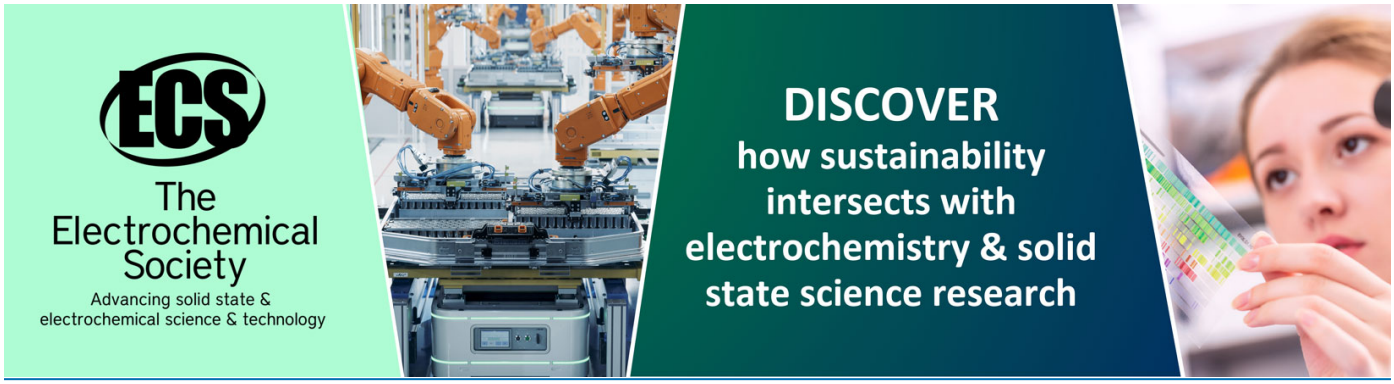
The effect of sulfur and *Rhizobium sp* application to the growth and nodulation of *Mucuna bracteata*



The effect of sulfur and *Rhizobium sp* application to the growth and nodulation of *Mucuna bracteata*

M Wahyuni[1*], M Sembiring[1], Wagino[1] and H G Sihite[2]

[1]Faculty of Agriculture, Universitas Sumatera Utara, Medan, Sumatera Utara, Indonesia.
[2]Sekolah Tinggi Ilmu Pertanian Agrobisnis Perkebunan, Medan, Sumatera Utara, Indonesia.

E-mail: *mardiana.wahyuni59@yahoo.co.id

Abstract. Planting of legumes cover crop, *Mucuna bracteata*, aims to reduce erosion, increase organic matter, improve soil structure and increase nutrient N. Factors that influence N fixation are food sources, rainfall, sunlight, pH, presence of toxic and competitors. The pH value of the soil is related to the Sulfur element S (sulfur). The study was conducted with 4x4 factorial design with 3 replications. The first factor in the dosage of sulfur consists of S0 (0g), S1 (30g), S2 (60g), S3 (90g) per plant. The second factor is the dose of *Rhizobium sp*, I0 (0ml), I1 (0.3ml), I2 (0.6ml), I3 (0.9ml), every 1 ml contains 10^6 cells. Observations were length of tendrils, number of leaves, number of nodules and levels of N. The results of treatment S significantly affected the length of tendrils, number of leaves, number of root nodules, most in S3, 127.50 and soil pH. . Treatment I significantly affected the increase in the number of root nodules, in I3 it increased 86.93%. N levels of all treatments in the high category. The highest N level is 4.17% for S3I2 treatment (90g sulfur and 0.6ml *Rhizobium sp*).

1. Introduction

In the management of oil palm plantations, the policy of planting legumes cover crop *Mucuna bracteata* aim was to reduce erosion, enrich organic matter, improve soil structure, reduce weed growth and increase N nutrient [1]. *Mucuna bracteata* fixates free N from the air through symbiosis with *Rhizobium sp*. Bacteria belong to the *Rhizobium* genus live freely in the soil and in the root region of legumes and non-legumes [2].

Several factors that influence the by *Rhizobium sp* activity are the presence of food sources, sunlight, soil pH, temperature, presence of toxic substances and other competitors [3]. The large population of *Rhizobium sp* bacteria in the soil varies depending on the type of soil, physical properties, chemical properties and the existence of previous legume planting [4].

The pH (acidity) condition of the soil is closely related to sulfur or sulfate compounds found in the soil. Sulfur acts as an essential nutrient and negatively affects soil pH reactions or lowers pH.

In this research a combination of sulfur (S) dose and *Rhizobium sp* dose was used with the aim of this study to determine the effect of sulfur (S), and *Rhizobium sp* application and its interaction on growth, nodule formation and N levels of *Mucuna bracteata* leaves.

2. Materials and Method

2.1. Experimental design

The research was conducted at STIPAP's experimental garden from April to August 2017. The tools used were soil sieves, scales, polybags, hoes and other supporting tools. The ingredients used are *Mucuna bracteata* seeds, top soil, sulfur, and *Rhizobium sp* from USU Biology Laboratory.

The design used was factorial randomized block design with 2 factors: The first factor is the dosage of sulfur with 4 levels, namely: S0, S1, S2, S3, each 0g, 30g, 60g and 90g sulfur/plant. The second factor is the inoculums of *Rhizobium sp* (10^6 cell/ ml), namely: I0, I1, I2 and I3 with a dose of application 0 ml, 0.3 ml, 0.6 ml and 0.9 ml of *Rhizobium sp*/ plant. The number of replications is 3x, the number of plants per treatment 2, the total number of plants is 96 plants.

Parameter testing with the F test and the treatment which significantly affected the Least Significant Different (LSD) with a level of 0.05 and 0.01.

2.2. Research implementation

Soil as a planting media that has been sieved as much as 6 kg of soil was inserted into a polybag with a base fertilizer of 10g RP / polybag. Seeds that have been seeded before are planted in polybags that have been filled with soil. The growth of the tendrils was directed upward by placing the slices of bamboo. Inoculation of *Rhizobium sp* bacteria was carried out when the seedlings were 2 weeks old by dropping the roots of *Mucuna bracteata* roots. Plants watering were done twice a day.

The observed parameters were: the length of *Mucuna bracteata* tendrils, number of leaves (trifoliate), soil pH, calculation of the number of root nodules at the end of the study and N-level analysis of leaves with the Kjehdahl method.

3. Results and Discussion

3.1. Length of tendrils

The results of observations and LSD tests are presented in Table 1.

Table 1. Recapitulation of observation length of tendrils (cm) *Mucuna bracteate.*

Treatment	4 WAP	6 WAP	8 WAP	10 WAP	%
S0	83.85	114.93	178.45 a	291.34 a	100
S1	88.57	128.12	213.30 b	326.68 b	126.97
S2	97.38	140.6	221.93 cb	327.56 cb	133.17
S3	104.98	151.57	235.49 cd	369.30 cd	146.8
I0	98.20	128.71	202.25	304.2	100
I1	91.47	135.74	213.84	321.38	126.97
I2	93.10	133.01	212.23	340.38	133.17
I3	92.03	137.77	220.85	348.93	146.8
Average	93.70	133.81	212.29	328.72	
+	0	40.11	78.49	116.43	
S	NS	NS	*	*	
I	NS	NS	NS	NS	
S X I	NS	NS	NS	NS	

*, ** significant at p<0.05 and 0.01 and NS: Not significant, means in a column followed by a common letter are not significantly different at 0.05 level by LSD and WAP: Week After Planting.

The increase of 4 weeks after planting (WAP) to 10 WAP was 328.72 cm, for 6 weeks it increased 235.02 cm or 5.59 cm/day. The treatment of sulfur (S) significantly affected the length of tendrils 8 WAP and 10 WAP observation, the highest value was S3 treatment, which was 369.30 cm increased by 56.82% compared to controls. This was in accordance with the research of Tuherkih et al [5] who reported that S fertilizer can increase plant height and fresh forage.

The Inoculum (I) treatment has no significant effect. The longest tendril length at 10 MST was I3 treatment which was 348.93 cm with an increase in tendrils of 46.8%. Rahmadhani [6] suggested that the *Rhizobium* had no significant effect on plant height. Statistical tests on the combination treatment of sulfur and *Rhizobium sp* did not have a significant effect. Wahyuni and Saragih [7] suggested the importance of good planting media (eg biocharcoal) to optimize tendril growth, number of leaves and formation of *Mucuna bracteata* root nodules.

3.2. Number of leaves (trifoliate)

The recapitulation and statistical analysis of the number of leaves is presented in Table 2.

Table 2. Recapitulation of observation results of *Mucuna bracteata* leaves.

Treatment	4 WAP	6 WAP	8 WAP	10 WAP	%
S0	12.67	19.33	26.68	36.42 A	100
S1	13.54	20.08	27.17	39.25 B	107.77
S2	13.33	19.46	28.00	42.83 C	117.6
S3	13.63	20.81	30.53	47.54 D	130.53
I0	13.13	19.25	27.19	40.25	100
I1	12.13	19.31	28.01	41.13	102.19
I2	14.25	21	28.46	42.21	104.87
I3	13.67	20.13	28.71	42.46	105.49
Average	13.30	19.92	28.09	41.51	
+	0	6.63	8.17	13.42	
S	NS	NS	**	**	
I	NS	NS	NS	NS	
S X I	NS	NS	NS	NS	

*, ** significant at p<0.05 and 0.01 and NS: Not significant, means in a column followed by a common letter are not significantly different at 0.05 level by LSD and WAP: Week After Planting.

The average rate of the leaves at 4 WAP was 13.30 stalks, and on the 10 weeks after planting was 41.51 growth stalks or 4.70 stalks/week.

Sulfur (S) application had a very significant effect on the number of leaves at 8 WAP and 10 WAP observations, with the highest number of leaves was S3 treatment, increased by 30.53% compared to controls. According to Goeswono [8]. S is a constituent of the amino acids methionine and cysteine. The protein structure is largely determined by the S cluster and this element is an important nutrient needed for the production of chlorophyll in the leaves.

Inoculum (I) treatment had no significant effect on the number of leaves. The highest number of leaves compared to control in the Inoculum treatment was I3 with an increase in the number of leaves by 5.49%. Combination treatment has no significant effect on the number of leaf parameters.

3.3. Number of root nodules, pH and levels of N Mucuna bracteate
Observation results are found in Table 3.

Table 3. Recapitulation of observations on the number of root nodules, pH, and levels of N *Mucuna bracteate*.

Treatment	Root Nodule		pH		N Level		
	Value	%	Value	%	Value	Categories	%
S0	40.57A	100	7.60	100	3.25	T	100
S1	46.08A	114	6.77	89	3.40	T	105
S2	67.04 B	165	6.27	83	3.43	T	106
S3	127.50 C	314	5.92	78	3.85	T	118
I0	55.08 a	100	6.67	100	3.41	T	100
I1	49.25 a	93	6.65	100	3.37	T	99
I2	71.96 b	131	6.67	100	3.73	T	109
I3	102.97 c	187	6.64	99	3.42	T	100
Average	79.39		6.64		3.48	T	
S	**						
I	*						
S X I	NS						

*, ** significant at p<0.05 and 0.01 and NS: Not significant, means in a column followed by a common letter are not significantly different at 0.05 level by LSD and WAP: Week After Planting, T = High.

The average number of root nodules was 79.39. Sulfur (S) application had a very significant effect on the number of root nodules, with the highest number was in S treatment, of 127.50, increased by 214% compared to controls. According to Tisdale et al [9]. Sulfur can also stimulate root and fruit formation and can reduce disease attacks.

In treatment I0 the number of root nodules was 55.08, indicated that inside the soil already contained Rhizobium bacteria. Yuwono [4] suggested that *Rhizobium sp* is a facultative symbiont, which is found in almost all agricultural land. The inoculum application in each plant were effective and nodule formation only takes 8 weeks after planting. In Wahyuni [10] research, the same *Rhizobium sp* inoculum (from the USU Laboratory) was also effective against root nodule formation in 12 WAP observations. The planting material used in this research was from seeds and was able to produce more root nodules than those from cuttings [11]. The highest number of root nodules was I3 treatment, which was 102.97 nodules with an increase of 86.93% compared to I0 (Figure 1).

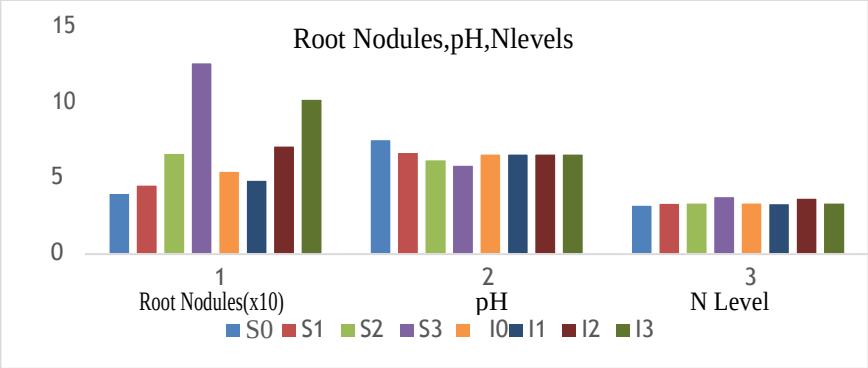


Figure 1. Effect of treatment on root nodules, pH, and levels of N.

According to Nugroho et al [12] the formation of *Mucuna bracteata* root nodules naturally is 2 years. Sari et al [13] also suggested the rule of Rhizobium on N Fixation The presence of isolates showed a very effective effect in producing a large number of root nodules. Combination treatment has no significant effect on root nodule formation.

The highest pH value was in S0 treatment which was 7.6 and the lowest pH was in S3 which was 5.9 values. High pH in planting media was caused by the application of RP basic fertilizer. Sulfur application lowered soil pH; in S3 treatment the pH value is 5.92. *Rhizobium sp* bacteria is resistant to mildly acidic conditions (pH 5.8) because the life ability of *Rhizobium sp* bacteria has the ability to maintain intracellular pH (pHi) between 7.2 and 7.4 when its external pH (pHe) low (pH 5.6). This is supported based on the results of Widyasari's [14] research from the endurance test of *Rhizobium sp* bacteria on the planting media pH (pH 5.5 and pH 7.0), the results showed that *Rhizobium sp* was more active at media pH of 5.8 compared to pH 7.0 which indicated that *Rhizobium sp* is able to form ATR (acid tolerance response).

The analysis results of N content of *Mucuna bractetata* leaves were all included in the high category. In line with what was stated by Harahap et al [15] *Mucuna bracteata* is a good source of nitrogen. *Mucuna bracteata* can absorb free N in the air through symbiosis with *Rhizobium sp* bacteria so that N can be available to plants. According to Ngoma et al [16] the use of inoculants has a direct impact on plants through increasing nutrient uptake and N2 mooring. Consistently with the observation of the number of root nodules, the highest N concentration was found in S3I2 treatment, which was 4.17%, increased by 27% compared to S0I0.

4. Conclusions

Based on this research, it can be concluded that the application of sulfur (S) had no significant effect on the length of tendrils and the number of leaves, but significantly affected the number of root nodules and soil pH. *Rhizobium sp* inoculum treatment significantly affected the number of root nodules. Leaves nutrient content were all included in the high category and the best treatment was S3I2, which was 4.17%, an increase of 27% compared to S0I0.

References

[1] Siagian N 2001 *Potensi dan Pemanfaatan Mucuna bracteata Sebagai Kacangan Penutup Tanah Di Perkebunan Karet [Potential and Utilization of Mucuna bracteata as Legumes Cover Crop in Rubber Plantation]* (Medan: Warta Rubber Research Institute) **20** 1-3 pp 32-43

[2] Ahemad M and Kibret M 2014 Mechanisme and application of plant growth promoting Rhizobacteria: current perspective *Journal of King Saud University-Science* **26** pp 1-20

[3] Subba R N S 1994 *Mikroorganisme tanah dan pertumbuhan tanaman [Soil microorganisms and plant growth] ed ii* (Jakarta: UI-Press)
[4] Yuwono T 2006 *Bioteknologi Pertanian [Agricultural Biotechnology]* (Yogyakarta: Gajah Mada University Press)
[5] Tuherkih E, Wigeno I G P, Purnomo J and Santoso D 1998 *Pengaruh pupuk belerang sifat kimia tanah dan hasil hijauan pakan ternak pada padang penggembalaan [Effect of sulfur fertilizer on chemical properties soil and biology soil]* (Bogor: Puslitbang) pp 283-91
[6] Rahmadani E 2009 *Respon pertumbuhan dan produksi kedelai (Glycine Max* (L) Merrill*) terhadap perbedaan waktu tanam dan inokulasi Rhizobium [Response of soybean on growth and production (Glycine Max* (L) Merrill*) to differences in planting time and Rhizobium inoculation]* (Medan, Seminar Hasil Fakultas Pertanian Universitas Sumatera Utara)
[7] Wahyuni M and Saragih M R 2017 Efektivitas biocharcoal dan Rhizobium sp terhadap nodulasi Mucuna bracteata asal biji dan stek [Effectiveness of biocharcoal and Rhizobium sp on nodulation of Mucuna bracteata from seeds and cuttings] *Proceeding SENASMUDI ISSN:978602039614* Universitas Asahan **1** 1 pp 344-49
[8] Goeswono S 1983 *Sifat dan ciri tanah [Properties and characteristics of soil]* (Bogor: Department of Soil Sciences Bogor Institut Pertanian Bogor)
[9] Tisdale S L, Nelson W L and Beaton J D 1985 *Soil fertility and fertilizers 4th ed* (New York: Mac Millan Publishing Company)
[10] Wahyuni M 2016 Efektivitas inokulasi bakteri *rhizobium sp* terhadap nodulasi mucuna bracteata monokultur dan biokultur dengan tanaman kacang hijau (*Phaseolus radiates L*) [Effectiveness of *Rhizobium sp* Bacteria Inoculation on Mucuna bracteata Nodulation of Monocultures and Biocultures with Green Bean Plants (*Phaseolus radiates L*)] *Jurnal Agro Estate* **7** 2 pp 94-106
[11] Wahyuni M and Sebayang E P 2018 Pengaruh pemberian bakteri *rhizobium sp* terhadap pertumbuhan dan pembentukan bintil akar mucuna bracteata asal biji dan stek [The influence of *rhizobium* sp dose on growth and nodulation of mucuna bracteata with seeds and cutting planting material] *Jurnal Agro Estate* **2** 1 pp 16-23
[12] Nugroho P A, Siagian I N and Karyudi 2006 Potensi *Mucuna bracteata* Dalam Pengambilan Unsur Hara Pada Areal Tanaman Karet Belum Menghasilkan [Potential of *Mucuna bracteata* in Uptake Nutrients in Immature Rubber Plant] *Proceeding Lokakarya Nasional Budidaya Tanaman Karet* Medan
[13] Sari, Ramdana. and Prayudyaningsih R 2015 *Rhizobium*: Pemanfaatannya sebagai bakteri penambat nitrogen *Jurnal Balai Penelitian Kehutanan Makassar* **12** 1 pp 61-4
[14] Widyasari M N 2013 *Pengaruh pH media pertumbuhan terhadap ketahanan dari Rhizobium sp pada tanah yang bersifat asam [Effect of pH media growth on resistance of Rhizobium sp to acidic soils]* (Bali: Universitas Udayana)
[15] Harahap Y, Taufik C H, Simangunsong G, Edy S S, Yusran P, Eka L and Suroso R 2011 Mucuna bracteata pengembangan dan pemanfaatannya di perkebunan kelapa sawit [Mucuna bracteata development and utilization in oil palm plantations] *Indonesia Oil Palm Research Institute* Medan
[16] Ngoma L, Babalola O O and Ahmad F 2012 Ecophysiology of plant growth promoting bacteria *Sci Res Essays* 7 pp 4003-013