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Impact of Organic NPK Nano Fertilizer on Growth and Physiological Parameters of Different Shallot Varieties

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ABSTRACT

The constant use of chemical fertilizers is associated with low nutrient-use efficiency and contributes to gas emissions, leading to global warming. Meanwhile, nano fertilizers represent an effort to increase nutrient-use efficiency. This study aimed to determine the effect of organic NPK nano fertilizers on growth performance and physiology of shallot plants. A completely randomized design was used in a factorial arrangement with 12 combinations and three replicates. The results showed that the best response was found in the treatment of Crok Kuning variety treated with 220 kg ha^{-1} Nitrogen, 160 kg ha^{-1} Phosphorus, and 120 kg ha^{-1} Potassium. This treatment increased plant height by 4.64%, leaf number by 8.89%, leaf area by 87%, plant fresh weight by 87%, stomatal aperture width by 33.20%, chlorophyll content by 23.7% and nitrate reductase activity by 9.6% compared to chemical fertilizers. The Tajuk variety treated with 160 kg/ha^{-1} Nitrogen, 100 kg ha^{-1} Phosphorus, and 60 kg/ha^{-1} Potassium fertilizers showed an increased in plant height by 4.80%, leaf number by 15.45%, leaf area by 60%, plant fresh weight by 60%, stomatal aperture width by 41.15%, chlorophyll content by 17.30% and nitrate reductase activity by 8.9% compared to chemical fertilizers. The application of organic nano-fertilizers has the potential to improve nutrient efficiency and physiological performance of shallots, and to promote sustainable agriculture by reducing the environmental impact of chemical fertilizers.

1. INTRODUCTION

Climate change is considered a critical global challenge, impacting various sectors of life, including agriculture, which plays a crucial role in meeting the world food demand [1]. The agriculture industry faces increasing instability due to climate-induced changes such as erratic weather, higher global temperatures, changing rainfall patterns, and frequent natural disasters. These changes have significantly disrupted agricultural productivity on a worldwide scale [2]. Increasing population and changing consumption habits have raised the demand for farm products such as shallots, leading to a gap between production and consumption [3, 4]. To bridge this gap, one widely practiced method is using inorganic fertilizer, which offers faster results in increasing crop growth and yield. However, the extensive use of fertilizer poses serious environmental problems. Inorganic fertilizer over time contributes to soil degradation, affecting the structure of microorganisms [5], the availability and uptake of elements in the soil [6, 7], and the eutrophication of water [8] bodies which can enter the food chain and ultimately interfere with human health [9]. In addition, using such fertilizers releases greenhouse gases such as nitrogen oxides (N_2O), which accelerate climate change.

Meeting the needs of shallots is achieved by applying organic fertilizer, which can be used as an option to increase nutrition and water resistance, improve soil structure, and support soil microbial activity [10]. Organic fertilizer can be obtained from agricultural waste and the slaughter industry. One of the farming wastes used is oil palm empty fruit bunches, which provide valuable nutrients reusable as an effective crop fertilizer, including 30-40% potassium [11, 12]. Generally, potassium is an essential macronutrient in plants, and it includes carbohydrate metabolism, enzyme activity, protein synthesis, assimilate translation, and osmotic adjustment [13-16]. It can be extracted from the fiber ash of oil palm empty fruit bunches after burning in open-air conditions. Furthermore, organic fertilizer provides essential nutrients that support crop productivity. An important waste from the slaughter industry is animal blood, which contains high nitrogen of about 10-13% making it suitable as organic N fertilizer [17]. Animal bones also contain a chemical composition of 16.85% phosphorus [18, 19]. The availability of some elemental content in bones can be used as fertilizer to improve soil fertility [20, 21].

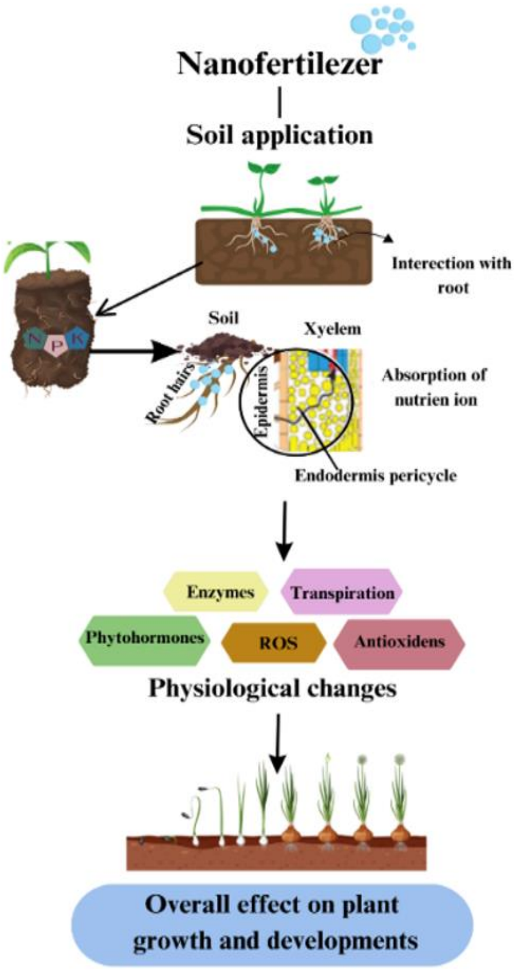

Figure 1. Absorption mechanism of nano fertilizer through soil application on plants

Fertilization of shallot plants can be achieved through the soil [22, 23], specifically when the particle size of fertilizer material is smaller than the root pore size (Figure 1). Soil applied nano fertilizer first enters the root through its surface, then passes through the root cuticle layer, and then penetrates the root epidermis after crossing the cuticle on the root surface. When nano fertilizers reached the root epidermis, they can take one of two routes: the apoplastic or symplastic pathway. However, in the symplastic pathway, nano fertilizers move from one cell to another through plasmodesmata. After traveling through the central cylinder, nano fertilizers reach the above-ground parts of the plant via the xylem transport flow [24]. Effective nutrient absorption requires technological innovation by reducing particle size through nanotechnology, which is a technique for creating materials and functional structures at the nanometer scale [25, 26]. Generally, fertilizer of the right size has the proper bonding and superior abilities to the initial material, such as being easily absorbed by plants with slow-release fertilizer [27-29]. Nanotechnology can also be used in agriculture to increase crop yields and reduce environmental disturbances [26]. Based on the type of nanoparticle, fertilizers containing N, P, K, Cu, Mn, Zn, Fe, Mo, and other agricultural inputs have been widely developed [24, 30]. The very small particle size allows fertilizers to be absorbed more quickly by plants and increases use efficiency. Furthermore, the application of fertilizer in the form of nano fertilizer has shown positive impacts on plant growth, production, and disease control.

Nanoparticles of blood powder, chicken bone ash, and palm empty fruit bunch ash are needed as a source of organic nano NPK fertilizer that can be used to meet nutritional needs in growth and physiology of shallot plants. Information on applying fertilizer nanoparticles to shallot plants is limited. Therefore, this study aimed to examine the effect of organic NPK nano fertilizer on plant growth performance and shallot plant physiology.

2. MATERIALS AND METHODS

2.1 Study location

This study was conducted in the greenhouse of Muhammadiyah Yogyakarta University. Nanotechnology was produced in the Production Laboratory 2, physiological analysis of plants was performed in the Agrobiotech Laboratory, while analysis of Nitrate Reductase Activity was conducted at the Production Management Laboratory of Gajah Mada University. The study location is sited at coordinates 7°33'32.9" South latitude and 110°51'24.3" East longitude, with an altitude of 950 meters past sea level.

2.2 Experimental design

Table 1. Treatment details of the field experiment

Treatment	Treatment Details
S1D0	Chemical fertilizer Nitrogen 180; Phosphorus 120; Potassium 60 kg ha^{-1}
S1D1	Nano fertilizer Nitrogen 140; Phosphorus 80; Potassium 20 kg ha^{-1}
S1D2	Nano fertilizer Nitrogen 160; Phosphorus 100; Potassium 40 kg ha^{-1}
S1D3	Nano fertilizer Nitrogen 180; Phosphorus 120; Potassium 60 kg ha^{-1}
S1D4	Nano fertilizer Nitrogen 200; Phosphorus 140; Potassium 80 kg ha^{-1}
S1D5	Nano fertilizer Nitrogen 220; Phosphorus 160; Potassium 100 kg ha^{-1}
S2D0	Chemical fertilizer Nitrogen 180; Phosphorus 120; Potassium 60 kg ha^{-1}
S2D1	Nano fertilizer Nitrogen 140; Phosphorus 80; Potassium 20 kg ha^{-1}
S2D2	Nano fertilizer Nitrogen 160; Phosphorus 100; Potassium 40 kg/ha^{-1}
S2D3	Nano fertilizer Nitrogen 180; Phosphorus 120; Potassium 60 kg ha^{-1}
S2D4	Nano fertilizer Nitrogen 200; Phosphorus 140; Potassium 80 kg ha^{-1}
S2D5	Nano fertilizer Nitrogen 220; Phosphorus 160; Potassium 100 kg ha^{-1}

Description: S1: Crok Kuning variety. S2: Tajuk variety. D0: Chemical fertilizer Nitrogen 180; Phosphorus 120; Potassium 60 kg ha^{-1}. D1: Nano fertilizer Nitrogen 140; Phosphorus 80; Potassium 20 kg ha^{-1}. D2: Nano fertilizer Nitrogen 160; Phosphorus 100; Potassium 40 kg ha^{-1}. D3: Nano fertilizer Nitrogen 180; Phosphorus 120; Potassium 60 kg ha^{-1}. D4: Nano fertilizer Nitrogen 200; Phosphorus 140; Potassium 80 kg ha^{-1}. D5: Nanofertilizer Nitrogen 220; Phosphorus 160; Potassium 100 kg ha^{-1}.

This study was conducted in polybags using a two-factor factorial CRD. The treatment factors were shallot variety and nano organic fertilizer dose. Animal bones and blood were obtained from a slaughterhouse in the Magelang area of Central Java, while oil palm empty fruit bunches were obtained from PT Merbaujaya Indaraya 2, Southeast Sulawesi. Shallot seed samples for the experiment were obtained from the Ngudi Makmur farmer group in Parangtritis village, Yogyakarta. The varieties used were Crok Kuning, and Tajuk, while the doses applied were chemical fertilizer 180 kg ha^{-1} Nitrogen; 120 kg ha^{-1} Phosphorus; 60 kg ha^{-1} Potassium, as well as organic NPK nano fertilizer 140 kg ha^{-1} Nitrogen; 80 kg ha^{-1} Phosphorus; 20 kg ha^{-1} Potassium; 160 kg ha^{-1} Nitrogen; 100 kg ha^{-1} Phosphorus; 40 kg ha^{-1} Potassium; 180 kg ha^{-1} Nitrogen; 120 kg ha^{-1} Phosphorus; 60 kg/ha^{-1} Potassium; 200 kg/ha^{-1} Nitrogen; 140 kg ha^{-1} Phosphorus; 80 kg/ha^{-1} Potassium; and 220 kg/ha^{-1} Nitrogen; 160 kg ha^{-1} Phosphorus; 100 kg ha^{-1} Potassium. From these 2 factors, 12 treatment varieties were received and repeated 3 times, resulting in 36 testing units. The experimental treatment specifications are shown in Table 1. Organic NPK nano fertilizer was directly applied to the soil 3 times at the age of 10, 25, and 40 days after sowing. The observed variables include plant growth (plant height, number of leaves, leaf area, plant fresh weight), chlorophyll content, number of stomata, stomatal opening width, and nitrate reductase activity.

2.3 The manufacturing procedure of nano fertilizer

Animal bone ash, palm empty fruit bunch ash, and animal blood meal were ground into powder using a hammer mill [31]. The powder was mixed with small steel balls and water in a ratio of 1:2:3 and milled using a ball mill for two hours [27]. The milling results were then screened to separate the steel balls and suspense. The suspense was sedimented for 2-3 days and dried using an oven at 30°C or dried in the sun for 3 days. The milling process is based on the principle of material deformation on the ball shell due to collision with other balls, resulting in the notification of bone ash powder, palm empty fruit bunch ash, and blood powder.

2.4 Parameter analysis

2.4.1 Leaf area

Measurement of leaf area was carried out using LAM (Leaf Area Meter). Leaf area observations were made on plants aged 21, 36, 52, and 70 DAP.

2.4.2 Chlorophyll content

Chlorophyll content was determined by extracting leaf samples collected 21, 36, 52, and 70 days after sowing, using acetone as the extraction agent. This measurement followed the method described by Arnon [32]. The chlorophyll analysis process was carried out by extracting 1 g of leaves using 20 ml of acetone, then filtered and analyzed using a spectrophotometer at wavelengths of 663 nm and 645 nm. Chlorophyll content was calculated using the formula:

$$\text{Chlorophyll a} = 1.07\ (\text{OD } 663) - 0.094\ (\text{OD } 644)$$
$$\text{Chlorophyll b} = 1.77\ (\text{OD } 644) - 0.28\ (\text{OD } 663)$$
$$\text{Total chlorophyll} = 0.79\ (\text{OD } 663) + 1.076\ (\text{OD } 644)$$

2.4.3 Stomatal aperture width

Observations were made at each growth phase of shallot plants at 21, 36, 52, and 70 days after transplanting. Sampling was carried out directly in the sun without picking the leaves to keep the stomatal cells open using the replica method and observed through an Olympus CX-22LEDRFS1 computer microscope at 400x magnification.

2.4.4 Nitrate reductase activity

Observations were made during each growth phase of shallot plants at 21, 36, 52, and 70 days after planting. The test was conducted based on Hari Hartiko's method. Before testing, the leaves to be tested were cleaned, and the bones were removed. The leaves were then cut with a width of approximately 1 mm, weighed up to 200 mg, placed in a film tube, and served with 5 mL of phosphate buffer solution with pH 7.0. The film tube filled with the sample was then incubated in a dark room for 24 hours. Subsequently, the solution in the film tube was scrapped and replaced with a new Phosphate Buffer of pH 7.0. In the film tube, 0.1 mL of 5 M $NaNO_3$ was added as a substrate, and this period represented the initial incubation time. A reagent solution was then prepared consisting of 0.2 mL 1% SA in 3 N HCl and 0.2 mL of 0.02% NED in a test tube. After the incubation time of 2 hours was completed, the film tube was removed from the dark room. A total of 0.1 mL filtrate was placed in a test tube previously prepared for the reagent, then waited for 15 minutes until a pink color appeared. After 15 minutes, distilled water of 2.5 mL was added. The solution was homogenized and repositioned into a cuvette to measure the absorbance with distilled water as a blank at a wavelength of 540 nm using a Genesis 10s UV-Vis spectrophotometer [33-35].

2.4.5 Plant tissue NPK uptake analysis

Plant tissue NPK analysis was also conducted to determine the content of NPK nutrients in plants so as to determine the NPK nutrients absorbed by plants. The analysis was carried out by the wet ashing method using a mixture of concentrated acids HNO_3 and $HClO_4$. The concentration of macro elements in the extract was then measured using an Atomic Absorption Spectrophotometer (AAS) and a visible light spectrophotometer.

2.5 Statistical analysis

The data received from this study were examined using the Statistical Analysis System (SAS) 9.0 application. The analytical method utilized was the analysis of variance (ANOVA) with a significance level of 5%. Mean comparisons between therapies were tested using the Duncan Multiple Range Test (DMRT) at 5%.

3. RESULTS AND DISCUSSION

Application of organic NPK nano fertilizer and shallot variety can increase shallot growth as illustrated in Figures 2, 3, 4, and 5. Although the treatment did not affect plant height (Figure 2), the Crok Kuning variety treatment with doses of Nitrogen 220, Phosphorus 160, and Potassium 120 kg ha^{-1} produced the best results. The chemical fertilizer doses of Nitrogen 180, Phosphorus 120, and Potassium 80 kg ha^{-1} had the lowest value. The treatment of Crok Kuning variety with nano fertilizer doses of Nitrogen 220, Phosphorus 160, and Potassium 120 kg ha^{-1} increased plant height by 4.64% compared to chemical fertilizer. Meanwhile, the Tajuk variety with nano fertilizer dose of Nitrogen 160, Phosphorus 100, and Potassium 60 kg ha^{-1} significantly increased the plant height by 4.80% compared to the chemical fertilizer. As shown in Figure 3, the Crok Kuning variety with nano fertilizer dose of Nitrogen 220, Phosphorus 160, and Potassium 120 kg ha^{-1} showed the highest increase in the number of leaves by 8.89% compared to chemical fertilizer. The Tajuk variety treatment with nano fertilizer dose of Nitrogen 160, Phosphorus 100, and

Potassium 60 kg ha^{-1} increased the number of leaves by 15.45% compared to chemical fertilizer. This result shows that the use of organic nano NPK can effectively replace the application of chemical fertilizer. A previous study [36] found a similar result where nano fertilizer increased growth of sesame (*Sesamum indicum* L) compared to chemical fertilizer. Using organic NPK nano fertilizer promotes plant growth [37] leading to improved crop yield and quality. The application in this study showed positive results on shallot plant growth. In particular, the Crok Kuning variety with nano fertilizer dose of Nitrogen 220, Phosphorus 160, and Potassium 120 kg ha^{-1} as well as the Tajuk variety with nano fertilizer dose of Nitrogen 160, Phosphorus 100, and Potassium 60 kg ha^{-1} were more effective in improving plant growth.

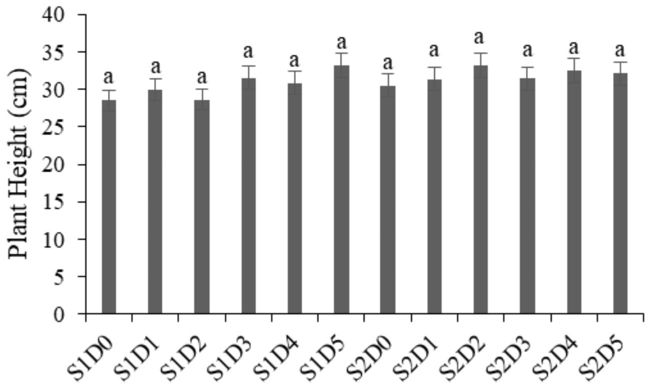

Figure 2. Effect of shallot variety and organic NPK nano fertilizer dose on plant height

where, S1: Crok Kuning variety. S2: Tajuk variety. D0: Chemical fertilizer Nitrogen 180; Phosphorus 120; Potassium 60 kg ha^{-1}. D1: Nano fertilizer Nitrogen 140; Phosphorus 80; Potassium 20 kg ha^{-1}. D2: Nano fertilizer Nitrogen 160; Phosphorus 100; Potassium 40 kg ha^{-1}. D3: Nano fertilizer Nitrogen 180; Phosphorus 120; Potassium 60 kg ha^{-1}. D4: Nano fertilizer Nitrogen 200; Phosphorus 140; Potassium 80 kg ha^{-1}. D5: Nano fertilizer Nitrogen 220; Phosphorus 160; Potassium 100 kg ha^{-1}.

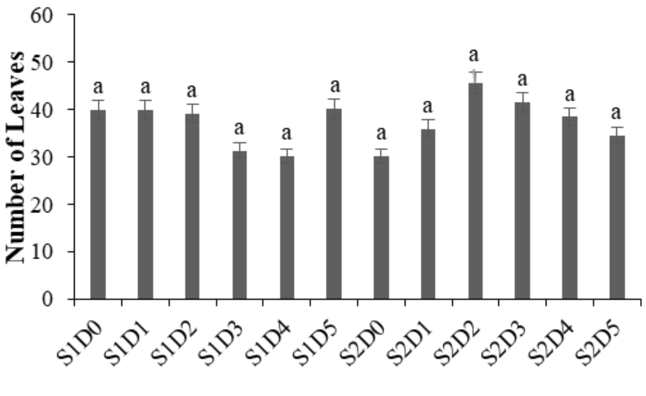

Figure 3. Effect of shallot variety and dose of organic NPK nano fertilizer on the number of leaves

As shown in Figure 3, the Crok Kuning variety treatment with nano fertilizer doses of Nitrogen 220, Phosphorus 160, and Potassium 120 kg/ha^{-1} increased leaf area by 87% compared to chemical fertilizer dose of Nitrogen 180, Phosphorus 120, and Potassium 80 kg ha^{-1}. Similarly, the Tajuk variety treatment with nano fertilizer dose of Nitrogen 160, Phosphorus 100, and Potassium 60 kg ha^{-1} increased leaf area yield by 60% compared to the chemical fertilizer. Leaf surface area is strongly influenced by nutrient availability. The increase in leaf area can occur due to the role of nano fertilizer in several metabolic activities, which elevate enzyme activity, positively impact plant production, and expand leaf area [38, 39]. The wider the leaves of a plant, the more sunlight that can be absorbed, thereby increasing the process of photosynthesis. The resulting photosynthate produced increases along with plant growth [40].

Figure 4 shows the effect of organic NPK nano fertilizer on fresh weight. The Crok Kuning variety with nano fertilizer doses of Nitrogen 220, Phosphorus 160, and Potassium 120 kg ha^{-1} has the effect of increasing the fresh weight by 87% compared to chemical fertilizer doses of Nitrogen 180, Phosphorus 120, and Potassium 80 kg ha^{-1}. Meanwhile, the Tajuk variety treatment with nano fertilizer dose of Nitrogen 160, Phosphorus 100, and Potassium 60 kg ha^{-1} increased plant fresh weight by 60% compared to the chemical fertilizer. Increasing the dose and amount of water applied enhances the water content in the cell used for metabolic activity, which will affect the fresh weight of the plant. Study [41] suggested that high water absorption will enhance cell elongation and cell enlargement, thereby increasing water content and wet weight.

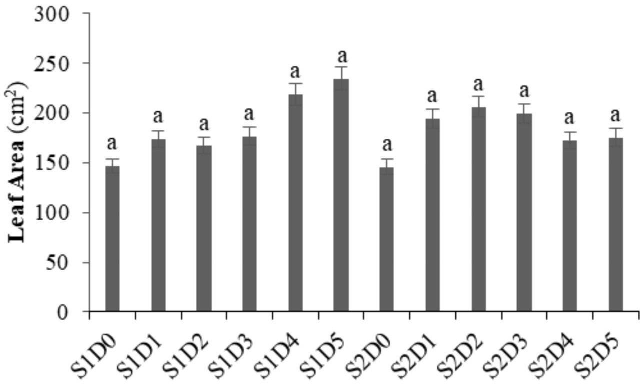

Figure 4. Effect of shallot variety and dose of organic NPK nano fertilizer on leaf area

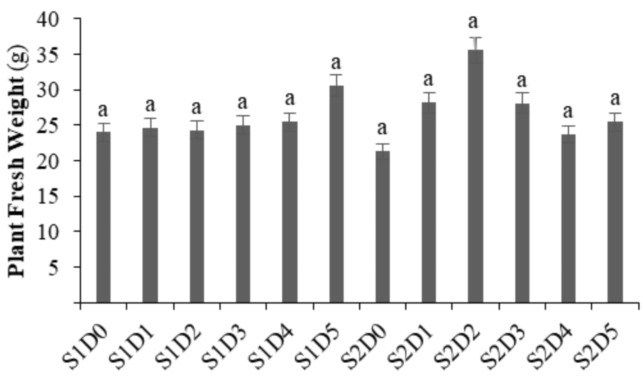

Figure 5. Effect of shallot variety and organic NPK nano fertilizer dose on plant fresh weight

The interaction between doses of organic NPK nano fertilizer and Tajuk variety requires less fertilizer in growth phase. A similar study [42] found that fertilizer application below the recommendations, increased plant growth. NPK is an important macro fertilizer that plays a role in plant growth. The lack of absorbed macro and micronutrients also affects the vegetative growth of plants [43, 44]. The provision of organic matter can advance the availability of nutrients in the soil, specifically N elements that function for the vegetative development of plants. In the vegetative phase, N plays a significant role in plant growth. Above-ground shallot growth responses, such as plant height, leaf area, number of leaves, and plant fresh weight, are affected by the application of organic NPK nano fertilizer (Figure 5). Furthermore, the application of organic material sources will produce equivalent or better effects on plant growth, development, and production [45]. Phosphorus also functions in plant metabolism, cellular energy transfer, respiration, and photosynthesis [46]. Potassium in plants has the function of being the most abundant cellular cation, contributing to stomatal movement regulation, osmotic adjustment, enzyme activation, and membrane protein transport [47]. The three nutrients will promote cell division and expansion, activate enzymes, as well as accelerate leaf formation, and plant growth.

The ANOVA results showed no significant interaction between shallot variety with the dose of organic NPK nano fertilizer. As shown in Table 2, shallot variety used had no significant effect on the weight of bulbs per plant. This result indicates that the Crok Kuning and Tajuk variety used had a negligible effect on the yield of shallot plants.

The use of organic NPK nano fertilizer dose did not significantly affect the weight of fresh bulbs per plant. This result shows that nano fertilizer is a potential nutrient source similar to inorganic fertilizer for plant yield. Applying organic NPK nano fertilizer at a dose of Nitrogen 140, Phosphorus 80, and Potassium 20 kg ha^{-1} achieved shallot yield comparable to the recommended chemical fertilizer with Nitrogen 180, Phosphorus 120, and Potassium 80 kg ha^{-1}. The achievement of high shallot yields is due to the availability of macronutrients such as K, N, P, Mg, and S that are sufficiently available to plants [48]. Therefore, adding nano nutrients of Nitrogen, Phosphorus, and Potassium directly to the soil is expected to meet plant growth needs and increase yields.

As shown in Table 3, the use of organic NPK nano fertilizer doses did not significantly affect the number of stomata in the vegetative stage and tuber maturation but had a significant effect on the tuber formation phase. The results of further tests showed that doses of Nitrogen 220, Phosphorus 160, and Potassium 120 kg ha^{-1}, had the highest number of stomata at 39 n/mm^2. The dose produced significantly different results from the chemical fertilizer treatment or control. This is because a high potassium concentration relatively increases stomatal density. Stomatal density affects two essential processes in plants, namely transpiration and photosynthesis. Plants with high stomatal density have a higher transpiration rate than those with low density. More stomata per unit area leads to increased CO_2 uptake [49].

The results showed that applying organic NPK nano fertilizer had no significant effect on the vegetative phase and bulb formation. The high number of stomata in shallot may be due to other factors aside from plant nutrition, such as surrounding environmental conditions. Studies [50, 51] reported that the number of stomata in plants can be influenced by the environment, in response to increased evaporation. For example, plants will be more susceptible to diseases such as bacteria or fungi that favor moist environments [52].

Table 2. Effect of variety and dose of organic NPK nano fertilizer on shallot yield

Treatment	Fresh Weight of Bulb per Plant (g)			
	21 DAP	36 DAP	52 DAP	70 DAP
Factor 1. Shallot Variety				
Variety Crok Kuning	1.78 a	6.40 a	9.19 a	25.45 a
Variety Tajuk	1.22 a	5.01 a	9.68 a	26.25 a
Factor 2. Dose of Organic NPK nano fertilizer				
Chemical fertilizer (N 180, P 120, K 80 kg/ha^{-1})	1,11 a	5.20 a	6.38 a	19.332 a
Nano fertilizer (N 140, P 80,K 80 kg/ha^{-1})	2.30 a	6.26 a	8.78 a	20.55 a
Nano fertilizer (N 160, P 100, K 60 kg/ha^{-1})	2.22 a	5.38 a	11.71 a	31.91 a
Nano fertilizer (N 180, P 120, K 80 kg/ha^{-1})	1.20 a	4.95 a	11.52 a	25.75 a
Nano fertilizer (N 200, P 140, K 100 kg/ha^{-1})	0.87 a	4.73 a	9.70 a	28.498 a
Nano fertilizer N 220, P 160,K 120 kg/ha^{-1})	1.30 a	7.71 a	8.57 a	29.073 a
Interaction	ns	ns	ns	ns

Note: The numbers values accompanied by different letters within the same column exhibit significant differences at the DMRT test level of α = 0.05; * = significant; ns = not significant.

Table 3. Effect of variety and dose of organic NPK nano fertilizer on the number of stomata

Treatment	Number of Stomata (n/mm^2)			
	21 DAP	36 DAP	52 DAP	70 DAP
Factor 1. Shallot Variety				
Variety Crok Kuning	61.37 a	57.90 a	60.17 a	58.84 a
Variety Tajuk	61.97 a	57.32 a	68.07 a	57.59 a
Factor 2. Dose of Organic NPK nano fertilizer				
Chemical fertilizer (N 180, P 120, K 80 kg/ha^{-1})	57.47 a	48.80 b	59.62 a	59.15 a
Nano fertilizer (N 140, P 80,K 80 kg/ha^{-1})	61.50 a	61.03 ab	65.72 a	68.31 a
Nano fertilizer (N 160, P 100, K 60 kg/ha^{-1})	54.69 a	53.52 ab	61.73 a	52.24 a
Nano fertilizer (N 180, P 120, K 80 kg/ha^{-1})	63.61 a	56.82 ab	67.84 a	49.79 a
Nano fertilizer (N 200, P 140, K 100 kg/ha^{-1})	69.95 a	66.43 a	63.14 a	54.22 a
Nano fertilizer N 220, P 160,K 120 kg/ha^{-1})	68.54 a	62.67 ab	66.67 a	61.97 a
Interaction	ns	ns	ns	ns

Note: The numbers values accompanied by different letters within the same column exhibit significant differences at the DMRT test level of α = 0.05; * = significant; ns = not significant.

Table 4. Effect of variety and dose of organic NPK nano fertilizer on plant physiology

Treatment	Stomatal Aperture Width (µm)	Chlorophyll Content (mg/g)	Nitrate Reductase (µ mol NO_2-/g/jam)
S1D0	8.42 b	67.47 b	0.65 e
S1D1	10.81 ab	68.35 ab	0.64 e
S1D2	10.77 ab	68.62 ab	0.74 de
S1D3	10.27 ab	69.15 ab	1.12 cd
S1D4	9.28 ab	68.84 ab	1.61 bc
S1D5	11.77 a	69.79 a	1.62 ab
S2D0	8.05 b	67.00 b	1.04 cd
S2D1	8.32 b	68.73 ab	1.74 ab
S2D2	12.20 a	68.17 ab	1.93 a
S2D3	9.09 ab	68.22 ab	1.63 ab
S2D4	8.91 ab	68.90 ab	1.71 ab
S2D5	10.01 ab	69.51 ab	1.34 bc
Interactiaon	ns	ns	ns

Note: The numbers values accompanied by different letters within the same column exhibit significant differences at the DMRT test level of α = 0.05; * = significant; ns = not significant. S1: Crok Kuning variety. S2: Tajuk variety. D0: Chemical fertilizer Nitrogen 180; Phosphorus 120; Potassium 60 kg/ha^{-1}. D1: Nano fertilizer Nitrogen 140; Phosphorus 80; Potassium 20 kg/ha^{-1}. D2: Nano fertilizer Nitrogen 160; Phosphorus 100; Potassium 40 kg/ha^{-1}. D3: Nano fertilizer Nitrogen 180; Phosphorus 120; Potassium 60 kg/ha^{-1}. D4: Nano fertilizer Nitrogen 200; Phosphorus 140; Potassium 80 kg/ha^{-1}. D5: Nano fertilizer Nitrogen 220; Phosphorus 160; Potassium 100 kg/ha^{-1}.

Table 5. Effect of shallot variety and doses of organic NPK nano fertilizer on nitrogen, phosphorus, potassium nutrient uptake

Treatment	Nutrient Uptake Nitrogen	Nutrient Uptake Phosphorus	Nutrient Uptake Potassium
S1D0	2.78 e	764.15 e	413.88 d
S1D1	2.83 e	853.33cd	515.82 cd
S1D2	2.99 de	869.16 bc	581.07 a
S1D3	3.39 ab	841.26 d	540.15 c
S1D4	3.68 a	891.15 ab	571.57 a
S1D5	2.91 de	894.29 a	585.55 a
S2D0	3.26 bc	728.44 b	528.27 cd
S2D1	3.43 ab	782.10 c	433.67 g
S2D2	3.53 ab	776.48 c	519.25 d
S2D3	3.27 bc	767.30 c	459.30 f
S2D4	3.42 ab	815.97 a	546.41b
S2D5	3.05 cd	757.42 c	500.64 e
Interactiaon	*	*	*

Note: The numbers values accompanied by different letters within the same column exhibit significant differences at the DMRT test level of α = 0.05; * = significant; ns = not significant. Note: S1: Crok Kuning variety. S2: Tajuk variety. D0: Chemical fertilizer Nitrogen 180; Phosphorus 120; Potassium 60 kg/ha^{-1}. D1: Nano fertilizer Nitrogen 140; Phosphorus 80; Potassium 20 kg/ha^{-1}. D2: Nano fertilizer Nitrogen 160; Phosphorus 100; Potassium 40 kg/ha^{-1}. D3: Nano fertilizer Nitrogen 180; Phosphorus 120; Potassium 60 kg/ha-1. D4: Nano fertilizer Nitrogen 200; Phosphorus 140; Potassium 80 kg/ha^{-1}. D5: Nano fertilizer Nitrogen 220; Phosphorus 160; Potassium 100 kg/ha^{-1}.

In this study, applying organic NPK nano fertilizer and shallot variety significantly affected the width of stomatal openings (Table 4). The widest stomatal width was obtained in the treatment of Crok Kuning variety with nano fertilizer doses of Nitrogen 220, Phosphorus 160, and Potassium 120 kg/ha^{-1} measuring 11.27 µm. This result differed from the treatment of Crok Kuning variety with chemical fertilizer doses of Nitrogen 180, Phosphorus 120, and Potassium 80 kg/ha^{-1}. The Tajuk variety with nano fertilizer doses of Nitrogen 160, Phosphorus 100, and Potassium 60 kg ha^{-1} also had the broadest stomatal opening width of 9.09 µm compared to the chemical fertilizer. Therefore, it was concluded that the width of stomatal openings can be increased by applying organic NPK nano fertilizer. This is due to the activity of guard cells that require potassium to maintain turgor pressure, keeping the stomata open and affecting the width. Entering K+ ions into protective cells triggers folds in water osmosis, resulting in increased cell turgor pressure. According to study [41], potassium plays a role in stimulating water absorption, affecting the increase in cell turgor pressure. When high cell turgor pressure is maintained, stomata can be opened optimally. Potassium has a role in the process of opening as well as closing stomata, which is influenced by several factors, namely the osmotic pressure, turgor mechanism, accumulation of potassium ions and abscisic acid, and environmental factors, such as sunlight, humidity, temperature [53].

Potassium ions are actively transported into the guard cells through potassium channels in the plasma membrane, driven by the activity of proton pumps (H^+-ATPase). This ion influx lowers the osmotic potential of the guard cells, causing water to move into the cells via osmosis. The resulting increase in water content leads to elevated turgor pressure in the guard cells, causing them to swell and bend outward, thereby opening the stomatal pore [54]. Nanofertilizers enhance this mechanism by ensuring an efficient and sustained supply of potassium and other essential nutrients, optimizing ionic balance and water uptake in guard cells. This maximizes stomatal width, as observed in certain shallot varieties and fertilizer treatments. High turgor pressure facilitates optimal stomatal function, supporting effective gas exchange and photosynthesis.

As shown in Table 4, the treatment of organic NPK nano fertilizer and shallot variety can increase chlorophyll content. The Crok Kuning variety with nano fertilizer dose of Nitrogen 220, Phosphorus 160, and Potassium 120 kg/ha^{-1} had the highest content of 61.37 mg/g compared to chemical fertilizer and Tajuk variety. The increase in chlorophyll content can be caused by the role of organic NPK nano fertilizer given to shallot plants. A similar study [55] that applying fertilizer to corn plants increased chlorophyll content compared to the control. Leaf chlorophyll content is related to N concentration in fresh plants and measures plant response to N availability in the soil.

The activity of Nitrate Reductase (ANR) content in shallot leaves increased in each treatment dose (Table 4). The Crok Kuning variety with nano fertilizer doses of Nitrogen 220, Phosphorus 160, and Potassium 120 kg/ha^{-1} produced an increase of 1.98% compared to chemical fertilizer. In this case, plants may interact with nitrogen, phosphorus, and potassium nutrients. Nitrogen is an essential plant organ component that makes up nucleic acids, amino acids, and proteins. It is absorbed by the roots and transported to the plant as nitrate (NO^{3-}), ammonium (NH^{4+}), and amino acids, which are subsequently transported and assimilated in plant tissues to support growth and development [56]. Soil phosphorus availability can affect plants uptake of nitrate or ammonium via regulating the phosphorylation status of nitrate or ammonium transporters. Nitrate reductase activity was identified as a distinct phosphoprotein in soybean roots in reaction to phosphorus deficiency [57]. Additionally, potassium plays a crucial role in osmoregulation, enzyme activation, and the maintenance of ionic balance, which facilitates efficient nutrient uptake, including nitrogen and phosphorus. Potassium also affects stomatal functioning, indirectly influencing photosynthetic efficiency and the allocation of assimilates toward amino acid and protein synthesis. These physiological interactions collectively enhance the nutrient utilization efficiency in plants, explaining the observed increase in ANR content with nano fertilizer application.

Nitrogen uptake efficiency is the ratio of the amount of nitrogen accumulated to the amount applied to plants [58, 59]. According to Table 5, the use of organic NPK nano fertilizer has a significant effect on nitrogen nutrient uptake. The dose of Nitrogen 220, Phosphorus 160, and Potassium 120 kg/ha^{-1} produced the highest nitrogen content in shallot plants. The results also showed a significant increase from the control plants, amounting to 90%. This is because plants absorb N elements according to a specific capacity. Excessive N fertilizer will cause a decrease in nutrient absorption efficiency. A previous study [60] reported that applying high doses of N fertilizer did not increase crop yields but caused a reduction. The application of nano N fertilizer through the soil can also stimulate nutrient absorption and increase the content in plants [61]. Another study [62] stated that applying nano N fertilizer to plants increased N content, reduced leaching, and controlled nitrogen release. The uptake depends on the root system and the efficiency of N use in the soil [63].

As shown in Table 5, there was an interaction effect between shallot variety and doses of organic NPK nano fertilizer on phosphorus uptake. Each treatment had significantly different results compared to control plants. In the Crok Kuning variety, nano fertilization with a dose of Nitrogen 220, Phosphorus 160, and Potassium 120 kg/ha^{-1} produced the highest yield, while the highest yield of Tajuk variety was produced by doses of Nitrogen 200, Phosphorus 140, and Potassium 80 kg/ha^{-1}. These results show that applying organic NPK nano fertilizer to shallot plants can increase the phosphorus content. A previous study [64] reported that the highest phosphorus content was produced in the leaves of cotton plants [65]. Applying nano phosphorus fertilizer can increase the element content in barley seeds. Plants can absorb more phosphorus with higher available amounts in the soil. Increased phosphorus uptake in plants enhances plant growth and yield [66].

Based on the results, there was an interaction effect between shallot variety and doses of organic NPK nano fertilizer on potassium uptake. Each treatment had significantly different results compared to control plants. Nano fertilizer doses Nitrogen 220, Phosphorus 160, and Potassium 120 kg ha^{-1} showed the highest potassium content. A significant increase was also found in the control plant, reaching 138%. Applying potassium can increase the content of shallot plants due to the ability to increase metabolism and nutrient absorption. Potassium also affects enzyme activity which increases nutrient absorption [67]. In particular, applying nano potassium fertilizer can increase nutrient absorption, affecting element uptake in shallot plants. According to a previous study [68], applying nano potassium fertilizer improved potassium content in shallot plants. Using nano fertilizer can minimize the leaching of nutrients applied to plants, resulting in maximum absorption [69].

4. CONCLUSIONS

In conclusion, applying organic NPK nano fertilizer up to a dose of Nitrogen 220, Phosphorus 160, and Potassium 120 kg/ha^{-1} increased growth and physiological variables of shallot plants. The Crok Kuning variety treated with Nitrogen 220, Phosphorus, and Potassium 120 kg/ha fertilizers increased plant height by 4.64%, leaf number by 8.89%, leaf area by 87%, plant fresh weight by 87%, stomatal aperture width 33.20%, chlorophyll content 23.7% and nitrate reductase activity 9.6% compared to chemical fertilizers. The Tajuk variety treated with Nitrogen 160 kg/ha, Phosphorus 100 kg/ha, and Potassium 60 kg/ha fertilizers increased plant height by 4.80%, leaf number by 15.45%, leaf area by 60%, plant fresh weight by 60%, stomatal aperture width by 41.15%, chlorophyll content by 17.30% and nitrate reductase activity by 8.9% compared to chemical fertilizers. However, no significant effect was observed on shallot yield and the number of stomata. Fertilizer doses and application techniques must be tailored to the specific requirements of each shallot variety, as demonstrated in this study. Additionally, integrating nano fertilizers with traditional organic or bio-fertilizers is suggested to support long-term soil fertility and sustainable farming practices. While this study highlights improvements in growth and physiological parameters, further research is needed to investigate yield-specific effects and to test the effectiveness of organic nano fertilizers across different varieties and agro-climatic conditions.

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