The effects of nanosilica fertilizer concentration and dose of rice hull ash on the characteristic of soybean leaves (Glycine max L. Merril)

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Abstract. Utilization of nanosilica fertilizer and rice hull ash in soybean cultivation is an environmentally friendly cultivation that supports sustainable agriculture. The purpose of the research was to analyze the effects of nanosilica fertilizer and dose of rice hull ash on characteristics of soybean leaves. The study was arranged as factorial design with two factors. The first factor was nanosilica fertilizer, consisted of four levels (0, 1.25, 2.5- and 3.75-ml l⁻¹). The second factor was dose of rice hull ash consisted of four levels (0, 1, 2 and 3-ton ha⁻¹). Each treatment combination was repeated three times. Concentration of nanosilica individually affect the number of leaves at 29 and 43 days, leaf area and the leaf area index at 43 days. There was interaction between nanosilica fertilizer and rice hull ash on leaf thickness, stomata density, trichome density and silica content in leaves. Combinations of 1.25 ml l⁻¹ nanosilica and 1-ton ha⁻¹ rice hull ash gave the highest leaf thickness, combination of 1.25 ml l⁻¹ nanosilica and 2-ton ha⁻¹ rice hull ash gave the highest stomata density and silica content in leaves. Combination of 1.25 ml l⁻¹ nanosilica fertilizer and 3-ton ha⁻¹ rice hull ash gave the highest trichome density.

1. Introduction

The organic farming system is an example of the application of sustainable agriculture. Organic farming is a crop production method that focuses on environmental protection, which avoids the use of chemical inputs, such as fertilizers and pesticides [1]. The techniques used in organic farming are approaches to sustainable agricultural systems that emphasize the preservation and conservation of natural resources to create a balance of ecosystems and contribute to increase agricultural productivity in the long period.

Soybean is one of the important commodities in Indonesia where the demand tends to increase from over years. In 2009, the demand of soybean was 1.9 million tons, while in 2011 it was 2 million tons and it increased in 2013 to 2.2 million tons [2]. The factor that constrains soybean farming in Indonesia is plant pest organisms. The problem of plant pest organisms can cause unsuccessful harvest or it just reaches 80% or even failure if it is not controlled properly and correctly [3]. To save soybean plants from plant pest organisms, farmers use very high chemical pesticides so that some pests have resistance.
The use of silicone fertilizers in the field of agriculture that is environmentally friendly can be done by utilizing natural silicon obtained from rice hull ash from the residual of brick combustion [4]. Rice hull ash not only contain SiO₂ of 87-97% but it also 1% N and 2% K nutrients [5]. In general, the distribution of silicone fertilizers can improve plant physiological functions and increase plant resistance to pests, diseases [6]. Distribution of rice hull ash with its silicone content significantly affects the growth rate of tomato plant and suppresses pests and diseases [7]. Fulfillment of plant needs for Si nutrients will improve the plant's natural protection system against diseases, insects and bad weather conditions [8]. Sufficient Si supply to cerealia provides good harvest because the addition of Si can increase strength and resilience of the cell, thus the use of silica fertilizers derived from rice hull ash can make the plant resistance from pest problem [10].

The characters of morpho physiology plant, such as leaf thickness and growth rate thought to affect the level of productivity, because it can affect the speed of photosynthesis. A high seed filling rate that lasts relatively long will produce a high seed weight as long as the seed as a sink can accommodate the assimilate results. Conversely, if the sink is quite a lot but the results of assimilate is low. It causes the emptiness seed. The limitation of source often occur in the period of filling soybean seeds, but the limitations of the sink occur in stress less conditions [10]. This research aimed to analyze the effect of nanosilica fertilizer concentration and dose of rice hull ash on the characteristic of soybean leaves (Glycine max (L.) Merrill).

2. Methods
The research was conducted from October 2017 to March 2018 at the Seed Crop Development Center (SCDC), Plumbon Subdistrict, Cirebon Regency, West Java Province which is located at an altitude of 17 m above sea level. This study used a Randomized Block Design (RBD) with two factors. The first factor was the concentration of nanosilica fertilizer (0, 1.25, 2.5- and 3.75-ml l⁻¹) and the second factor was rice hull ash (0, 1, 2 and 3 tons ha⁻¹). Each treatment combination was repeated 3 times, so that there were 48 treatment plots with a plot size of 3 m × 3 m. The variables observed included the number of trifoliate leaves, leaf area, the leaf area index (LAI), leaf thickness, stomata density, trichome density and silica content in leaves. The data were analyzed by ANOVA then it is tested using 5% DMRT.

3. Results and discussion
3.1. The number of trifoliate leaves (pieces).
The results of the variance analysis showed that there was no interaction between the concentration of nanosilica fertilizer and dose of rice hull ash on the number of trifoliate leaves per plant aged 15, 29 and 43 days after planting (dap). The individual effect of nanosilica fertilizer application was evident at the age of 15, 29 and 43 dap, while the application of rice hull ash fertilizer did not give significant effect on the number of trifoliate leaves per plant in all observation periods.

Figure 1 shows the individual effect of the treatment of nanosilica fertilizer concentration on the number of trifoliate leaves of soybean plants at all ages of observation, it is assumed that the application of Si through leaves can be directly absorbed by soybean plants to support physiological processes, especially photosynthesis. Energy formed from photosynthesis can be beneficial for the growth of soybean plants so that optimum result is obtained. Furthermore, the application of Si by spraying through leaves would be more effective compared to the application of roots in increasing the resistance of vines to pests and leaf diseases [11].
The application of nanosilica fertilizer concentration up to 2.50 ml l⁻¹ increased the number of trifoliate leaves, this is in line with previous research where nanosilica fertilizer affected height, number of leaves and number of roots of tomato plants. Whereas if the nanosilica concentration was increased to 3.75 ml l⁻¹, the number of trifoliate leaves decreased [12]. While the dose of rice hull ash had no significant effect on the number of trifoliate leaves, it was hypothesized that the application of rice hull ash up to 3 tons ha⁻¹ was not sufficient to increase the availability of nutrients for plants.

3.2. Leaf area (LA) (cm²)
There is no interaction effect between the treatment of nanosilica fertilizer concentration and the dose of rice hull ash on leaf area (LA) of soybean plants at various ages of observation.

Table 1. Average leaf area of soybean plants in various application of nanosilica fertilizer and rice hull ash

| Treatments | Average Leaf Area (cm²) |
|------------|-------------------------|
|            | 15 dap                  |
|            | 29 dap                  |
|            | 43 dap                  |
| s₀ (0 ml l⁻¹) | 32.76                   |
| s₁ (1.25 ml l⁻¹) | 33.90                   |
| s₂ (2.50 ml l⁻¹) | 42.72                   |
| s₃ (3.75 ml l⁻¹) | 32.76                   |
| p₀ (0 ton ha⁻¹) | 41.46                   |
| p₁ (1 ton ha⁻¹) | 37.68                   |
| p₂ (2 ton ha⁻¹) | 29.76                   |
| p₃ (3 ton ha⁻¹) | 33.24                   |

The average number with the same lowercase letters in the same column or uppercase in the line shows no significant difference based on the 5% DMRT test.

Leaf area is one of the important parameters needed to determine the growth of soybean plants (Glycine max L.). Nanosilica fertilizer concentration had a significant individual effect on leaf area at 43 dap. Concentration of nanosilica fertilizer 2.50 ml l⁻¹ generated the highest influence on leaf area, which was equal to 2,364.22 cm² compared to other treatments. Si elements might stimulate photosynthesis and translocation of carbon dioxide (CO₂). Silica can accumulates in the leaves functions to keep the leaves upright so it can help to capture sunlight in the process of photosynthesis and CO₂ translocation [13]. Leaf area is not constant with time, but it decreases with increasing age [14].

3.3. Leaf area index (LAI)
There is no interaction effect between the application of nanosilica fertilizer and rice hull ash on the leaf area index (LAI) at the age of 15, 29 and 43 dap. The application of nanosilica fertilizer has significant individual effect on the LAI at 43 dap.
Figure 2. The leaf area index (LAI) of soybean at 15, 29 and 43 dap

Figure 2 shows that the treatment of 2.50 ml l⁻¹ nanosilica fertilizer concentration gave the highest effect on LAI of 3.94. The treatment of nanosilica fertilizer concentrations up to 2.50 ml l⁻¹ increased LAI and if the nanosilica fertilizer concentration is increased, the LAI decreased. The LAI increased in relation to increasing plant age, then it decreased and maximum LAI was achieved when the number of leaves and maximum leaf size [15].

The leaf area index is influenced by leaf wide and distance spacing plants. Soybean plants with a large number of leaves provide a large assimilate supply on the condition that the leaves receive sufficient intensity to carry out photosynthesis [16]. The application of nanosilica fertilizer increased the availability of P which plays a role in photosynthetic processes as a ready-to-use energy source in the form of ATP, and can initiate an increase in photosynthesis and increase carbohydrate production.

3.4. Leaf thickness (μm)

The results of the variance analysis show that there is an interaction effect between application of nanosilica fertilizer concentration and dose of rice hull ash to the leaf thickness of soybean. Figure 3 shows that leaf thickness in treatments p₀ and p₁ showed a relatively similar pattern which increased when accompanied by nanosilica fertilizer at the level of s₁, then thickness decreased when nanosilica fertilizer exceeded the level of s₁. On the other hand, on the application of rice hull ash fertilizer with a level of p₂, leaf thickness decreases if it is accompanied by the applications of nanosilica fertilizer of s₁. Whereas at the level of giving rice hull ash fertilizer as much as p₃, the thickness did not change at various levels of nanosilica fertilizer application.

The combination of 1.25 mm l⁻¹ nanosilica fertilizer and 1 ton ha⁻¹ dose of rice hull ash produced the highest effect on leaf thickness of 222.22 μm. Silica fertilizer accumulate in the leaves to form a silica
gel layer on the epidermal cell wall [17]. Thickness of the epidermis is one of the structural defenses found in plants, even before pathogens come and contact with plants [18]. The thickness and strength of the outer walls of epidermal cells is an important factor in the resistance of certain types of plants to certain pathogens [19]. Sufficient Si supply to cerealia is able to produce good plants because the addition of Si can increase cell strength and endurance [9].

Furthermore, leaf thickness would have a higher dry period per unit area and have thicker palisade tissue and more chloroplasts, this leaf structure is more conducive to transport CO₂, so that thicker leaf has strong photosynthetic capacity and higher biomass accumulation [20].

3.5. Stomata density (pieces.cm⁻²)

There is an interaction effect between the treatment of nanosilica fertilizer and rice hull ash on stomata density. The high stomata density was obtained from the highest treatment of nanosilica fertilizer and without rice hull ash fertilizer (s₃p₀). It shows that if you want to use nanosilica fertilizer without rice hull ash fertilizer, nanosilica fertilizer must be at the highest level to get high stomata density. Table 1 also shows that to get a high stomata density, if you want to use nanosilica fertilizer with a level below s₃, it must be accompanied by the application of rice hull ash fertilizer with a level of p₂ (s₁p₂) of 385.56 pieces.cm⁻². A study by sugarcane plants showed that the application of silica increased the density of stomata which causes more stomata numbers, thus it increases the rate of transpiration and CO₂ absorption for photosynthesis [21].

Table 2. Average stomata density of soybean plants in various treatment of concentration nanosilica fertilizer and dose of rice hull ash

| Treatment | s₀ (0 mm l⁻¹) | s₁ (1.25mm l⁻¹) | s₂ (2.50 mm l⁻¹) | s₃ (3.75ml l⁻¹) |
|-----------|--------------|-----------------|-----------------|-----------------|
| p₀ (0 tons ha⁻¹) | 314.23         | 290.45          | 225.90          | 373.67          |
| p₁ (2 tons ha⁻¹) | 304.03         | 205.52          | 295.54          | 264.97          |
| p₂ (3 tons ha⁻¹) | 322.72         | 385.56          | 317.62          | 287.05          |
| p₃ (4 tons ha⁻¹) | 285.35         | 334.61          | 290.45          | 281.95          |

Description: The average number with the same lowercase letters in the same column or uppercase in the line shows no significant difference based on the 5% DMRT test

This shows that silica plays a role in the process of cell differentiation. Differentiation is one form of plant adaptation to adjust its function to the environment. Increasing the number of stomata is a process of adaptation of plants to their environmental conditions. Plants treated with nanosilica fertilizer had a greater stomata size than the control. The increasing number of stomata can be assumed as a form of adaptation of plants to the surrounding environment due to greater evaporation needs.

3.6. Trichome density (pieces.cm⁻²)

The results of the variance analysis show that there is an interaction effect between the applications of nanosilica and rice hull ash on trichome density (Table 3). Application of rice hull ash with levels p₁ and p₂, it is obtained that the trichome density will decrease if it is accompanied by the applications of nanosilica fertilizer with level s₁. On the other hand, in the treatment of rice hull ash at the level of p₃, the trichome density was the highest when it was coupled with the applications of nanosilica fertilizer with a level of s₁ (s₁p₃) which was 14.35 pieces.cm⁻². Trichome density will decrease if the application of nanosilica fertilizer exceeds s₁. Unlike without giving rice hull ash fertilizer (p₀), trichome density was not affected either without or with the application of nanosilica fertilizer.
Table 3. Average of trichome density of soybean plants on various treatments of nanosilica fertilizer and rice hull ash.

| Treatment | Trichome Density (pieces.cm$^{-2}$) |
|-----------|------------------------------------|
|           | s0 (0 mm l$^{-1}$) | s1 (1.25 mm l$^{-1}$) | s2 (2.50 mm l$^{-1}$) | s3 (3.75 mm l$^{-1}$) |
| p0 (0 tons ha$^{-1}$) | 8.57$^a$ | 9.15$^a$ | 7.70$^a$ | 7.13$^a$ |
| p1 (2 tons ha$^{-1}$) | 6.74$^a$ | 6.55$^a$ | 8.86$^a$ | 7.03$^a$ |
| p2 (3 tons ha$^{-1}$) | 10.01$^a$ | 7.80$^a$ | 10.01$^a$ | 6.45$^a$ |
| p3 (4 tons ha$^{-1}$) | 8.76$^a$ | 14.35$^b$ | 7.13$^a$ | 7.41$^a$ |

Description: The average number with the same lowercase letters in the same column or uppercase in the line shows no significant difference based on the 5% DMRT test.

This result was in line with a study where soybeans with tighter trichomes tend to have resistance to whitefly [22]. A tight trichome prevents plants from being attacked by whitefly so as to reduce leaf damage. In addition to trichome density, the trichome position (upright or not) also affects the whitefly population in soybeans [23]. This can be understood because with a tight trichome it will prevent infestation of whitefly and reduce leaf damage, so that plants can grow well and be able to produce optimally. In two types of trichomes in strawberry plants, it was found that the content of oxidative enzymes produced by the trichome gland acted as a resistance factor for strawberries against mite pests [24]. In soybean plants, composition of the chemical substance lupeol (triterpene) affects the eating behavior of whitefly [23]. In this study, the control of the pest did not use chemical pesticides, it only relied on nanosilica fertilization and it was proven that soybean plants did not get a problem from pest.

3.7. Silica content in leaves (%)

There is an interaction effect between nanosilica application and rice hull ash on the nanosilica content in plant leaves. Table 4 below show that the content of nanosilica in leaves was high if the application of rice hull ash fertilizer with a level of p2 was accompanied by the applications of nanosilica with a level of s1 (treatment of s1p2) of 2.22%. On the other hand, the nanosilica content in the leaves was low if the applications of nanosilica with S1 level was accompanied by the fertilization of lower rice hull ash, which is level p1 (treatment of s1p1) which is 1.17%. Silica in the leaves of plants will accumulate in the cell wall and cuticle forms a silica layer. Under the cuticle, silica will be associated with cellulose it causes the silica layer become thicker so that it can increase the thickness of the leaves. According to [25] the accumulation of silica leaves will form cuticle-silica double layer which is located between the cuticle and the cell wall of the epidermis.

The ability of plants to absorb Si elements in different soils which causes differences in Si which accumulates in plants. Si is a micro nutrient element for plants that are important in plant tissues. In non-collector, Si is in the group of dicotyledons, for example soybeans, the Si content in the system is only 0.5% which forms a silica layer under epidermal system. In the plant body Si elements are found as silica gel or formless biogenic opal (SiO$_2$·nH$_2$O) on cell walls or in intercellular spaces or as monosilic acids, colloidal silicic acids, or organosilicon components in plant systems [26]. The Silica element is absorbed by plants in the form of silicic acid (H$_4$SiO$_4$), which is very important in strengthening the walls of the epidermis and vascular tissue, reducing water shortages and avoiding fungal infections.
Table 4. Average silica content in leaves of soybean plants in various treatments of nanosilica fertilizer and rice hull ash

| Treatment | p0 (0 tons ha⁻¹) | p1 (2 tons ha⁻¹) | p2 (3 tons ha⁻¹) | p3 (4 tons ha⁻¹) |
|-----------|-----------------|-----------------|-----------------|-----------------|
| s₀ (0 mm l⁻¹) | 1.41a           | 1.39a           | 1.16a           | 1.45a           |
| s₁ (1.25 mm l⁻¹) | 1.72ab         | 1.17a           | 2.22a           | 1.69ab          |
| s₂ (2.50 mm l⁻¹) | 1.89a           | 1.38a           | 1.72a           | 1.54a           |
| s₃ (3.75 mm l⁻¹) | 1.58a           | 2.04a           | 1.92a           | 1.49a           |

Description: The average number with the same lowercase letters in the same column or uppercase in the line shows no significant difference based on the 5% DMRT test

4. Conclusion
Nanosilica fertilizer concentration and rice hull ash dose significantly affected on the characteristics of soybean leaves (*Glycine max* (L.) Merrill): (a) nanosilica fertilizer concentration 1.25 mm l⁻¹ influences leaf thickness, while 2.50 mm l⁻¹ influence on leaf area leaf and LAI; (b) the combination of nanosilica concentration 1.25 mm l⁻¹ and 1 tons ha⁻¹ rice hull ash dose influences the thickness of the leaves; (c) nanosilica concentration 1.25 mm l⁻¹ and 2 tons ha⁻¹ rice hull ash dose influence the density of stomata and silica content in the leaves; and (d) the combination of nanosilica concentration 1.25 mm l⁻¹ and 3 tons ha⁻¹ rice hull ash dose influence the trichome density.

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References
[1] Theocharopoulos A, Aggelopoulos S, Papanagiotou P, Melfou K and Papanagiotou E 2012 Sustainable farming systems vs conventional agriculture: A socioeconomic approach in Sustainable development-Education, business and management-Architecture and building construction-Agriculture and food security (InTech)
[2] Malau 2013 National Soybean Needs Tribun News 2013
[3] Marwoto dan Bejo 1996 Leaf Caterpillar Pest Resistance To Insecticides In The Center Of Soybean Production In Eastern Java. Technical Report 1996–1997 (Malang: Research Centers For Legumes And Tubers)
[4] Putro A L and Prasetyoko D 2007 *Akta Kimindo* 3 33-36
[5] Kiswondo S 2011 *Embryo* 8 9–17
[6] Takahashi E 1995 *Sci. Rice Plant* 2 58–71
[7] Martanto E A 2001 *Irian Jaya Agro* 8 37–40
[8] Snyder G H, Matichenkov V V and Datnoff L E 2016 *Handbook of plant nutrition* (CRC Press) 567–584.
[9] Sumida H 2002 *Proceedings of the Second Silicon in Agriculture Conference, 22-26 August 2002* (Tsuruoka, Yamagata, Japan) pp. 43–49
[10] Egli D B 1999 *Crop Sci* 39 1361–1368
[11] Bowen, P, Menzeis J, Ehret D 1992 *Journal of the American Society for Horticultural Science* 117 906-912
[12] Fitriani H P and Haryanti S 2016 *Bul. Anat. DAN Fisiol. dh SELLULA* 24 34–41
[13] Husnain 2011 *War. Penelit. dan Pengemb. Pertan.* **33** 12–13
[14] Indrananda H K 1986 *Soil Fertility Management* (Jakarta: PT. Bina Aksara)
[15] Blad B L and Baker D G 1972 *Agron. J.* **64** pp 26–29
[16] Surtinah 2013, Correlation of Growth of Vegetative Organs with Soybean (*Glycine max*, (L) Merill), Production in Mitigation and Climate Change Impact Adaptation Strategies in Indonesia
[17] Davidson 2013 Plant Cell Vacuoles
[18] Agrios G N *Plant Disease Science* (Yogyakarta: Gadjah Mada Univ Press) *Busnia, M penerjemah. Yogyakarta Gadjah Mada Univ. Press. Terjem. dari Plant Pathol.* **3**
[19] Mariana 2004 *Rice Plant Resistance to Blast (Pyricularia oryzae Cav.) Disease in Tidal Rice Fields in South Kalimantan* (Malang: Program Pascasarjana Universitas Brawijaya)
[20] Wu G, Zhou Z, P Chen, Tang X, Shao H and H. Wang 2014 *Sci. World J.*
[21] Arista Y and Wijaya K A *Morphology and Physiology of two sugarcane (Saccharum officinarum L.) Varieties*
[22] Tama O H 2011 *Trichome Density Analysis and Bemisia Tabaci Preference for Soybean Resistance CPMMV and its use as Integrated Pest Management Teaching Materialas Thesis* (Malang: Disertation dan Thesis Progr. Pascasarja UM)
[23] Lambert A L, McPherson R M and Espeliei K E 1995 *Environ. Entomol.* **24** 1381–1386
[24] Steinite I and Ievinsh G 2003 *Acta Univ. Latv.* **662**
[25] Yoshida S, Ohnishi Y and Kitagishi K 1962 *Soil Sci. Plant Nutr.* **8** 1–5
[26] Yoshida S 1985 *Techn. Bull* **25** 1–27