Physiological Response, Growth, and Yield of Edamame Soybean (*Glycine Max* L. Merr.) Under Foliar Application of Nano Cattle Bone Ash in Entisol

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ABSTRACT

Nano cattle bone ash can reduce requirement for SP-36 and increase pod yield. This study aimed to examine the physiological response, growth, and yield of edamame soybean in entisol of concentration and frequency of foliar application of nano cattle bone ash and SP-36 fertilizer.The research was conducted an experimental method which arranged in a Randomized Completely Block Design (RCBD) which consists of factor concentration (0.15, 0.30, and 0.45%) and frequency (2, 3, and 4 times). In control treatment was given 100% recommended dose SP-36, whereas between concentration and frequency foliar were given 50%. The findings demonstrated that foliar application of nano cattle bone ash with 50% SP-36 resulted in higher leaf dry weight and pod yield per plant as compared to control, increasing pod production per plant by 21.7%. The results of this study concluded that nano cattle bone ash can replace a half dose of SP-36 and increase pod yield. In the future, it is expected that the use of nano cattle bone ash can be applied into soybean cultivation and reduce use of SP-36 fertilizer.

Keywords: Nano cattle bone ash, Foliar application, Phosphate, Edamame

ABSTRAK

Abu tulang sapi nano dapat menurunkan kebutuhan SP-36 dan meningkatkan hasil polong. Penelitian ini bertujuan untuk menguji pengaruh konsentrasi dan frekuensi aplikasi abu tulang sapi nano dan pupuk SP-36 terhadap sifat fisiologis, pertumbuhan dan hasil kedelai edamame pada entisol. Penelitian dilaksanakan dengan metode percobaan yang diatur dalam Rancangan Acak Kelompok Lengkap, yang terdiri dari faktor konsentrasi (0,15%, 0,30% dan 0,45%) dan frekuensi (2, 3 dan 4 kali). Pada perlakuan kontrol diberikan 100% dosis anjuran SP-36 sedangkan antara konsentrasi dan frekuensi diberikan 50%. Hasil penelitian menunjukkan bahwa aplikasi abu tulang sapi nano dengan penambahan 50% dosis rekomendasi SP-36 memberikan bobot kering daun dan bobot polong per tanaman yang lebih tinggi, meningkatkan hasil polong per tanaman sebesar 21,7% dibandingkan kontrol. Hasil penelitian ini menyimpulkan bahwa abu tulang sapi nano dapat menggantikan SP-36 setengah dosis dan meningkatkan hasil polong. Kedepan diharapkan penggunaan abu tulang sapi nano dapat diterapkan pada budidaya kedelai untuk menurunkan penggunaan pupuk SP-36.

Kata kunci: *Abu tulang sapi nano, Aplikasi foliar, Fosfat, Edamame*

INTRODUCTION

Indonesia has not been able to fulfill the needs of domestically produced soybeans. Even during the last decade, soybean imports averaged 1.49 million tons per year, or 67 percent of national soybean needs. In other words, domestic production is only able to meet 33% of the needs (Suhartini, 2018). In an irrigated cropping system, edamame can be grown four to six times a year as a short-cycle income crop, and its production can reach up to 10 tons per ha of marketable fresh pods (Mahoussi et al., 2020). Edamame soybeans can be grown in a variety of soil types, including Entisol. Entisol has a relatively low organic matter content, such as N, P, and K content because it is still a primary mineral that has not been mineralized. Loose consistency, sensitivity to erosion, low aggregation rate, and high nutrient total but low availability are the properties of Entisol soils (Putra et al., 2016).

Entisol soil is poor in organic matter, so the ability to store water and nutrients is very low.

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It needs efforts to improve the fertility of entisol soil so that it can be used for the cultivation of edamame soybeans. Efforts to improve entisol soil fertility can be done by adding organic matter or fertilizing with fertilizers containing N, P and K. Sources of P fertilizers that have been used by farmers are TSP or SP-36 fertilizers. Nutrient-rich organic fertilizer non-acid-2 (raw material using sulfur as much as 5%) has an acid reaction and high content of organic matter, N, P, K, and S (Kuntyastuti et al., 2018). This fertilizer raw material comes from phosphate rock because it is a non-renewable or non-renewable material, so at one time, there may be a shortage of P fertilizer (Putinella, 2014).

One of the renewable sources of phosphate is cattle bone waste. To be used as fertilizer, cattle bone waste must first be roasted by burning at a temperature of 800 °C (Yusnita et al., 2012). To make a smaller size of cattle bone ash, a milling process was carried out for 4 hours with a ball mill tool that produces particles with a size of 118-300 nm. Foliar application through the leaves with a concentration of 0.3% on corn plants can increase leaf area, root dry weight, NAR, the weight of one hundred seeds, and the weight of cobs (Mulyono & Hidayat, 2020).The results of milling still produce a particle size above 100 nm, so we need to study the process of milling to obtain particle sizes below 100 nm by increasing the duration of milling to 6 hours. The source of phosphate can be obtained from hydroxy apatite rocks, which are processed into a nano size. The use of nano hydroxy apatite in soybean plants can increase yields and reduce the use of urea fertilizer (Kottegoda et al., 2017). Hydroxy apatite compounds can be obtained from cattle bone waste because beef bones contain 93% hydroxy apatite and 7% -tricalcium phosphate (Cranney et al., 2007). The phosphorus contained in cattle bones needs to be processed further, one of which is by ashing and changing the particle size into nanoparticles.

One of the efforts that can be done to improve the fertility of entisol soil for the cultivation of edamame soybeans is through fertilization. In this study, the effectiveness of the application of nanoparticles of cattle bone ash on the growth and yield of edamame soybeans in entisol soils was evaluated. The application of nano cattle bone ash through leaves (foliar application) on soybeans has never been studied, especially the dose, concentration, or frequency of spraying.

MATERIALS AND METHODS

Experimental design

The research was was carried out in Green House and Agriculture Laboratory, Universitas Muhammadiyah Yogyakarta from October to December 2020. This experimental was conducted with factorial treatment (3×3) + 1. First, there was concentration, which had three levels: 0.15%, 0.30%, and 0.45%. Secondly, there was frequency, which had three levels: 2, 3, and 4 times. The control treatment was given SP-36 fertilizer according to the Edamame recommended dose of 343 kg ha^{-1}, while the combination treatments were given 50% SP-36 (171.5 kg ha^{-1}) (Miles et al., 2000).

The process of making cattle bone ash

The bone of cattles were obtained in slaughterhouses. They were changed into ashes by being burnt at a temperature of 800 °C so that the ashes were complete (Yusnita et al., 2012). However, it turned out that the ashes of the cattle bones were still large, and many were still intact like their original size. To reduce into nano size, milling with a ball mill for six hours.

Application

Nano cattle bone ash was then dissolved in 1 liter of water according to the treatment of concentration. To prevent deposition, each solution was added with a CMC (*Carboxymethyl cellulose*) emulsifier of as much as 1/10 of the solution concentration. Foliar spraying at the age 12, 22, 32, and 42 days after planting.

Data Analysis

The data were analyzed using ANOVA at a 5% of significance level, the SAS portable software was used. To compare controls and treatmens a contrast test was performed and mean comparison was determined using Duncan's Multiple Range Test (DMRT) at a 5% significance level.

RESULTS AND DISCUSSION

Entisol characteristics

Entisol soil was taken from farmer's rice fields in Tamantirto, Kasihan, Bantul, and Yogyakarta. According to the findings of the chemical property investigation, the soil fertility was average and had a very low organic C content of less than 1%. The content of organic matter was low, total N content was moderate because it was in the moderate range of 0.21%-0.25 %, P content was very low so that it is necessary to add P to cultivate Edamame soybean, K content was very low, the CEC value was low, ranging from 5 to16 me %, and the soil pH was neutral. The soil used has limiting factors, in which the content of N, P, K, and C shows that Entisol soil has low chemical properties, with organic levels of 0.94%, Nitrogen of 70.95 ppm, pH of 6.24, and soil CEC of 6.04 me % (Sonbai, 2013).

Characteristics

Based on results of milling for six hours at a speed of 125 rpm produced particles with a nano-size (<100 nm) of 98.92% (Figure 1).

Based on the EDX test, it was found that the elemental content in nano cattle bone ash was the highest, respectively, namely oxygen 45.20%,

Table 1. Effect of Treatment on Agronomy Parameters

Treatments	Width of stomatal (mm)	Chloro phyll a	Chloro Phyll b	Total Chloro phyll	Leaf fresh weight (g)	Fresh weight of stems (g)	Root Fresh Weight (g)	Leaf dry weight (g)	Stem dry weight (g)	Root dry weight (g)	Number of pods	Number of bunches	Weight 100 pods (g)	Number of seeds per plant (grain)	Seed weight per plant
Contras test															
Control	8.24 y	0.031 y	0.022 y	0.053 y	7.16 y	5.89 y	4.66 y	2.11 y	1.69 y	2.02 y	28.45 y	9.11 y	134.51 y	165.67 y	18.80 y
Treatment	8.22 y	0.031 y	0.022 y	0.052 y	7.54 y	5.30 y	6.00 y	2.06 y	1.79 y	1.77 y	30.90 y	15.93 x	259.72 x	170.22 y	20.63 y
Foliar application concentration (%)															
0.15	10.0 a	0.030 a	0.020 a	0.051 a	8.31 a	8.16 a	6.15 a	1.47 a	1.47 a	1.50 a	28.07 a	15.40 a	238.11 b	170.22 a	19.89 a
0.30	7.16 b	0.029 a	0.019 a	0.048 a	8.16 a	8.31 a	6.08 a	1.98 a	1.98 a	1.82 a	33.07 a	16.55 a	260.73 ab	196.67 a	21.38 a
0.45	7.50 b	0.032 a	0.026 a	0.057 a	6.52 a	6.51 a	5.78 a	2.17 a	1.92 a	2.00 a	29.26 a	15.82 a	280.32 a	178.56 a	20.62 a
Foliar application frequency (times)															
2	8.81 p	0.030 p	0.021 p	0.051 p	7.97 p	6.10 p	6.10 p	2.06 p	1.70 p	1.67 p	33.15 p	15.74 p	247.04 p	204.22 p	20.62 p
3	7.42 p	0.030 p	0.021 p	0.051 p	7.62 p	6.27 p	6.27 p	2.36 p	1.69 p	1.63 p	30.92 p	15.77 p	275.49 p	189.56 p	20.34 p
4	8.45 p	0.032 p	0.021 p	0.051 p	7.41 p	5.63 p	5.63 p	2.32 p	1.98 p	2.01 p	30.92 p	16.26 p	256.63 p	151.56 p	20.93 p
Interaction	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
CV (%)	19.93	7.58	17.40	9.97	9.07	7.22	14.73	9.76	9.80	9.20	18.47	18.28	13.19	18.18	11.80

Remarks: Means followed by the different letters are significantly different based on DMRT at 5% significance level. Signs - indicate that there is no interaction between foliar application of concentration and the frequency

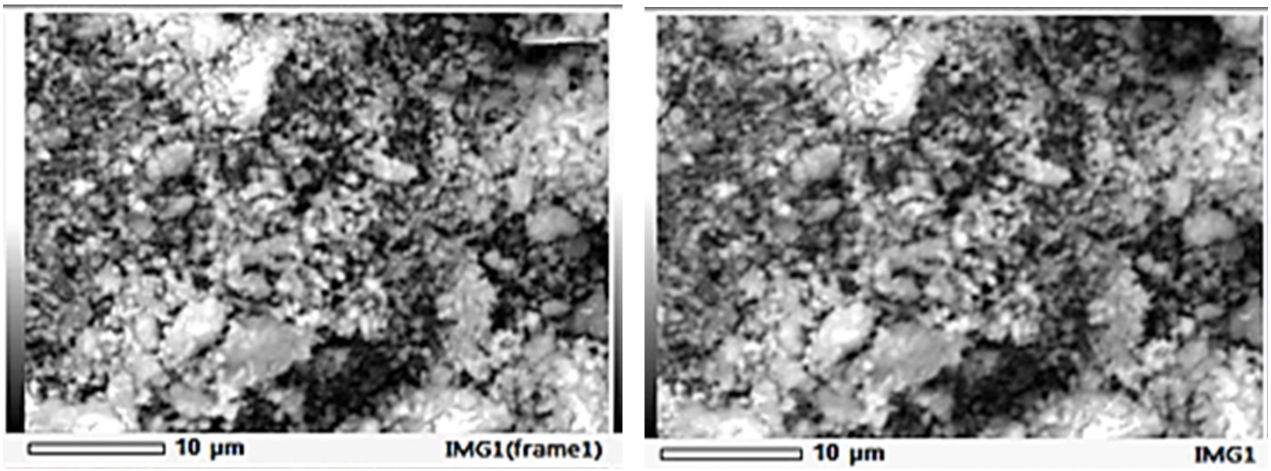

Figure 1. SEM results with magnifications of 5.000 and 50.00

calcium 22.66%, phosphorus 16.42%, carbon 7.27%, sodium 0.82%, and magnesium 0,63%. The high oxygen content was due to the elements contained in cattle bones undergoing an oxidation process during the combustion process to become ash. It was suspected that these elements form oxide compounds, such as CaO, MgO, NaO, and PO_4. These oxides compounds when dissolved in water will form ions that are easily absorbed by plants (Figure 2).

Width of stomatal opening (mm)

Foliar application concentration showed a significant effect on the width of stomatal openings (Table 1). Increasing the concentration of foliar application from 0.15% to 0.30% and 0.45% resulted in a decrease in the width of the stomatal opening, and this was thought to be influenced by the high Ca content in nano cattle bone ash. The width of the stomatal opening plays a role in the entry of CO_2 used in the process of photosynthesis and transpiration process through the leaves. The frequency of application of nano cattle bone ash has no effect on the opening of the stomata. The opening of the stomata is more affected by temperature. The stomata's breadth and length as well as the leaf proline content both slightly increase in response to drought (Fatimah et al., 2020).

Leaf chlorophyll content (mg g^{-1})

Based on the contrast test of chlorophyll a, b, and total chlorophyll. The combination of treatment and control had the same effect on the chlorophyll content, with no discernible change. (Table 1). There was no interaction between concentration and foliar application frequency, and each treatment did not give a significant effect. Nano cattle bone ash contains relatively high Ca and P elements, which with the foliar application, it would enter through the stomatal and affect metabolic processes. Ca plays a role in maintaining tissue structure and spurring the development of new root and shoot tissue. As a result, a low calcium level weakens the cell wall, which is important for expansion during tip growth, for instance, in the case of pollen tubes or root hairs (Bascom et al., 2018).

Fresh weight of plant (gram)

There was no significant different in the combined effects of treatment and control according to the contrast test on the fresh weight of leaves, stems, and roots (Table 1). There was no interaction, and there was no significant difference in the effect of concentration and foliar application frequency. From these results, it can be explained that the application of nano cattle bone ash can

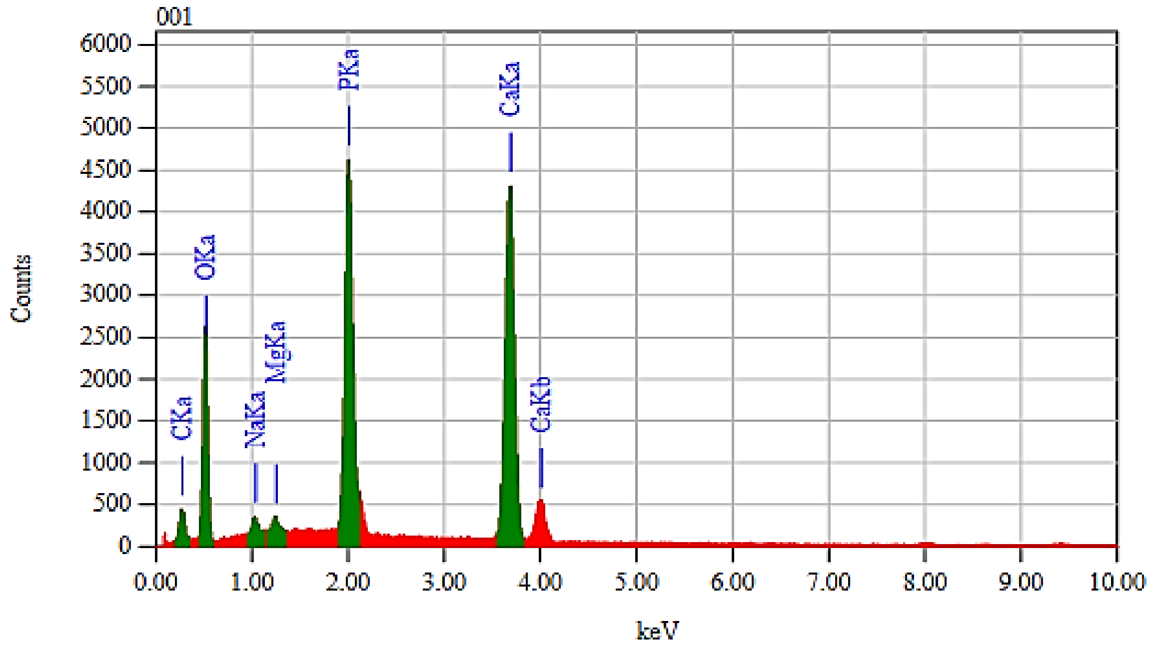

Figure 2. EDX results of nano cattle bone ash

replace 50% of the recommended dose of SP-36 fertilizer with the addition of nano cattle bone ash.

Dry weight of plant (gram)

Based on the contrast test, there was no significant difference in the effect of the combination of treatment and control on the dry weight of leaves, stems, and roots of plants (Table 1). There was no interaction effect of concentration and frequency. The use of nano cattle bone ash can replace 50% of the recommended amount of SP-36 fertilizer, according to these results, which can be explained. In absorbing nutrients, the process of photosynthesis occurs in the leaves and produces photosynthesis. The photosynthate produced is distributed throughout the plant, one of which was the roots so that root biomass increases. Determination of the amount of water absorbed by plant roots is used as an indication of root dry weight. In biomass, the amount of water absorbed determines the success of the roots in translocating throughout the plant body (Hizbi & Ghulamahdi, 2019).

Number of pods, number of bunches, and weight of 100 pods

Edamame plants fertilized with P at half of the control dose added with nano cattle bone ash sprayed onto leaves showed a different result. These results indicate that foliar application of nano-cattle bone ash was more efficient than soil application because fertilizer applied through leaves can be directly absorbed through the stomata and can be utilized in the photosynthesis process.

The effects of frequency and concentration on the number of pods were not significantly different, as shown in Table 1, and there was no interaction between the two variables. A different outcome was obtained by foliar spraying nano cattle bone ash at a concentration of 0.15 percent, but an increase in the concentration to 0.30% and 0.45% did not result in an increase in the number of bunches per plant. The number of bunches was not significantly impacted by the foliar applications of nano cattle bone ash 2, 3, or 4. Research demonstrates that a two-time foliar treatment of nano cattle bone ash

Table 2. Pod weight per plant (gram)

Frequency	Concentration (%)			
	0.15	0.30	0.45	Average
2	74.55 bc	75.33 bc	89.55 a	79.81
3	71.77 c	84.44 ab	85.11 ab	80.44
4	67.88 c	84.89 ab	72.50 c	75.09
Average	71.40	81.55	82.39	(+)
Contrast test				
Control				78.45 y
Treatment				90.50 x

Remarks: Means followed by the different letters are significantly different based on DMRT at 5% significance level. Signs + indicate that there is interaction between foliar application of concentration and the frequency.

was successful in providing the P that edamame soybean plants require. Following direct calcium contact with flowers, a favorable impact on pollen tube growth or even cell division may lead to an increase in the number of pods or even seeds. (Fioreze et al., 2017).

The combination of treatment and control had a significantly different impact on the weight of 100 pods based on the contrast test. When compared to the control treatment, foliar spray of nano cattle bone ash treated with 50% of the SP-36 fertilizer's authorized dose could raise the weight of 100 by 93%. Phosphorus is one of the macro elements needed by soybean plants in the pod-filling phase. Nano cattle bone ash applied through leaves was thought to be directly absorbed through the leaf stomatal and immediately used in the photosynthesis process, thereby increasing the weight of 100 pods.

Number of seeds per plant

Based on table 1, nano cattle bone ash was applied to the leaves at different rates and concentrations, but it had no discernible impact on how many seeds each plant. P has the ability to boost grain production, hasten root growth, hasten flowering and the ripening of fruit and seeds, and all of these functions in plants. (Murtilaksono et al., 2019). Research indicates that the P requirements of edamame soybeans could be met by foliar applications made at a frequency of twice at a concentration of 0.15%.

Pod weight per plant

Compared to the control treatment, the weight of the pods in the treatment plants was higher.. Plants that were given a 50% dose of P fertilizer and replaced with nano cattle bone ash fertilizer were able to give heavier pods weight than plants fertilized with the recommended P of 21%. One of the fertilization methods that has shown great potential gains in P for plants over conventional soil is a foliar application (Bindraban et al., 2020).

The pod weight per plant was impacted by the interplay of the concentration and frequency of application of the nano cattle bone ash fertilizer (Table 2). Increasing the frequency of foliar application from two to four times on plants sprayed with a concentration of 0.15% to 0.30% did not cause a significant change in pod weight. Different things happened when the concentration given was higher, namely 0.45%, in which an increase in foliar application frequency caused a significant decrease in pod weight.

At a high concentration of 0.45% and just two foliar applications, a substantial pod weight could be produced. When the foliar spray was made three times, a heavy pod weight was attained when a medium concentration of 0.30% to a high concentration of 0.45% was utilized. If the foliar application was carried out four times, the weight of heavy pods could be obtained with a moderate concentration of 0.30%, while with a high concentration of 0.45%, the weight of the pods obtained was lower. This is interesting to be studied further.

Seed weight per plant

Edamame plants fertilized with P at half of the control dose combined with nano cattle bone ash through foliar application showed no significant differences. One of phosphorus' crucial functions in plants is to store and transmit the energy generated during photosynthesis, which is then used for expansion and reproduction. Foliar application of nano cattle bone ash can supply plant P needs and can replace 50% of recommended SP-36 fertilizer dose. An important macronutrient called phosphorus (P) restricts plant productivity. (Carstensen et al., 2018). The application of foliar application of P can increase the growth and yield of wheat crop seeds which is not different from the application of P fertilizer through the soil (Talboys et al., 2016).

CONCLUSIONS

As comparison to 100% fertilization with SP-36 at the approved dose, foliar application of nano cattle bone ash mixed with SP-36 at 50% of the recommended dose produced higher leaf dry weight and pod weight per plant. Nano cattle bone ash applied topically could replace 50% of the SP-36 fertilizer dose and result in a 21.7% increase in pod production per plant. The best results were achieved with three time-foliar sprays at a concentration of 0.30% at 22, 32, and 42 days after planting and for two treatments with a 0.45% concentration, made 32 and 42 days after planting.

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