therefore, metabolic processes

The observation results of the distribution pattern of the root nodules in Table 2 showed the best treatment of R_2. The nodule distribution pattern determines the effectiveness of the nodule. Nodules scattered on the root neck indicate nodules effective in fixing N. The distribution pattern of root nodules explains the effectiveness of root nodules in fixing nitrogen free from the air. Effective root nodules are located on the taproots, while ineeffective root nodules are scattered on lateral roots or root branches and are pale in color. The activity of N_2 fixation by bacteria is essential for overall N balance because the continuous form of fixed N can be lost due to denitrification and permeation (Howie & Echandi, 1983).

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Nitrogen, phosphorus and potassium uptake

The ANOVA results showed that cultivars (V)had a significant effect on nitrogen (N) and potassium (K) uptake but had no significant effect on Phosphorus (P) uptake. The combinations of *Rhizobium* strain *Nod⁺Fix⁺*, $CaCO_3$ and NH_4 -molybdate (R) significantly affected N, P, and K up-take (Table 3). There was no interaction betweenthe cultivars and the combinations of *Rhizobium* strain *Nod⁺Fix⁺*, $CaCO_3$, and NH -molybdate. The Baluran cultivar (V1) produced higher N and K uptake, significantly different from the Willis cultivar (V2). However, the P uptake of treatments was not significantly different. According to

Table 3 shows that N, P, and K uptake increased with increasing doses of the combinations ($CaCO_3$ + NH_4-molybdate). The highest increase was obtained in the combination of *Rhizobium* strain *Nod⁺ Fix⁺*+ $CaCO_3$ 1.5 tons/ha + NH_4-molybdate 500 g/ha of 852.7 mg/plant, 52.4 mg/plant, and 442.9 mg/plant, respectively. However, the results for the combination of *Rhizobium* strain *Nod⁺ Fix⁺* + $CaCO_3$ 2.0 tons/ha + NH_4-molybdate 750 g/ha were decreased statistically significantly. The increased uptake of N, P,and K was thought to be the result of the ability of soybean cultivars to form root nodules in response to Rhizobium strain Nod+ Fix+ inoculation. In addition, it was supposed that the number of Rhizobium strain Nod+ Fix+ in that treatment was more suitable than others. The root nodules were formed if there was a suitability between *Rhizobium* strain *Nod⁺ Fix⁺* and its host.

The results of Duncan's test in Table 3 showed that the highest nitrogen uptake was found in the R_2 treatment compared to other treatments (R_0, R_1 and R_3). The significant effect of the R treatment (bacterial strain *Nod⁺ Fix⁺* + MoCo (1.0: 0.6) kg/ha) was thought to result from the soybean's ability to form root nodules in response to the bacterial strain *Nod⁺ Fix⁺*inoculation. In addition, it is suspected that the number of bacterial strains *Nod⁺ Fix⁺* in this treatment is more suitable than the other treatments, and effective root nodules would form if there were a suitability between the bacteria and its host.

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Table 3. Average nutrient uptake of N, P and K in soybean cultivars and combinations of *Rhizobium* strain *Nod⁺Fix⁺*, $CaCO_3$, and NH_4 -molybdate

	nutrient uptake (mg/plant)		
Treatment	N	P	K
Cultivars			
V1	710.6 ^{x}	43.1 ^{x}	370.0 ^{x}
V2	641.1^y	40.5 ^{x}	364.7 ^{y}
Sub-plots			
R0	385.6 ^{a}	33.1 ^{a}	244.8 ^{a}
R1	737.3 ^{b}	41.2 ^{b}	377.3 ^{b}
R2	852.7 ^{c}	52.4 ^{c}	442.9 ^{bc}
R3	727.8 ^{b}	40.6 ^{b}	405.4 ^{b}

Note: numbers followed by the same letter in each column are not significantly different at Duncan's test level = 0.05.

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Leaf color scale

The observation results of the leaf color scalein Table 4 showed that the treatment of bacterial strain *Nod⁺Fix⁺* with Mo and CaCo lime supple-ments on soybeans using ultisol soils affected theleaf color scale. Table 4 shows that the *Willis* andBaluran varieties treated with the *Nod⁺ Fix⁺* bacterial strain, Mo and $CaCo_3$ lime supplements hadvarious leaf color scales.

Table 4. Leaf Color Scale.

Varietas	Treatments	leaf color scales. *) 1	2	3	4
V_1	R_0	x			
	R_1		x		
	R_2			x	
	R_3			x	
V_2	R_0	x			
	R_1				x
	R_2			x	
	R_3			x	

Note: * Criteria based on color scale, diagnosis of nutrient status from leaf color. The strengthening of legumes in relation to cropping system research project. Japan International Cooperation Agency (JICA): 1 – yellowish green, 2 – light green, 3 – green,4 – slightly dark green.

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Table 4 showed that the results on the leaf color scale was the best treatment of R_1 for the Baluran cultivar compared to other treatments. This showed that the fixed N element was usedto meet the N required by plants so the availability of N for plants increases. An effective fixation, 50–75% of the total N requirement by plants canbe fulfilled. In general, this study showed the pat-tern of increasing plant height, root dry weight, number of root nodules, N uptake, root nodule distribution pattern, leaf color scale, weight of 100 seeds, and dry seed yield. It was found that the treatment of the bacterial strain *Nod⁺ Fix⁺* with supplements of microelements (MoCo), lime ($CaCO_3$), and Cobalt, simultaneously increased the availability of Mo in the soil so as to increase the uptake of Mo by plants. Jaiswal et al. (2021) stated that the elementMo functions as an activator of nitrogenation and reducing enzymes which activate *Rhizobium* performance in the N fixation process so that N binding increases. Nitrogen can stimulate cell elongation, vegetative growth, enlarge pods, slow down the initiation phase, and increaseds seeds yield and content (Ohyama et al., 2013). The re- sults of Duncan's test in Table 4 showed that the highest weight of 100 seeds was obtained in the R treatment (bacterial strain *Nod⁺ Fix⁺* + MoCo (1.0 : 0.6) kg/ha) compared to other treatments (R_0, R_1 and R_3). This revealed that the treatment isvital for the growth and production of soybeans.

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Table 5. The average weight of 100 seeds, number of pods per plant, and dry seed weight per plant

Treatment	Weight 100 seeds (g)	Number of pods per plant (piece)	Dry seed weight per plant (g)
Cultivars			
V1	13,75 ^{a}	28,66 ^{a}	16,53 ^{a}
V2	11,34 ^{a}	26,51 ^{a}	15,17 ^{a}
Sub-plots			
R0	10,37 ^{a}	19,18 ^{a}	10,99 ^{a}
R1	14,04 ^{b}	26,94 ^{b}	17,97 ^{bc}
R2	14,49 ^{b}	35,89 ^{c}	19,45 ^{c}
R3	11,88 ^{a}	28.34 ^{b}	15,00 ^{b}

Note: numbers followed by the same letter in each column are not significantly different at Duncan's test level = 0.05.

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This was due to the Cofactor Mo (MoCo) forming the active site of all eukaryotic molybdenum enzymes.

Yield components

The ANOVA results showed that cultivars (V)had no significant effect; while the combinations of *Rhizobium* strain *Nod^{+}Fix^{+}*, $CaCO_3$, and NH_4 – molybdate had a significant effect on the weight of 100 seeds, number of pods per plant, and dry seed weight per plant. There was no interaction between the cultivars and the combinations of Rhizobium strain Nod+Fix+, CaCO ,and NH -molybdate (Table 5).

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Typo

Table 5 shows that the weight of 100 seeds, number of pods per plant, and dry seed weight per plant were

REFERENCES

Author

Please, delete brackets in whole reference

Reply

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5. PENGIRIMAN REVISI ARTIKEL KE JOURNAL OF ECOLOGICAL ENGINEERING
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Dear Editor Team,

We would like to thank you for your review of our article entitled: Utilization of Dry Land using Molybdenum, Lime, and Rhizobium Strains to Increase Soybean Yield. The manuscript has been revised and edited as reviewer's request.

Regards,
Muhibuddin

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REVIEWER'S SPECIFIC COMMENTS PER SECTION OF MANUSCRIPT

	Reviewer	Author
Title	The title is mostly appropriate	
Abstract	The authors should correct all grammatical and typo	We apologise for the error. We have corrected the grammatical and typographical errors
Keywords	Keywords are appropriate	
Introduction	The authors should briefly discuss the relevance of the research methods to the research. Authors should correct all grammatical, spellings, italic and typo.	We have added relevant matters related to research methods to be researched. We have corrected the grammatical, spellings, italic and typographical errors. Hopefully this revised manuscript can be more comfortable to read.
Materials and Methods	The authors should improve the table to make it easy to read. Authors should clarified using what statistical analysis. All grammatical, spellings and typo should be corrected.	We have fixed all the existing tables. hopefully the tables can be read properly. We have described the statistical analysis used. Hopefully this revised manuscript can be more comfortable to read.
Results and Discussion	The authors should improve the table to make it easy to read. All grammatical, spellings and typo should be corrected.	We have fixed all the existing tables. We have corrected the grammatical and typographical errors
Conclusion	Conclusion are appropriate	
References	The authors should delete brackets in whole reference	We have removed all the brackets

Utilization of Dry Land Using Molybdenum, Lime, and *Rhizobium* Strains to Increase Soybean Yield

Zulkifli Maulana[1], Andi Muhibuddin[1*], Muhammad Arief Nasution[1], Abri Abri[1], Amiruddin Amirudin[1], Baharuddin Baharuddin[2], Andi Tenri Fitriyah[2], Sitti Nurani Sirajuddin[3], Abdel Razzaq M. Al Tawaha[4]

[1] Department of Agrotechnology, Faculty of Agriculture, Bosowa University, Makassar, 90245, Indonesia

[2] Department of Agribusiness, Faculty of Agriculture, Bosowa University Makassar, 90245, Indonesia

[3] Hasanuddin University, Socio-Economics Husbadry Department, Faculty of Animal Science, Makassar, Indonesia

[4] Department of Crop Science, Faculty of Agriculture, Universiti Putra Malaysia 43400 UPM Serdang, Selangor, Malaysia

* Corresponding author's e-mail: muhibuddin@universitasbosowa.ac.id

ABSTRACT

Ultisol is a type of soil with low organic matter, pH, and nutrient content, including molybdenum, leading to low productivity. This study aimed to investigate the use of dry land using molybdenum and lime ($CaCO_3$) inoculated with *Rhizobium* strain *Nod⁺Fix⁺* to increase the soybean production of *Willis* and *Baluran* cultivars. This research was conducted from May to September 2021 in Pallangga Subdistrict, Gowa Regency, South Sulawesi, Indonesia. The study used a split-plot design with three replications for each treatment. The first factor was soybean varieties, consisting of Baluran and *Willis* cultivars. The second factor was the composition of the bacterial strain *Nod^+ Fix^+*, lime $CaCO_3$ and NH_4-molybdate, which consisted of without (*Rhizobium* strain *Nod^+ Fix^+* + $CaCO_3$ + NH_4-molybdate); *Rhizobium* strain *Nod^+ Fix^+* + $CaCO_3$ 1.0 ton/ha + NH_4-molybdate 250 g/h); *Rhizobium* strain *Nod^+ Fix^+* + $CaCO_3$ 1.5 tons/ha + NH_4-molybdate 500 g/h); and *Rhizobium* strain *Nod^+ Fix^+* + $CaCO_3$ 2.0 tons/ha + NH_4-molybdate 750 g/h). The results showed that treating the bacterial strain *Nod^+ Fix^+* + MoCo (1.0:0.6) kg/ha achieved the best results on growth, nutrient uptake (Nitrogen, Phosphorus and Potassium), and soybean yields, both for *Willis* and *Baluran* varieties on ultisol soils.

Keywords: Nod factor; nodulation; legume symbiosis; biological nitrogen fixation; rhizobium strain nod^+ fix^+.

INTRODUCTION

Indonesia's soybean demand continues to increase along with population growth. This is due to the need for soybeans in the community for consumption, sothe increase in soybean production is one indicator of the success of the agricultural sector. The high demand for soybeans is because it is one of the basic needs and the main source of vegetable protein for most Indonesians. In addition, soybeans are a group of leguminous plants that can fix nitrogen from the air that occurs in root nodules (Al-Tawaha et al., 2022a; Al-Tawaha et al., 2022b; Saranraj et al., 2021; Shiri Janagard and Ebadi-Segherloo, 2015) with an average protein content of 40% based on dry matter (Al-Tawaha and Al-Tawaha, 2017; Cruz-Suárez et al., 2009; Banaszkiewicz, 2011), soybean consumption in Indonesia is 2.2 million tons per year. This figure is far from the average production level of only 819,442 tons per year. As a result, the government must import soybeans as much as 2–2.6 million tons per year. Domestic soybean production is currently only able to meet about 35% of the consumption (Ningrum et al., 2018). The government has made several attempts to increase domestic soybean production, such as by clearing new farming land. Ultisol soil is one of the alternative lands for developing soybean areas in Indonesia, whose distribution reaches 45,794,000 ha or about 25% of the total land area of Indonesia (Syahputra et al., 2015), with great potential to be developed into productive land. However, ultisol soils are poor in organic matter (<5%), low pH, high Al saturation, low base saturation, and organic C, causing nutrient deficiencies, especially molybdenum (Mo) (Fitriatin et al., 2014). On the other hand, the element Mo is needed in the nitrate reductase enzyme to reduce nitrogen in nitrogen fixation in the root nodule tissue of soybean plants (Stiens et al., 2007). The element Mo is a component of the nitrate reductase enzyme, which plays a significant role in the assimilation of nitrogen nitrate (Mmbaga et al., 2015). High acidity (low pH) in ultisol soils and high Al saturation values can be neutralized by liming. Lime ($CaCO_3$) increases pH, neutralizes Al and Fe, and increases soil Ca levels so that it can affect soil potassium levels to achieve balanced nutrient adequacy for soybean plants (Rahman et al., 2018). With a decrease in soil pH, *Rhizobium* bacteria can adapt, i.e., in the low pH range, around pH 5.0–6.5 (Cieśla et al., 2016). This is because root nodules of leguminous plants will only form when *Rhizobium* bacteria are present in the place where legumes grow (Wang et al., 2018). However, air nitrogen fixation does not always occur efficiently due to competition between inoculums which are efficient and inefficient specific *Rhizobium* that exist naturally in the soil (Mitsch et al., 2007; Mengel et al., 2012).

According to Reuhs et al. (2005), several types of *Rhizobium* bacteria are known based on their ability to form nodules, i.e., a) *Rhizobium* which does not have a nodule-forming character on plant roots, so that no symbiosis occurs, is known as a *Nod*$^-$ strain; b) *Rhizobium* which has nodule-forming character but does not have air nitrogen fixing character, known as *Nod*$^+$*Fix*$^-$ strain; and c) *Rhizobium* which has two characters at once as nodule forming and as air nitrogen fixer is known as *Nod*$^+$*Fix*$^+$ strain (Salih et al., 2015). Research on the inoculation of *Rhizobium* strains and the application of molybdenum to soybean plants has been carried out by several previous researchers (Lande et al., 2019). However, no research has combined the treatment of molybdenum, $CaCO_3$, NH_4-molybdate with *Rhizobium*Strain *Nod*$^+$*Fix*$^+$in ultisol soils.

The urgency of this research was the breakthrough of soybean production technology innovation by combining molybdenum, $CaCO_3$, NH_4-molybdate with *Rhizobium*strain *Nod*$^+$*Fix*$^+$ on ultisol soils which have not been widely used in Indonesia because it is a marginal soil group. In addition, this research can reduce Indonesia's dependence on imported soybeans. Indonesia is currently still importing 2.6 million tons of soybeans from abroad.

This study aimed to investigate the use of dry land using molybdenum and $CaCO_3$ inoculated with *Rhizobium* strain *Nod*$^+$*Fix*$^+$ to increase the soybean production of *Willis* and *Baluran* cultivars.

MATERIALS AND METHODS

Experimental design and treatments

This research was conducted in the greenhouse of the Faculty of Agriculture, University of Bosowa, Pallangga Subdistrict, Gowa Regency, South Sulawesi, Indonesia, from May to September 2021. The study was arranged according to a split-plot design with two factors, with three replications for 24 plots, and each plot had 20 plants. Five plants were measured as the samples for each plot. The first factor was soybean varieties consisting of V_1 = *Baluran* and V2 = Willis. The second factor was the composition of the bacterial strain *Nod*$^+$ *Fix*$^+$, lime $CaCO_3$and NH_4-molybdates follows: R_0 = without (*Rhizobium* strain *Nod*$^+$*Fix*$^+$+ $CaCO_3$ + NH_4-molybdate); R_1 = *Rhizobium* strain *Nod*$^+$ *Fix*$^+$ + $CaCO_3$24 g/pot + NH_4-molybdate 6 g/pot; R_2 = *Rhizobium* strain *Nod*$^+$*Fix*$^+$+ $CaCO_3$ 36 g/pot + NH_4-molybdate 12 g/pot; dan R_3 = *Rhizobium* strain *Nod*$^+$ *Fix*$^+$ + $CaCO_3$48 g/pot + NH_4-molybdate 18 g/pot.

Preparation of growing media, administration of Rhizobium strain Nod$^+$Fix$^+$, and treatment of $CaCO_3$ and NH_4-molybdate

The soil used was ultisol soil which was taken compositely at a depth of 0–25 cm and had never been planted with soybeans. The soil was then air-dried and crushed and sieved with a 0.5 cm diameter sieve. The sieved soil was taken sufficiently to determine its physical and chemical properties in the laboratory before the treatments (Table 1). After that, 10 kg of ultisol soil was put into a black polybag with a size of 60 cm and a diameter of 40 cm and $CaCO_3$ was added (according to the treatment). The polybags were arranged in rows with wooden supports, with a distance between rows of 40 cm and between

Table 1. Chemical and physical properties of ultisol soils before and after treatments

Parameters	Content before treatment (R_0)	Content after treatment (R_1)	Content after treatment (R_2)	Content after treatment (R_3)
pH (1:2,5) H_2O	4.9	5.50	5.65	5.70
C-organic (%)	0.82	0.83	0.83	0.84
N-Total (%)	0.18	0.28	0.36	0.38
Available-P (ppm)	10.00	10.20	10.37	10.48
K-dd (me/100 g)	0.32	0.43	0.47	0.51
CEC (me/100 g)	15. 07	15.10	16.10	16.20
Base saturation (%)	15.15	16.10	16.12	16.40
Al-dd (me/100 g)	1.92	10.20	10.20	10.20
Mo (ppm)	0.35	1.51	1.69	2.10
Al saturation	22.64	22.12	21.78	21.60
Fraktion – sand (%)	55.23	55.21	55.21	55.10
Dust (%)	24.10	24.12	24.13	24.32
Clay (%)	20.67	20.67	20.66	20.58
Tekstur	Sandy clay loam	Sandy clay loam	Sandy clay loam	Sandy clay loam

rows of 25 cm. One week later, 50 g/pot. Then pots were given with basic fertilizers of Urea, TSP and KCl each dose of 0.25; 50 and 0.25 g/pot (50, 100 and 50 kg/ha). 7.5 mL *Rhizobium* strain *Nod*$^+$ *Fix*$^+$ was mixed with 1 kg of soybean seeds. The mixture was then moistened with water, drained, mixed well, and stored in a shady place. It was left for 15 minutes and then was planted. The treatment of NH_4-molybdate as a source of Mo (containing 82% acid MoO_3) (according to the treatment) was given at the planting time by dissolving each in 10 ml of water. The solution was sprinkled into polybags around the plants and then sprayed on the plants once every two weeks. After one week, the plants were thinned so only one plant/polybag was maintained until the plants were harvested. After the plants were harvested, the soil residue from the polybags was taken compositely at a depth of 0–25 cm to analyze its physical and chemical properties in the laboratory.

Maintenance and harvest

Watering was performed daily by maintaining the groundwater condition at around 80% of field capacity. Thinning was carried out after the plants were 7 days old by removing one plant per polybag. Plant maintenance included embroidery, weeding, and hoarding. Plants were harvested after about three months of age. The characteristics of soybean plants that were ready to harvest were that, after all, the plant leaves were old or yellow.

Observation and measurement

Vegetative growth observation included plant height, age of flower appearance, number of root nodules, percentage of effective root nodules, and nitrogen (N) and potassium(K) uptake. Meanwhile, observations for the generative phase were the weight of 100 seeds, the number of pods per plant, and dry seed weight per plant.

Data analysis

The data from the experiment were statistically analyzed using SPSS version 16 software. The differences among treatments were compared using analysis of variance (ANOVA) with the least significant difference (LSD) posthoc test at a 5% probability level.

RESULTS AND DISCUSSION

Soil chemical and physical properties

The soil analysis results before (without treatment) and after the experiment (soil treatment results of mixed R_1, R_2 and R_3) showed a change in the chemical properties of the soil. In general, there was a change in the chemical properties of the soil, i.e., pH, organic matter, total nitrogen (N), available phosphorus (P), potassium (K), Cation-exchange capacity (CEC), Ca, Mg, Mo, and Al. This was due to the treatment of *Rhizobium* strain

Nod⁺ Fix⁺, supplementation of microelements Mo (NH_4-molybdate), and lime $CaCO_3$ in ultisol soils which contributed to improving the chemical properties of the soil. Application of NH_4-molybdate increased Mo from 0.35 ppm to 0.47 ppm. The application of lime $CaCO_3$ increased soil pH from 5.1 to 5.5. This occurred due to the ability of lime $CaCO_3$ to neutralize soil acidity through the mechanism of H^+ substitution by Ca^{2+}. This condition provides a way for the availability of phosphorus freely through mineralization by soil biota in the solution. The basic reason for liming is to increase soil pH. As a result of changes in pH, changes in soil chemistry and biology occur, such as increased availability of phosphorus, nitrogen, potassium, calcium, and magnesium.

Vegetative growth

The ANOVA results showed that cultivars (V) and the combinations of *Rhizobium* strain *Nod^+Fix^+*, $CaCO_3$ and NH_4-molybdate (R) had a significant effect on plant height, age of flower appearance, and number of root nodules, and percentage of effective root nodules (Table 2). There was no interaction between the cultivars and the combinations of *Rhizobium* strain *Nod^+Fix^+*, $CaCO_3$ and NH_4-molybdate. The *Baluran* cultivar (V1) produced higher plant height, age of flower emergence, number of root nodules, and percentage of effective nodules. This was significantly different from the *Willis* cultivar (V2). These differences were due to the different characteristics of *Baluran* and *Willis* cultivars that affect photosynthetic activity, which determines plant height growth, flower appearance age, number of root nodules, and percentage of effective root nodules. Pregitzer et al. (2013) explained that some factors that influence plant growth and production are the environment, species or types of plants related to the genotype.

Table 2 shows that plant height, age of flower appearance, number of root nodules, and percentage of effective root nodules were increased with increasing doses of the combinations ($CaCO_3$+ NH_4-molybdate). The highest increase was obtained in the combination of *Rhizobium* strain *Nod^+ Fix^+*+ $CaCO_3$ 1.5 tons/ha + NH_4 -molybdate 500 g/ha of 90.16 cm, 35.66 days, 57.22 seeds, and 79.76 (%), respectively. However, the results for the combination of *Rhizobium* strain *Nod^+ Fix^+*+ $CaCO_3$ 2.0 tons/ha+NH_4 -molybdate 750 g/ha were decreased statistically significantly. The increase in plant height, age of flower appearance, number of root nodules, and percentage of effective root nodules was supported by the availability of Mo for *Rhizobium* strain *Nod^+ Fix^+*by Mo administration. According to Schwarz and Mendel (2006), Mo is not only essential for plants, but also essential for microorganisms. Furthermore, more than 40 Mo enzymes are found in nature, most of which are found in bacteria, but only four Mo enzymes are found in plants. The increase in the availability of Mo in the soil has stimulated the growth, development, and activity of *Rhizobium* bacteria. Therefore,it increased the ability of *Rhizobium* bacteria to infect root nodules, resulting in a higher percentage of effective root nodules formed. Effective root nodules contain *Rhizobium* bacteria that can fix air N_2.

Based on the results of Duncan's test in Table 2, the observation of plant height showed that the *Willis* cultivar achieved a higher plant height and was significantly different compared to the *Baluran* cultivar, both at the age of 2, 4, and 6 (weeks after planting) WAP, as well as significant differences in each treatment (R_0, R_1, R_2, and R_3). This was because the effectiveness of each treatment (bacterial strain *Nod^+ Fix^+*, Lime ($CaCO_3$), Molybdenum ($(NH_4)_6Mo_7O_{24}.4H_2O$) and Cobalt (CoC_{l2})) also depends on the type of variety used.

Table 2. Average plant height, age of flower appearance, number of root nodules, and percentage of effective root nodules on soybean cultivars and the combinations of *Rhizobium* strain *Nod^+Fix^+*, $CaCO_3$ and NH_4-molybdate

Treatment		Plant height (cm)	Age of flower appearance (days)	Number of root nodules (seeds)	Percentage of effective root nodules (%)
Cultivars	V1	83.96^x	32.78^x	40.22^x	62.21^x
	V2	75.62^y	38.96^y	34.64^y	58.28^y
Sub-plots	R0	66.04^a	35.38^a	09.80^a	27.23^a
	R1	82.59^b	36.05^a	26.14^b	67.76^b
	R2	90.16^c	35.66^a	57.22^{cd}	79.76^{cd}
	R3	80.37^b	36.10^a	48.47^c	73.74^c

Note: Numbers followed by the same letter in each column are not significantly different at Duncan's test level =0.05.

One factor affecting growth and production is the species or types of plants related to genotype (Pregitzer et al., 2013).

The results of Duncan's test in Table 2 on the observation of the number of root nodules showed that different treatments gave different results on the number of root nodules. Treatment R_2 showed the highest number of root nodules. This was due to the suitability of the needs for bacterial strain*Nod*$^+$ *Fix*$^+$, Lime ($CaCO_3$), Molybdenum ($(NH_4)_6Mo_7O_{24}.4H_2O$), and Cobalt (CoC_{l2}). Therefore, metabolic processes were increased due to increased assimilate formation, the influence of microelements Molybdenum and Cobalt, which play an essential role in enzyme systems (Bhattacharya et al., 2016). However, the soil analysis results before the study showed that Mo levels were quite low (0.97 ppm. In contrast, the requirement for soybeans was generally around 0.51 - 1.00 ppm (Ibañez et al., 2020). If the concentration of Mo in the soil is low, it will interfere with the formation of root nodules and inhibit N fixation by *Rhizobium*(Egamberdieva et al., 2018). This can be observed from the ability of plants to form effective root nodules. Ismunadji and Mahmud (1993) stated that applying Mo to soybean plantswould increase the number of root nodules.

The increasing in the number of root nodules was caused by an increase in the number of nitrogenation enzymes and nitrate reductase, thereby increasing N fixation. (Imran et al., 2019) stated that Mo is the main component of the nitrogenation enzyme and nitrate reductase, whose preparation mechanism occurs due to a change in valence. Meanwhile, the process of forming root nodules occurs through cooperation between plants and N-fixing bacteria. Nodules that are active in symbiotic N binding, when split, will turn red due to the presence of leghaemoglobin. If the center of the root nodule is not red, it means it is not effective. Furthermore, (Zheng et al., 2020) and (Koskey et al., 2018) explained that root nodules are distinguished into effective and ineffective root nodules, in which the differences between the two root nodules are in size or shape, color, and location on the root.

The results of Duncan's test in Table 2 showed that the R_2 treatment (bacterial strain *Nod*$^+$ *Fix* + MoCo (1.0 : 0.6) kg/ha) produced the highest number of red nodules compared to other treatments. The high results indicated that the R_2 treatment impacted the formation of red root nodules. Therefore, it can infect plant roots in the form of root nodules which are effective in fixing nitrogen from the air. This is presumably due to the ability of inoculation to meet the optimal N requirement for plant growth forming an effective red nodule. In addition, root nodules that are effective at fixing N can be recognized by their reddish color on the inside when the nodules are split. The red color is due to the presence of the pigment leghaemoglobin.

The observation results of the distribution pattern of the root nodules in Table 2 showed the best treatment of R_2. The nodule distribution pattern determines the effectiveness of the nodule. Nodules scattered on the root neck indicate nodules effective in fixing N. The distribution pattern of root nodules explains the effectiveness of root nodules in fixing nitrogen free from the air. Effective root nodules are located on the taproot, while ineffective root nodules are scattered on lateral roots or root branches and are pale in color. The activity of N_2 fixation by bacteria is essential for overall N balance because the continuous form of fixed N can be lost due to denitrification and permeation (Howie & Echandi, 1983).

Nitrogen, phosphorus and potassium uptake

The ANOVA results showed that cultivars (V) had a significant effect on nitrogen (N) and potassium (K) uptake but had no significant effect on Phosphorus (P) uptake. The combinations of *Rhizobium* strain *Nod*$^+$*Fix*$^+$, $CaCO_3$ and NH_4-molybdate (R) significantly affected N, P, and K uptake (Table 3). There was no interaction between the cultivars and the combinations of *Rhizobium* strain *Nod*$^+$*Fix*$^+$, $CaCO_3$, and NH_4-molybdate.

The *Baluran* cultivar (V_1) produced higher N and K uptake, significantly different from the *Willis* cultivar (V_2). However, the P uptake of treatments was not significantly different. According to

Table 3 shows that N, P, and K uptake increased with increasing doses of the combinations ($CaCO_3$ + NH_4-molybdate). The highest increase was obtained in the combination of *Rhizobium* strain *Nod*$^+$ *Fix*$^+$+ $CaCO_3$ 1.5 tons/ha + NH_4-molybdate 500 g/ha of 852.7 mg/plant, 52.4 mg/plant, and 442.9 mg/plant, respectively. However, the results for the combination of *Rhizobium* strain *Nod*$^+$ *Fix*$^+$ + $CaCO_3$ 2.0 tons/ha + NH_4-molybdate 750 g/ha were decreased statistically significantly. The increased uptake of N, P, and K was thought to be the result of the ability of soybean cultivars to form root nodules in

Table 3. Average nutrient uptake of N, P and K in soybean cultivars and combinations of *Rhizobium* strain *Nod^+Fix^+*,$CaCO_3$, and NH_4-molybdate

Treatment		Nutrient uptake (mg/plant)		
		N	P	K
Cultivars	V1	710.6^x	43.1^x	370.0^x
	V2	641.1^y	40.5^x	364.7^y
Sub-plots	R0	385.6^a	33.1^a	244.8^a
	R1	737.3^b	41.2^b	377.3^b
	R2	852.7^c	52.4^c	442.9^{bc}
	R3	727.8^b	40.6^b	405.4^b

Note: numbers followed by the same letter in each column are not significantly different at Duncan's test level = 0.05.

response to *Rhizobium* strain *Nod^+ Fix^+* inoculation. In addition, it was supposed that the number of *Rhizobium* strain *Nod^+ Fix^+* in that treatment was more suitable than others. The root nodules were formed if there was a suitability between *Rhizobium* strain *Nod^+ Fix^+* and its host.

The results of Duncan's test in Table 3 showed that the highest nitrogen uptake was found in the R_2 treatment compared to other treatments (R_0, R_1 and R_3). The significant effect of the R_2 treatment (bacterial strain *Nod Fix* + MoCo (1.0 : 0.6) kg/ha) was thought to result from the soybean's ability to form root nodules in response to the bacterial strain *Nod^+ Fix^+*inoculation. In addition, it is suspected that the number of bacterial strains *Nod^+ Fix^+* in this treatment is more suitable than the other treatments, and effective root nodules would form if there were a suitability between the bacteria and its host.

Leaf color scale

The observation results of the leaf color scale in Table 4 showed that the treatment of bacterial strain *Nod^+Fix^+* with Mo and CaC_O lime supplements on soybeans using ultisol soils affected the leaf color scale. Table 4 shows that the *Willis* and *Baluran* varieties treated with the *Nod^+ Fix^+* bacterial strain, Mo and $CaCo_3$ lime supplements had various leaf color scales.

Table 4 showed that the results on the leaf color scale was the best treatment of R_1 for the *Baluran* cultivar compared to other treatments. This showed that the fixed N element was used to meet the N required by plants so the availability of N for plants increases. An effective fixation, 50–75% of the total N requirement by plants can be fulfilled. In general, this study showed the pattern of increasing plant height, root dry weight, number of root nodules, number of red nodules,

Table 4. Leaf color scale

Varietas	Treatments	Leaf color scales *			
		1	2	3	4
V_1	R_0	x			
	R_1		x		
	R_2			x	
	R_3			x	
V_2	R_0	x			
	R_1				x
	R_2			x	
	R_3			x	

Note: * Criteria based on color scale, diagnosis of nutrient status from leaf color. The strengthening of legumes in relation to cropping system research project. Japan International Cooperation Agency (JICA): 1 – yellowish green, 2 – light green, 3 – green, 4 – slightly dark green.

N uptake, root nodule distribution pattern, leaf color scale, weight of 100 seeds, and dry seed yield. It was found that the treatment of the bacterial strain *Nod^+ Fix^+* with supplements of microelements (MoCo), lime ($CaCO_3$), and Cobalt, simultaneously increased the availability of Mo in the soil so as to increase the uptake of Mo by plants. Jaiswal et al. (2021)stated that the element Mo functions as an activator of nitrogenation and reducing enzymes which activate *Rhizobium* performance in the N fixation process so that N binding increases. Nitrogen can stimulate cell elongation, vegetative growth, enlarge pods, slow down the initiation phase, and increase seeds yield and content (Ohyama et al., 2013). The results of Duncan's test in Table 4 showed that the highest weight of 100 seeds was obtained in the R_2treatment (bacterial strain *Nod^+ Fix^+* + MoCo (1.0 : 0.6) kg/ha) compared to other treatments (R_0, R_1 and R_3). This revealed that the treatment is vital for the growth and production of soybeans.

Table 5. The average weight of 100 seeds, number of pods per plant, and dry seed weight per plant

Treatment		Weight 100 seeds (g)	Number of pods per plant (piece)	Dry seed weight per plant (g)
Cultivars	V1	13.75ᵃ	28.66ᵃ	16.53ᵃ
	V2	11.34ᵃ	26.51ᵃ	15.17ᵃ
Sub-plots	R0	10.37ᵃ	19.18ᵃ	10.99ᵃ
	R1	14.04ᵇ	26.94ᵇ	17.97ᵇᶜ
	R2	14.49ᵇ	35.89ᶜ	19.45ᶜ
	R3	11.88ᵃ	28.34ᵇ	15.00ᵇ

Note: numbers followed by the same letter in each column are not significantly different at Duncan's test level = 0.05.

This was due to the Cofactor Mo (MoCo) forming the active site of all eukaryotic molybdenum enzymes.

Yield components

The ANOVA results showed that cultivars (V) had no significant effect; while the combinations of *Rhizobium* strain *Nod⁺Fix⁺*, $CaCO_3$, and NH_4-molybdate had a significant effect on the weight of 100 seeds, number of pods per plant, and dry seed weight per plant. There was no interaction between the cultivars and the combinations of *Rhizobium* strain *Nod⁺Fix⁺*, $CaCO_3$, and NH_4-molybdate (Table 5).

Table 5 shows that the weight of 100 seeds, number of pods per plant, and dry seed weight per plant were increased with increasing doses of the combinations ($CaCO_3$+ NH_4-molybdate), the highest increase was achieved in the combination of *Rhizobium* strain *Nod⁺ Fix⁺*+ $CaCO_3$ 1.5 tons/ha + NH_4-molybdate 500 g/ha of 14.49 g, 35.89 pieces, and 19.45 g, respectively. However, the results for the combination of *Rhizobium* strain *Nod⁺ Fix⁺* + $CaCO_3$ 2.0 tons/ha + NH_4-molybdate 750 g/ha decreased statistically significantly. Increased vegetative growth continues in the generative growth of plants. The excess photosynthate, as a result of the photosynthesis process,, was utilized to form pods, then stored in the form of seeds. In addition, the increase in N produced by plants will increase the synthesis of amino acids and proteins and plant organic compounds. These organic compounds are deposited on the seeds, increasing the dry seed weight per plant.

At the combination dose of *Rhizobium* strain *Nod⁺ Fix⁺* + $CaCO_3$ 2.0 tons/ha + NH_4-molybdate 750 g/ha, there was a tendency to decrease growth and yield of soybeans, presumably due to the excess of Mo and $CaCO_3$. Based on Table 5, the highest dry seed yield was obtained in treatment R_2 and was significantly different compared to other treatments (R_0 and R_3). This was because the R_2 treatment will increase nitrogen fixation to meet nitrogen needs for plants so that the availability of nitrogen for plants increases. In the R_3 treatment, there was a tendency to decrease the growth and production of soybeans, presumably due to excess (toxicity) of Mo and Co. The elements Mo and Co are micronutrients that are required in relatively low amounts with a narrow optimum range (Maggini et al., 2018). Therefore, its slightly excessive amounts will interfere with plant physiological processes and can even be toxic to plants. According to (Glass et al., 2012), the addition of excessive Mo will not affect the fixation of N.

The results of soil analysis before and after the study showed a change/increase in the content of physical/chemical properties of the soil, including parameters of soil pH, organic matter; N-total; Phosphor; Potassium; CEC; Ca; Mg; Mo and Co. This was due to the administration of the bacterial strain *Nod⁺ Fix⁺* with Micro (MoCo) and Lime ($CaCo_3$) supplementations. In the podzolic marginal soil, the chemical properties were generally low/moderate (Table 1), and in each of these treatments, the *Nod⁺ Fix⁺* strain bacteria, Lime ($CaCO_3$), Molybdenum $(NH_4)_6Mo_7O_{24}.4H_2O$), and Cobalt (CoC_{l2}) contributed to improving the chemical properties of the podzolic soil.

CONCLUSION

This study showed that treating the bacterial strain *Nod⁺ Fix⁺*+ MoCo (1.0 : 0.6) kg/ ha gave the best results on soybean growth and production, both for *Willis* and *Baluran* varieties on Ultisol marginal soils. Increasing the Mo dose will not significantly increase plant growth and yield.

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Dear Authors,

Thank you for the revision manuscript, entitled: Utilization of Dry Land using Molybdenum, Lime, and Rhizobium Strains to Increase Soybean Yield. The following number has been assigned to it JEENG-04241-2023-02.

The manuscript will be rated once again by the Editors and then sent to the Reviewers.

You will be informed by email about any further decision on this article.

Kindest regards,
Justyna Kujawska
Managing Editor
Journal of Ecological Engineering

7. KONFIRMASI PENERIMAAN ARTIKEL

29 MEI 2023

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May 29, 2023
JEENG-04241-2023-02
Utilization of Dry Land using Molybdenum, Lime, and Rhizobium Strains to Increase Soybean Yield

Dear Authors,

I am pleased to inform you that your manuscript, entitled: Utilization of Dry Land using Molybdenum, Lime, and Rhizobium Strains to Increase Soybean Yield, has been accepted with revision references, a few typo and others details.

Thank you for submitting your work to us.

Kindest regards,
Justyna Kujawska
Managing Editor
Journal of Ecological Engineering

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Utilization of Dry Land Using Molybdenum, Lime, and *Rhizobium* Strains to Increase Soybean Yield

Zulkifli Maulana[1], Andi Muhibuddin[1*], Muhammad Arief Nasution[1], Abri Abri[1], Amiruddin Amirudin[1], Baharuddin Baharuddin[2], Andi Tenri Fitriyah[2], Sitti Nurani Sirajuddin[3], Abdel Razzaq M. Al Tawaha[4]

[1] Department of Agrotechnology, Faculty of Agriculture, Bosowa University, Makassar, 90245, Indonesia

[2] Department of Agribusiness, Faculty of Agriculture, Bosowa University Makassar, 90245, Indonesia

[3] Hasanuddin University, Socio-Economics Husbadry Department, Faculty of Animal Science, Makassar, Indonesia

[4] Department of Crop Science, Faculty of Agriculture, Universiti Putra Malaysia 43400 UPM Serdang, Selangor, Malaysia

* Corresponding author's e-mail: muhibuddin@universitasbosowa.ac.id

ABSTRACT

Ultisol is a type of soil with low organic matter, pH, and nutrient content, including molybdenum, leading to low productivity. This study aimed to investigate the use of dry land using molybdenum and lime ($CaCO_3$) inoculated with *Rhizobium* strain *Nod⁺Fix⁺* to increase the soybean production of *Willis* and *Baluran* cultivars. This research was conducted from May to September 2021 in Pallangga Subdistrict, Gowa Regency, South Sulawesi, Indonesia. The study used a split-plot design with three replications for each treatment. The first factor was soybean varieties, consisting of Baluran and *Willis* cultivars. The second factor was the composition of the bacterial strain *Nod⁺ Fix⁺*, lime $CaCO_3$ and NH_4-molybdate, which consisted of without (*Rhizobium* strain *Nod⁺ Fix⁺* + $CaCO_3$ + NH_4-molybdate); *Rhizobium* strain *Nod⁺ Fix⁺* + $CaCO_3$ 1.0 ton/ha + NH_4-molybdate 250 g/h); *Rhizobium* strain *Nod⁺ Fix⁺* + $CaCO_3$ 1.5 tons/ha + NH_4-molybdate 500 g/h); and *Rhizobium* strain *Nod⁺ Fix⁺* + $CaCO_3$ 2.0 tons/ha + NH_4-molybdate 750 g/h). The results showed that treating the bacterial strain *Nod⁺ Fix⁺* + MoCo (1.0:0.6) kg/ha achieved the best results on growth, nutrient uptake (Nitrogen, Phosphorus and Potassium), and soybean yields, both for *Willis* and *Baluran* varieties on ultisol soils.

Keywords: Nod factor; nodulation; legume symbiosis; biological nitrogen fixation; rhizobium strain nod⁺fix⁺.

INTRODUCTION

Indonesia's soybean demand continues to increase along with population growth. This is due to the need for soybeans in the community for consumption, sothe increase in soybean production is one indicator of the success of the agricultural sector. The high demand for soybeans is because it is one of the basic needs and the main source of vegetable protein for most Indonesians. In addition, soybeans are a group of leguminous plants that can fix nitrogen from the air that occurs in root nodules (Al-Tawaha et al., 2022a; Al-Tawaha et al., 2022b; Saranraj et al., 2021; Shiri Janagard and Ebadi-Segherloo, 2015) with an average protein content of 40% based on dry matter (Al-Tawaha and Al-Tawaha, 2017; Cruz-Suárez et al., 2009; Banaszkiewicz, 2011), soybean consumption in Indonesia is 2.2 million tons per year. This figure is far from the average production level of only 819,442 tons per year. As a result, the government must import soybeans as much as 2–2.6 million tons per year. Domestic soybean production is currently only able to meet about 35% of the consumption (Ningrum et al., 2018). The government has made several attempts to increase domestic soybean production, such as by clearing new farming land. Ultisol soil is one of the alternative lands for developing soybean areas in Indonesia, whose distribution reaches 45,794,000 ha or about 25% of the total land area of Indonesia (Syahputra et al., 2015), with great potential to be developed into productive land. However, ultisol soils are poor in organic matter (<5%), low pH, high Al saturation, low base saturation, and organic C, causing nutrient deficiencies, especially molybdenum (Mo) (Fitriatin et al., 2014). On the other hand, the element Mo is needed in the nitrate reductase enzyme to reduce nitrogen in nitrogen fixation in the root nodule tissue of soybean plants (Stiens et al., 2007). The element Mo is a component of the nitrate reductase enzyme, which plays a significant role in the assimilation of nitrogen nitrate (Mmbaga et al., 2015). High acidity (low pH) in ultisol soils and high Al saturation values can be neutralized by liming. Lime ($CaCO_3$) increases pH, neutralizes Al and Fe, and increases soil Ca levels so that it can affect soil potassium levels to achieve balanced nutrient adequacy for soybean plants (Rahman et al., 2018). With a decrease in soil pH, *Rhizobium* bacteria can adapt, i.e., in the low pH range, around pH 5.0–6.5 (Cieśla et al., 2016). This is because root nodules of leguminous plants will only form when *Rhizobium* bacteria are present in the place where legumes grow (Wang et al., 2018). However, air nitrogen fixation does not always occur efficiently due to competition between inoculums which are efficient and inefficient specific *Rhizobium* that exist naturally in the soil (Mitsch et al., 2007; Mengel et al., 2012).

According to Reuhs et al. (2005), several types of *Rhizobium* bacteria are known based on their ability to form nodules, i.e., a) *Rhizobium* which does not have a nodule-forming character on plant roots, so that no symbiosis occurs, is known as a *Nod⁻* strain; b) *Rhizobium* which has nodule-forming character but does not have air nitrogen fixing character, known as *Nod^+Fix^-* strain; and c) *Rhizobium* which has two characters at once as nodule forming and as air nitrogen fixer is known as *Nod^+Fix^+* strain (Salih et al., 2015). Research on the inoculation of *Rhizobium* strains and the application of molybdenum to soybean plants has been carried out by several previous researchers (Lande et al., 2019). However, no research has combined the treatment of molybdenum, $CaCO_3$, NH_4-molybdate with *Rhizobium*Strain *Nod^+Fix^+* in ultisol soils.

The urgency of this research was the breakthrough of soybean production technology innovation by combining molybdenum, $CaCO_3$, NH_4-molybdate with *Rhizobium*strain *Nod^+Fix^+* on ultisol soils which have not been widely used in Indonesia because it is a marginal soil group. In addition, this research can reduce Indonesia's dependence on imported soybeans. Indonesia is currently still importing 2.6 million tons of soybeans from abroad.

This study aimed to investigate the use of dry land using molybdenum and $CaCO_3$ inoculated with *Rhizobium* strain *Nod^+Fix^+* to increase the soybean production of *Willis* and *Baluran* cultivars.

MATERIALS AND METHODS

Experimental design and treatments

This research was conducted in the greenhouse of the Faculty of Agriculture, University of Bosowa, Pallangga Subdistrict, Gowa Regency, South Sulawesi, Indonesia, from May to September 2021. The study was arranged according to a split-plot design with two factors, with three replications for 24 plots, and each plot had 20 plants. Five plants were measured as the samples for each plot. The first factor was soybean varieties consisting of V_1 = *Baluran* and V2 = Willis. The second factor was the composition of the bacterial strain *$Nod^+ Fix^+$*, lime $CaCO_3$ and NH_4-molybdates follows: R_0 = without (*Rhizobium* strain *$Nod^+ Fix^+$* + $CaCO_3$ + NH_4-molybdate); R_1 = *Rhizobium* strain *$Nod^+ Fix^+$* + $CaCO_3$ 24 g/pot + NH_4-molybdate 6 g/pot; R_2 = *Rhizobium* strain *$Nod^+ Fix^+$* + $CaCO_3$ 36 g/pot + NH_4-molybdate 12 g/pot; dan R_3 = *Rhizobium* strain *$Nod^+ Fix^+$* + $CaCO_3$ 48 g/pot + NH_4-molybdate 18 g/pot.

Preparation of growing media, administration of Rhizobium strain Nod^+Fix^+, and treatment of $CaCO_3$ and NH_4-molybdate

The soil used was ultisol soil which was taken compositely at a depth of 0–25 cm and had never been planted with soybeans. The soil was then air-dried and crushed and sieved with a 0.5 cm diameter sieve. The sieved soil was taken sufficiently to determine its physical and chemical properties in the laboratory before the treatments (Table 1). After that, 10 kg of ultisol soil was put into a black polybag with a size of 60 cm and a diameter of 40 cm and $CaCO_3$ was added (according to the treatment). The polybags were arranged in rows with wooden supports, with a distance between rows of 40 cm and between

Table 1. Chemical and physical properties of ultisol soils before and after treatments

Parameters	Content before treatment (R_0)	Content after treatment (R_1)	Content after treatment (R_2)	Content after treatment (R_3)
pH (1:2,5) H_2O	4.9	5.50	5.65	5.70
C-organic (%)	0.82	0.83	0.83	0.84
N-Total (%)	0.18	0.28	0.36	0.38
Available-P (ppm)	10.00	10.20	10.37	10.48
K-dd (me/100 g)	0.32	0.43	0.47	0.51
CEC (me/100 g)	15. 07	15.10	16.10	16.20
Base saturation (%)	15.15	16.10	16.12	16.40
Al-dd (me/100 g)	1.92	10.20	10.20	10.20
Mo (ppm)	0.35	1.51	1.69	2.10
Al saturation	22.64	22.12	21.78	21.60
Fraktion – sand (%)	55.23	55.21	55.21	55.10
Dust (%)	24.10	24.12	24.13	24.32
Clay (%)	20.67	20.67	20.66	20.58
Tekstur	Sandy clay loam	Sandy clay loam	Sandy clay loam	Sandy clay loam

rows of 25 cm. One week later, 50 g/pot. Then pots were given with basic fertilizers of Urea, TSP and KCl each dose of 0.25; 50 and 0.25 g/pot (50, 100 and 50 kg/ha). 7.5 mL *Rhizobium* strain *Nod*$^+$ *Fix*$^+$ was mixed with 1 kg of soybean seeds. The mixture was then moistened with water, drained, mixed well, and stored in a shady place. It was left for 15 minutes and then was planted. The treatment of NH_4-molybdate as a source of Mo (containing 82% acid MoO_3) (according to the treatment) was given at the planting time by dissolving each in 10 ml of water. The solution was sprinkled into polybags around the plants and then sprayed on the plants once every two weeks. After one week, the plants were thinned so only one plant/polybag was maintained until the plants were harvested. After the plants were harvested, the soil residue from the polybags was taken compositely at a depth of 0–25 cm to analyze its physical and chemical properties in the laboratory.

Maintenance and harvest

Watering was performed daily by maintaining the groundwater condition at around 80% of field capacity. Thinning was carried out after the plants were 7 days old by removing one plant per polybag. Plant maintenance included embroidery, weeding, and hoarding. Plants were harvested after about three months of age. The characteristics of soybean plants that were ready to harvest were that, after all, the plant leaves were old or yellow.

Observation and measurement

Vegetative growth observation included plant height, age of flower appearance, number of root nodules, percentage of effective root nodules, and nitrogen (N) and potassium(K) uptake. Meanwhile, observations for the generative phase were the weight of 100 seeds, the number of pods per plant, and dry seed weight per plant.

Data analysis

The data from the experiment were statistically analyzed using SPSS version 16 software. The differences among treatments were compared using analysis of variance (ANOVA) with the least significant difference (LSD) posthoc test at a 5% probability level.

RESULTS AND DISCUSSION

Soil chemical and physical properties

The soil analysis results before (without treatment) and after the experiment (soil treatment results of mixed R_1, R_2 and R_3) showed a change in the chemical properties of the soil. In general, there was a change in the chemical properties of the soil, i.e., pH, organic matter, total nitrogen (N), available phosphorus (P), potassium (K), Cation-exchange capacity (CEC), Ca, Mg, Mo, and Al. This was due to the treatment of *Rhizobium* strain

Nod⁺ Fix⁺, supplementation of microelements Mo (NH_4-molybdate), and lime $CaCO_3$ in ultisol soils which contributed to improving the chemical properties of the soil. Application of NH_4-molybdate increased Mo from 0.35 ppm to 0.47 ppm. The application of lime $CaCO_3$ increased soil pH from 5.1 to 5.5. This occurred due to the ability of lime $CaCO_3$ to neutralize soil acidity through the mechanism of H^+ substitution by Ca^{2+}. This condition provides a way for the availability of phosphorus freely through mineralization by soil biota in the solution. The basic reason for liming is to increase soil pH. As a result of changes in pH, changes in soil chemistry and biology occur, such as increased availability of phosphorus, nitrogen, potassium, calcium, and magnesium.

Vegetative growth

The ANOVA results showed that cultivars (V) and the combinations of *Rhizobium* strain *Nod^+Fix^+*, $CaCO_3$ and NH_4-molybdate (R) had a significant effect on plant height, age of flower appearance, and number of root nodules, and percentage of effective root nodules (Table 2). There was no interaction between the cultivars and the combinations of *Rhizobium* strain *Nod^+Fix^+*, $CaCO_3$ and NH_4-molybdate. The *Baluran* cultivar (V1) produced higher plant height, age of flower emergence, number of root nodules, and percentage of effective nodules. This was significantly different from the *Willis* cultivar (V2). These differences were due to the different characteristics of *Baluran* and *Willis* cultivars that affect photosynthetic activity, which determines plant height growth, flower appearance age, number of root nodules, and percentage of effective root nodules. Pregitzer et al. (2013) explained that some factors that influence plant growth and production are the environment, species or types of plants related to the genotype.

Table 2 shows that plant height, age of flower appearance, number of root nodules, and percentage of effective root nodules were increased with increasing doses of the combinations ($CaCO_3$+ NH_4-molybdate). The highest increase was obtained in the combination of *Rhizobium* strain *Nod^+ Fix^+* + $CaCO_3$ 1.5 tons/ha + NH_4 -molybdate 500 g/ha of 90.16 cm, 35.66 days, 57.22 seeds, and 79.76 (%), respectively. However, the results for the combination of *Rhizobium* strain *Nod^+ Fix^+* + $CaCO_3$ 2.0 tons/ha+NH_4-molybdate 750 g/ha were decreased statistically significantly. The increase in plant height, age of flower appearance, number of root nodules, and percentage of effective root nodules was supported by the availability of Mo for *Rhizobium* strain *Nod^+ Fix^+* by Mo administration. According to Schwarz and Mendel (2006), Mo is not only essential for plants, but also essential for microorganisms. Furthermore, more than 40 Mo enzymes are found in nature, most of which are found in bacteria, but only four Mo enzymes are found in plants. The increase in the availability of Mo in the soil has stimulated the growth, development, and activity of *Rhizobium* bacteria. Therefore,it increased the ability of *Rhizobium* bacteria to infect root nodules, resulting in a higher percentage of effective root nodules formed. Effective root nodules contain *Rhizobium* bacteria that can fix air N_2.

Based on the results of Duncan's test in Table 2, the observation of plant height showed that the *Willis* cultivar achieved a higher plant height and was significantly different compared to the *Baluran* cultivar, both at the age of 2, 4, and 6 (weeks after planting) WAP, as well as significant differences in each treatment (R_0, R_1, R_2, and R_3). This was because the effectiveness of each treatment (bacterial strain *Nod^+ Fix^+*, Lime ($CaCO_3$), Molybdenum ($(NH_4)6Mo_7O_{24}.4H_2O$) and Cobalt (CoC_{l2})) also depends on the type of variety used.

Table 2. Average plant height, age of flower appearance, number of root nodules, and percentage of effective root nodules on soybean cultivars and the combinations of *Rhizobium* strain *Nod^+Fix^+*, $CaCO_3$ and NH_4-molybdate

Treatment		Plant height (cm)	Age of flower appearance (days)	Number of root nodules (seeds)	Percentage of effective root nodules (%)
Cultivars	V1	83.96^{x}	32.78^{x}	40.22^{x}	62.21^{x}
	V2	75.62^{y}	38.96^{y}	34.64^{y}	58.28^{y}
Sub-plots	R0	66.04^{a}	35.38^{a}	09.80^{a}	27.23^{a}
	R1	82.59^{b}	36.05^{a}	26.14^{b}	67.76^{b}
	R2	90.16^{c}	35.66^{a}	57.22^{cd}	79.76^{cd}
	R3	80.37^{b}	36.10^{a}	48.47^{c}	73.74^{c}

Note: Numbers followed by the same letter in each column are not significantly different at Duncan's test level =0.05.

One factor affecting growth and production is the species or types of plants related to genotype (Pregitzer et al., 2013).

The results of Duncan's test in Table 2 on the observation of the number of root nodules showed that different treatments gave different results on the number of root nodules. Treatment R_2 showed the highest number of root nodules. This was due to the suitability of the needs for bacterial strain*Nod⁺ Fix⁺*, Lime ($CaCO_3$), Molybdenum ($NH_4$6Mo_7O_{24}.4H_2O), and Cobalt (CoC_{l2}). Therefore, metabolic processes were increased due to increased assimilate formation, the influence of microelements Molybdenum and Cobalt, which play an essential role in enzyme systems (Bhattacharya et al., 2016). However, the soil analysis results before the study showed that Mo levels were quite low (0.97 ppm. In contrast, the requirement for soybeans was generally around 0.51 - 1.00 ppm (Ibañez et al., 2020). If the concentration of Mo in the soil is low, it will interfere with the formation of root nodules and inhibit N fixation by *Rhizobium*(Egamberdieva et al., 2018). This can be observed from the ability of plants to form effective root nodules. Ismunadji and Mahmud (1993) stated that applying Mo to soybean plantswould increase the number of root nodules.

The increasing in the number of root nodules was caused by an increase in the number of nitrogenation enzymes and nitrate reductase, thereby increasing N fixation. (Imran et al., 2019) stated that Mo is the main component of the nitrogenation enzyme and nitrate reductase, whose preparation mechanism occurs due to a change in valence. Meanwhile, the process of forming root nodules occurs through cooperation between plants and N-fixing bacteria. Nodules that are active in symbiotic N binding, when split, will turn red due to the presence of leghaemoglobin. If the center of the root nodule is not red, it means it is not effective. Furthermore, (Zheng et al., 2020) and (Koskey et al., 2018) explained that root nodules are distinguished into effective and ineffective root nodules, in which the differences between the two root nodules are in size or shape, color, and location on the root.

The results of Duncan's test in Table 2 showed that the R_2 treatment *(*bacterial strain *Nod⁺ Fix* + MoCo (1.0 : 0.6) kg/ha) produced the highest number of red nodules compared to other treatments. The high results indicated that the R_2 treatment impacted the formation of red root nodules. Therefore, it can infect plant roots in the form of root nodules which are effective in fixing nitrogen from the air. This is presumably due to the ability of inoculation to meet the optimal N requirement for plant growth forming an effective red nodule. In addition, root nodules that are effective at fixing N can be recognized by their reddish color on the inside when the nodules are split. The red color is due to the presence of the pigment leghaemoglobin.

The observation results of the distribution pattern of the root nodules in Table 2 showed the best treatment of R_2. The nodule distribution pattern determines the effectiveness of the nodule. Nodules scattered on the root neck indicate nodules effective in fixing N. The distribution pattern of root nodules explains the effectiveness of root nodules in fixing nitrogen free from the air. Effective root nodules are located on the taproot, while ineffective root nodules are scattered on lateral roots or root branches and are pale in color. The activity of N_2 fixation by bacteria is essential for overall N balance because the continuous form of fixed N can be lost due to denitrification and permeation (Howie & Echandi, 1983).

Nitrogen, phosphorus and potassium uptake

The ANOVA results showed that cultivars (V) had a significant effect on nitrogen (N) and potassium (K) uptake but had no significant effect on Phosphorus (P) uptake. The combinations of *Rhizobium* strain *Nod⁺Fix⁺*, $CaCO_3$ and NH_4-molybdate (R) significantly affected N, P, and K uptake (Table 3). There was no interaction between the cultivars and the combinations of *Rhizobium* strain *Nod⁺Fix⁺*, $CaCO_3$, and NH_4-molybdate. The *Baluran* cultivar (V_1) produced higher N and K uptake, significantly different from the *Willis* cultivar (V_2). However, the P uptake of treatments was not significantly different. According to

Table 3 shows that N, P, and K uptake increased with increasing doses of the combinations ($CaCO_3$ + NH_4-molybdate). The highest increase was obtained in the combination of *Rhizobium* strain *Nod⁺ Fix⁺* + $CaCO_3$ 1.5 tons/ha + NH_4-molybdate 500 g/ha of 852.7 mg/plant, 52.4 mg/plant, and 442.9 mg/plant, respectively. However, the results for the combination of *Rhizobium* strain *Nod⁺ Fix⁺* + $CaCO_3$ 2.0 tons/ha + NH_4-molybdate 750 g/ha were decreased statistically significantly. The increased uptake of N, P, and K was thought to be the result of the ability of soybean cultivars to form root nodules in

Table 3. Average nutrient uptake of N, P and K in soybean cultivars and combinations of *Rhizobium* strain *Nod⁺Fix⁺*, $CaCO_3$, and NH_4-molybdate

Treatment		Nutrient uptake (mg/plant)		
		N	P	K
Cultivars	V1	710.6ᵃ	43.1ᵃ	370.0ᵃ
	V2	641.1ᵇ	40.5ᵃ	364.7ᵇ
Sub-plots	R0	385.6ᵃ	33.1ᵃ	244.8ᵃ
	R1	737.3ᵇ	41.2ᵇ	377.3ᵇ
	R2	852.7ᶜ	52.4ᶜ	442.9ᵇᶜ
	R3	727.8ᵇ	40.6ᵇ	405.4ᵇ

Note: numbers followed by the same letter in each column are not significantly different at Duncan's test level = 0.05.

response to *Rhizobium* strain *Nod⁺ Fix⁺* inoculation. In addition, it was supposed that the number of *Rhizobium* strain *Nod⁺ Fix⁺* in that treatment was more suitable than others. The root nodules were formed if there was a suitability between *Rhizobium* strain *Nod⁺ Fix⁺* and its host.

The results of Duncan's test in Table 3 showed that the highest nitrogen uptake was found in the R_2 treatment compared to other treatments (R_0, R_1 and R_3). The significant effect of the R_2 treatment (bacterial strain *Nod⁺ Fix⁺* + MoCo (1.0 : 0.6) kg/ha) was thought to result from the soybean's ability to form root nodules in response to the bacterial strain *Nod⁺ Fix⁺*inoculation. In addition, it is suspected that the number of bacterial strains *Nod⁺ Fix⁺* in this treatment is more suitable than the other treatments, and effective root nodules would form if there were a suitability between the bacteria and its host.

Leaf color scale

The observation results of the leaf color scale in Table 4 showed that the treatment of bacterial strain *Nod⁺Fix⁺* with Mo and $CaCo_3$ lime supplements on soybeans using ultisol soils affected the leaf color scale. Table 4 shows that the *Willis* and *Baluran* varieties treated with the *Nod⁺ Fix⁺* bacterial strain, Mo and $CaCo_3$ lime supplements had various leaf color scales.

Table 4 showed that the results on the leaf color scale was the best treatment of R_1 for the *Baluran* cultivar compared to other treatments. This showed that the fixed N element was used to meet the N required by plants so the availability of N for plants increases. An effective fixation, 50–75% of the total N requirement by plants can be fulfilled. In general, this study showed the pattern of increasing plant height, root dry weight, number of root nodules, number of red nodules,

Table 4. Leaf color scale

Varietas	Treatments	Leaf color scales *			
		1	2	3	4
V_1	R_0	x			
	R_1		x		
	R_2			x	
	R_3			x	
V_2	R_0	x			
	R_1				x
	R_2			x	
	R_3			x	

Note: * Criteria based on color scale, diagnosis of nutrient status from leaf color. The strengthening of legumes in relation to cropping system research project. Japan International Cooperation Agency (JICA): 1 – yellowish green, 2 – light green, 3 – green, 4 – slightly dark green.

N uptake, root nodule distribution pattern, leaf color scale, weight of 100 seeds, and dry seed yield. It was found that the treatment of the bacterial strain *Nod⁺ Fix⁺* with supplements of microelements (MoCo), lime ($CaCO_3$), and Cobalt, simultaneously increased the availability of Mo in the soil so as to increase the uptake of Mo by plants. Jaiswal et al. (2021)stated that the element Mo functions as an activator of nitrogenation and reducing enzymes which activate *Rhizobium* performance in the N fixation process so that N binding increases. Nitrogen can stimulate cell elongation, vegetative growth, enlarge pods, slow down the initiation phase, and increase seeds yield and content (Ohyama et al., 2013). The results of Duncan's test in Table 4 showed that the highest weight of 100 seeds was obtained in the R_2 treatment (bacterial strain *Nod⁺ Fix⁺* + MoCo (1.0 : 0.6) kg/ha) compared to other treatments (R_0, R_1 and R_3). This revealed that the treatment is vital for the growth and production of soybeans.

Table 5. The average weight of 100 seeds, number of pods per plant, and dry seed weight per plant

Treatment		Weight 100 seeds (g)	Number of pods per plant (piece)	Dry seed weight per plant (g)
Cultivars	V1	13.75^{a}	28.66^{a}	16.53^{a}
	V2	11.34^{a}	26.51^{a}	15.17^{a}
Sub-plots	R0	10.37^{a}	19.18^{a}	10.99^{a}
	R1	14.04^{b}	26.94^{b}	17.97^{bc}
	R2	14.49^{b}	35.89^{c}	19.45^{c}
	R3	11.88^{a}	28.34^{b}	15.00^{b}

Note: numbers followed by the same letter in each column are not significantly different at Duncan's test level = 0.05.

This was due to the Cofactor Mo (MoCo) forming the active site of all eukaryotic molybdenum enzymes.

Yield components

The ANOVA results showed that cultivars (V) had no significant effect; while the combinations of *Rhizobium* strain *Nod⁺Fix⁺*, $CaCO_3$, and NH_4-molybdate had a significant effect on the weight of 100 seeds, number of pods per plant, and dry seed weight per plant. There was no interaction between the cultivars and the combinations of *Rhizobium* strain *Nod⁺Fix⁺*, $CaCO_3$,and NH_4-molybdate (Table 5).

Table 5 shows that the weight of 100 seeds, number of pods per plant, and dry seed weight per plant were increased with increasing doses of the combinations ($CaCO_3$+ NH_4-molybdate), the highest increase was achieved in the combination of *Rhizobium* strain *Nod⁺ Fix⁺*+ $CaCO_3$ 1.5 tons/ha + NH_4-molybdate 500 g/ha of 14.49 g, 35.89 pieces, and 19.45 g, respectively. However, the results for the combination of *Rhizobium* strain *Nod⁺ Fix⁺* + $CaCO_3$ 2.0 tons/ha + NH_4-molybdate 750 g/ha decreased statistically significantly. Increased vegetative growth continues in the generative growth of plants. The excess photosynthate, as a result of the photosynthesis process,, was utilized to form pods, then stored in the form of seeds. In addition, the increase in N produced by plants will increase the synthesis of amino acids and proteins and plant organic compounds. These organic compounds are deposited on the seeds, increasing the dry seed weight per plant.

At the combination dose of *Rhizobium* strain *Nod⁺ Fix⁺* + $CaCO_3$ 2.0 tons/ha + NH_4-molybdate 750 g/ha, there was a tendency to decrease growth and yield of soybeans, presumably due to the excess of Mo and $CaCO_3$. Based on Table 5, the highest dry seed yield was obtained in treatment R_2 and was significantly different compared to other treatments (R_0 and R_3). This was because the R_2 treatment will increase nitrogen fixation to meet nitrogen needs for plants so that the availability of nitrogen for plants increases. In the R_3 treatment, there was a tendency to decrease the growth and production of soybeans, presumably due to excess (toxicity) of Mo and Co. The elements Mo and Co are micronutrients that are required in relatively low amounts with a narrow optimum range (Maggini et al., 2018). Therefore, its slightly excessive amounts will interfere with plant physiological processes and can even be toxic to plants. According to (Glass et al., 2012), the addition of excessive Mo will not affect the fixation of N.

The results of soil analysis before and after the study showed a change/increase in the content of physical/chemical properties of the soil, including parameters of soil pH, organic matter; N-total; Phosphor; Potassium; CEC; Ca; Mg; Mo and Co. This was due to the administration of the bacterial strain *Nod⁺ Fix⁺* with Micro (MoCo) and Lime ($CaCo_3$) supplementations. In the podzolic marginal soil, the chemical properties were generally low/moderate (Table 1), and in each of these treatments, the *Nod⁺ Fix⁺* strain bacteria, Lime ($CaCO_3$), Molybdenum $(NH_4)_6Mo_7O_{24}.4H_2O)$, and Cobalt ($CoC_{l2)}$ contributed to improving the chemical properties of the podzolic soil.

CONCLUSION

This study showed that treating the bacterial strain *Nod⁺ Fix⁺*+ MoCo (1.0 : 0.6) kg/ ha gave the best results on soybean growth and production, both for *Willis* and *Baluran* varieties on Ultisol marginal soils. Increasing the Mo dose will not significantly increase plant growth and yield.

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