Growth and yield of edamame soybean in post-tin mining land with application of Rhizobium bacteria and organic fertilizer

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Abstract. Post tin mining land in Bangka Island can be utilized as agricultural land with the application of organic fertilizer and Rhizobium. Organic fertilizer and Rhizobium are the right combination to provide a good planting environment for edamame soybean. The purpose of this study was to study the effects of organic fertilizer and Rhizobium the growth and yield of edamame soybean in post tin mining land. This research was conducted in a post-tin mining land, Bangka, from January to May 2020. This research used a split-plot randomized complete block design. The main plot was an application of Rhizobium (legin) consisting of without Rhizobium (R0) and Rhizobium (R1). The subplot was the application of organic fertilizer consisting of empty palm fruit bunch (P1), husk (P2), and commercial compost (P3). The results showed that commercial compost gave the best effect on plant height, the number of leaves, chlorophyll content, weight of 100 seeds, number and weight of pods per plant, number and volume of roots. Rhizobium gave the best effect on the numbers of roots, root volume, and percentage of effective root nodules. The combination of husk and Rhizobium legin gave the best effect on the flowering day of edamame soybean in post tin mining land.

1. Introduction
Bangka Belitung province is one of tin major producers in Indonesia. The use of post tin mining land in Bangka as agricultural land is not optimal because there are several obstacles. Post tin mining land was dominated by tailings sand with landscapes destructions, highly acidic soil (low pH), low cation exchange capacity, and low fertility due to lack of N, P, K, and C-organic nutrients level [1]. [2] Soil content of C-organic 0.097% (very low), total N-0.001% (very low), CEC 10.88 cmol kg⁻¹ (very low), soil texture of 51.78% sand, 40.69% dust, and 7.53% clay.

Cultivation of edamame soybean in post-tin mining land can be utilized by application of organic fertilizer and Rhizobium. Edamame soybean requires much nitrogen for the forming of nodules and proteins. Available N in the soil can be derived from fertilizer and free nitrogen fixation results by Rhizobium bacteria. [3] Legume plants are able to utilize N₂ in the air results from symbiosis with Rhizobium bacteria. Application of organic material can also be used in legume plants as macronutrients essential for plants that are utilized as a source of energy for Rhizobium. The purpose of this study was to study organic fertilizer and Rhizobium that affect on growth and yield of edamame soybean in post tin mining land.
2. Methodology
The research was conducted in a post-tin mining land, Bangka, from January to May 2020. The tools and materials consisted of cuttings, hoes, digital scales, digital cameras, chlorophyll meter, measuring glass, stationery, water, Rhizobium legin, commercial compost, husk, empty palm fruit bunch, and edamame soybean seed variety of Ryoko 75. The research used a split-plot randomized complete block design. The main plot was an application of Rhizobium legin, consisting of without Rhizobium (R0) and application of Rhizobium legin (R1). The subplot was the application of organic fertilizer consisting of empty palm fruit bunch (P1), husk (P2), and commercial compost (P3). The experiment used 4 replications and each plot consisted of 10 samples.

The procedures include land preparation, application of organic fertilizer, seed preparation, application of Rhizobium bacteria, planting, plant maintenance, and harvest. The first step was land clearance from weeds, then 24 plots of 1 m x 2 m were made. Organic fertilizer was then applied on plot surface with determined dose, namely empty palm fruit bunch: 3000 g/plot, husk: 900 g/plot, and commercial compost 1000 g/plot. The process of seed preparation is done by soaking edamame seeds (Ryoko 75 variety) in one liter of water at 45°C for 10 minutes. The application of Rhizobium bacteria in plants was carried out by legin weighing 10 g/kg of edamame soybean seeds [4][5]. The process of planting seeds is done by entering 2 seeds per planting hole. Watering was done twice a day. Harvesting is done after edamame soybean pods according to harvest criteria by cutting pods from the stem.

The parameters used plant height, number of leaves, chlorophyll content, flowering day, number and weight of pods per plot, number of seeds per pod, weight of 100 seeds, roots number, roots length, roots volume, and percentage of effective root nodules. The data analysis used the F test, and if treatment was significant at $\alpha=0.05$, comparisons of the means were performed using the least significant difference.

3. Results and Discussion
The result of variance analysis showed that the application of organic fertilizer gave no significant effect on the growth and yield of edamame soybean. Application of Rhizobium gave the best effect on the numbers of roots, root volume, and percentage of effective root nodules. The combination of husk and Rhizobium legin gave the best effect on the flowering day of edamame soybean in post tin mining land (Table 1).

Table 1. Analysis of variation on growth and yield of edamame soybean in post-tin mining land

| Trait                  | Organic fertilizer Mean | CV (%) | Pr >F | Rhizobium Mean | CV (%) | Pr >F | Interaction Mean | CV (%) | Pr >F |
|------------------------|-------------------------|--------|-------|----------------|--------|-------|------------------|--------|-------|
| Plant height           | 1.04 0.3923**           | 9.73   |       | 0.17 0.6860**  | 3.90   |       | 0.21 0.8140**    | 9.38   |       |
| Number of leaves       | 3.12 0.0934**           | 13.58  |       | 0.31 0.5902**  | 11.63  |       | 1.26 0.3286**    | 13.63  |       |
| Chlorophyll content 4 WAP | 0.47 0.6408**        | 15.63  |       | 4.16 0.0718**  | 25.73  |       | 0.03 0.9687**    | 21.70  |       |
| Chlorophyll content 5 WAP | 1.89 0.2058**        | 19.80  |       | 0.00 0.9865**  | 13.06  |       | 0.88 0.4471**    | 14.48  |       |
| Flowering day          | 0.76 0.4955**           | 9.62   |       | 0.36 0.5633**  | 3.35   |       | 4.68 0.0404*     | 8.06   |       |
| Number of pods per plant | 3.07 0.0963**        | 43.75  |       | 0.12 0.7360**  | 9.30   |       | 3.96 0.0585**    | 27.25  |       |
| Number of seeds per pod | 4.05 0.0557**        | 11.19  |       | 0.88 0.3736**  | 11.73  |       | 0.07 0.9302**    | 9.32   |       |
| Weight of pods per plant | 1.44 0.2867**        | 38.86  |       | 1.93 0.1978**  | 14.47  |       | 3.04 0.0980**    | 35.38  |       |
| Weight of 100 seeds    | 0.33 0.7281**           | 20.22  |       | 0.76 0.4055**  | 24.94  |       | 1.08 0.3800**    | 21.55  |       |
| Length of roots        | 0.75 0.4995**           | 18.77  |       | 0.85 0.3794**  | 2.82   |       | 0.85 0.3794**    | 18.54  |       |
| Number of roots        | 0.15 0.8662**           | 17.47  |       | 7.22 0.0249**  | 15.16  |       | 0.04 0.9631**    | 16.02  |       |
| Volume of roots        | 0.18 0.8392**           | 16.92  |       | 8.95 0.0152*   | 7.98   |       | 0.44 0.6575**    | 13.90  |       |
| Percentage of effective root nodules | 0.27 0.7730** | 20.82  | 10.10 0.0112* | 11.40  | 0.83 0.4673** | 18.84  |       |

CV = Coefficient of variation; * = Significant at 5% level; ns = Not significant; Pr>F = Probability value
The application of commercial compost gave a better effect than empty palm fruit bunch compost and husk on plant height, the number of leaves, chlorophyll content, number and weight of pods per plot, weight of 100 seeds, the number and volume of roots (Figure 1).

Figure 1. Application of organic fertilizer on (a) plant height, (b) number of leaves, (c) chlorophyll content, (d) flowering day, (e) number of pods per plot, (f) number of seeds per pod, (g) weight of seeds per pod, (h) weight of 100 seeds, (i) length of roots, (j) number of roots, (k) volume of roots, (l) percentage of effective root nodules of edamame soybean

The application of Rhizobium on edamame soybean yields had a significantly different effect than without Rhizobium on the number of roots, root volume, and percentage of effective root (Table 2).

Table 2. Mean of growth and yield of edamame soybean on the control and Rhizobium treatment.

| Trait                        | Rhizobium Without | Rhizobium With |
|------------------------------|-------------------|----------------|
| Number of roots              | 23.72 b           | 28.29 a        |
| Volume of roots (cm³)        | 2.66 b            | 3.15 a         |
| Percentage of effective root nodules (%) | 65.11 b     | 83.23 a        |

Numbers followed by the same letter in the same row indicate non-significant differences based on the LSD test at 0.05 level.
The application of \textit{Rhizobium} on edamame soybean yields gave a higher result than without \textit{Rhizobium} on flowering day, chlorophyll content, the number and weight of pods per plant, number of seeds per pod, and root length of soybean plants edamame. (Figure 2).

The combination of husk + \textit{Rhizobium} (R\textsubscript{1}P\textsubscript{2}) gave the best flowering age is 27.25 HST. This combination produced a significantly different flowering age from the combination of husk + without \textit{Rhizobium} (23.25 HST), but not significantly different from TKKS + commercial compost treatment (Table 3).

**Table 3.** Mean flowering days for each combination treatment of organic fertilizer and \textit{Rhizobium} on edamame soybean

| Trait                  | Treatment combination |
|------------------------|------------------------|
|                        | R\textsubscript{0}P\textsubscript{1} | R\textsubscript{1}P\textsubscript{1} | R\textsubscript{0}P\textsubscript{2} | R\textsubscript{1}P\textsubscript{2} | R\textsubscript{0}P\textsubscript{3} | R\textsubscript{1}P\textsubscript{3} |
| Flowering day (day)    | 26.25ab                | 25.75ab                | 23.25b                | **27.25a**                | 25.75ab                | 23.75ab                |

Numbers followed by the same letter in the same row indicate non-significantly different at 0.05 level.

Plant height increased from 1 to 3 weeks after planting (WAP), but relatively consistent at 4 and 5 WAP (Figure 3).

The number of leaves of the plant shows significant improvement from 1 to 3 WAP and relatively consistent at 4 and 5 WAP. However, plant height commercial compost + treatment without \textit{Rhizobium} still increasing to 5 MST (Figure 4).
Figure 4. Average a number of leaves plant by application organic fertilizer and Rhizobium of edamame soybean

The results of the LSD test showed that application of husk + Rhizobium (R1P2) gave the best interaction on flowering day, chlorophyll content, number, and length of root edamame soybean in post-tin mining land.

Commercial compost application (cow manure) was able to provide essential nutrients needed for growing plants. High potential crop yields that support growth and plant development are influenced by nutrients available in capable soil live microorganisms [6]. After being described microorganisms in embedded manure in the soil will produce certain compounds and growth regulatory substances such as gibberellins, auxins, and cytokines [7].

The Rhizobium application helps the nitrogen absorption efficiency and the therefore may increase plant growth in terms of the number of roots, root volume, and percentage of effective root nodules [8]. Effective inoculants are capable to nitrogen efficiently. They can optimally infect the plant roots and make the plant grow better. On a contrary, ineffective inoculants are less able to fix nitrogen so that the plant growth is less well [9]. Rhizobium helps the formation of root nodules in soybean plants. The more root nodules, the more it will help to supply N nutrients for the stem, leaf, and roots growth process. Soybean plants have a number of root nodules. Some species only utilize available N in the soil; only a small percentage is obtained from the air as a result of N$_2$ fixation [10].

Figure 5. Application of organic fertilizer on a (number of pods per plant), b (weight of pods per plant), c (number of seeds per pod), d (weight of 100 seeds), e (length of roots), f (number of roots), g (volume of roots), h (percentage of effective root nodules) of edamame soybean
Application of husk was able to provide an environment suitable for *Rhizobium* as an aerobic bacterium because they contain organic material for improving soil porosity. Husk has micro and macro pores almost balanced in number, so the air circulation is quite well and the water absorption is high [11]. P and K in the husk are quite high compared to other organic fertilizers. These elements are needed by plants in the vegetative and generative phases. [12] The P element functions in the flowering process and fertilization, while the K functions as an activator of essential enzymes in photosynthesis and respiration which affects flowering. [13] The environmental factors affect plant physiology which is needed to increasing plant metabolisms such as photosynthesis, assimilation, and nutrient accumulation to resemble energy in flowering. Water stress in plants as a trigger for flower initiation. [14] N nutrient functions to repair the vegetative growth of plants and P play an important role in accelerating the growth of seedlings and plants, to become mature, as well as speeding up flower formation and seed maturity.

4. Conclusions
The application of organic fertilizer gave no significant effect on the growth and yield of edamame soybean. Commercial compost gave the best effect on plant height, number of leaves, chlorophyll content, weight of 100 seeds, number and weight of pods per plant, number and volume of roots. *Rhizobium* gave the best effect on the number of roots, root volume, and percentage of effective root nodules. The combination between husk and *Rhizobium* lignin gave the best effect on the flowering day of edamame soybean in post tin mining land.

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