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Optimization of rabbit urine liquid organic fertilizer and reduced NPK fertilizer doses for improving growth and physiology of shallot (*Allium ascalonicum* L.)

Abstract. Rabbit urine liquid organic fertilizer (LOF) can be used as an alternative fertilizer to reduce the use of inorganic fertilizers, especially for shallots, as a national food commodity. This study aims to determine the best concentration of rabbit urine LOF, the best dose of NPK fertilizer dose reduction, and a combination of rabbit urine LOF and NPK fertilizer dose reduction on the physiological characters of shallot plants. The observed variables consisted of net assimilation rate, crop growth rate, stomatal density, stomatal aperture, and chlorophyll content. The experimental results showed that a concentration of 200 mL L^{-1} of rabbit urine LOF had the best effect on several physiological characteristics studied. In addition, a 50% reduction in the NPK dose had the best effect on physiological characteristics compared to other treatments. Rabbit urine LOF 200 mL L^{-1} and a 50% reduction in NPK doses had a significant interaction effect on the physiological characters of shallot plants.

Keywords: Organic fertilizer · Rhizomes · Shallot · Sustainable

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Introduction

Horticultural commodities are an important source of providing nutritional needs and have a high potential to increase a country's income through export activities. One of the potential horticultural commodities is shallots (*Allium ascalonicum* L.). Shallot production in Indonesia peaked in July 2023 at 223.17 thousand tons from a harvested area of 18.31 thousand hectares. East Java, Central Java, and West Sumatra were the top-producing provinces. East Java contributed 24.41% to national production (484.67 thousand tons; 51.02 thousand hectares harvested), followed closely by Central Java at 24.13% (479.09 thousand tons; 46.80 thousand hectares) (Badan Pusat Statistik, 2024).

Brebes Regency is the largest shallot production center in Indonesia, providing 75% of the shallot needs in all parts of Central Java Province and supplying 23% of the national needs in Indonesia (Badan Pusat Statistik, 2015). The Bima Brebes shallot variety is an adaptive, superior variety with high production yields compared to other varieties. The results of research by Kartinaty et al. (2019), that of the five superior varieties of shallots tested showed that Bima Brebes and Katumi varieties produced the highest production of 9.37 tons ha^{-1} and 9.09 tons ha^{-1}; besides that the Bima Brebes variety had a high value compared to other varieties in each research variable in bulb diameter 9.55 mm; weight 25 g; yield per plot 68.5 kg followed by Katumi varieties with bulb diameter 9.47 mm; weight 22.5 g; yield per plot 54.5 kg.

The Bima Brebes shallot variety is adaptive to acidic soil. The Bima Brebes shallot variety has a high percentage of survival for planting on acidic soil, compared to other varieties (Ayu, 2022). The Bima shallot variety is adaptive to bulb rot disease, making it a preference for the interests of farmers and markets in Indonesia. Consumer interest in shallots with a strong aroma makes the Bima Brebes variety of shallots one of the preferred choices to be cultivated by farmers in Indonesia. The shallot cultivation carried out by farmers in Indonesia, especially in Brebes, Central Java, uses inorganic fertilizers at a high dose. Shallot farmers in Brebes tend to apply phosphorus (P) and potassium (K) fertilizers in high doses exceeding the recommended doses, although the application does not always increase crop yields (Trisnaningsih et al., 2023). NPK fertilizer supplies the primary macronutrients: nitrogen (N), phosphorus (P_2O_5), and potassium (K_2O) in varying compositional ratios (Sinha & Tandon, 2020).

In fact, long-term excessive chemical fertilizer application induces soil degradation, loss of beneficial soil microorganisms, and associated detrimental impacts (Vitousek et al., 1997). Efforts to reduce the accumulation of chemical residues in inorganic fertilizers are carried out by switching to the use of rabbit urine liquid organic fertilizer integrated with various other organic materials that form a solution medium, making it an innovation that has the potential to overcome the problems of cost and chemical residues.

The use of rabbit urine as an alternative organic fertilizer shows promise, particularly when fermented with additional natural ingredients that work synergistically to enhance nutrient content. The high nitrogen content in rabbit urine supports its use as a very prospective fertilizer. According to Rosniawaty et al. (2018), when compared with other manure, fertilizer from rabbit urine contained three times more nitrogen and urea than other livestock. Nitrogen demand in plants plays a role in plant development in important processes such as growth, leaf expansion, and biomass generation. These processes play a crucial role in the formation of physiological characteristics in plants. When nutrient requirements are met, essential primary macronutrients (N, P, and K) drive plant growth and physiological improvements (Ye et al., 2019; Wang et al., 2021). The few studies that have currently been conducted focus more on the use of rabbit urine as a single element in fertilization. Limited studies have added rhizomes and pineapple peels to the fermentation process of making rabbit urine LOF.

Materials and Methods

Plant material. This research was conducted on experimental land at coordinates 7º21'23 "S 109º15'35" E (Google Earth) at an altitude of 220 meters above sea level in Banjarsari Wetan Village, Sumbang District, Banyumas Regency, Central Java Province, from March to August 2023. The general condition of the average temperature in the morning, afternoon, and evening at the research site ranged from 23.3 – 32.2 °C, 28.9 – 35.3 °C, and 25.4 – 30.9 °C, respectively. Humidity in the morning, afternoon, and evening at the research site ranged

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from 55 – 99%, 49 – 89%, and 60 – 93%, respectively. Sunlight intensity on experimental land in the morning, afternoon, and evening at the research site ranged from 5900 – 73700 lux, 9920 – 83300 lux, and 11280 – 32700 lux, respectively. Daily rainfall ranged from 0 – 6.12 mm day^{-1}. Seeds of Bima Brebes shallot were collected from Brebes, Central Java. LOF is produced by fermenting for three months a mixture of rabbit urine, water, sambiloto (*Andrographis paniculata*) leaves, turmeric, galangal, temu ireng (*Curcuma aeruginosa* Roxb), brown sugar, pineapple peel waste, and Effective Microorganism 4 (EM4). The inorganic fertilizer used in this study is NPK (16-16-16).

Experimental design and treatments. This research was arranged in a complete randomized group design consisting of two factors. The first factor of rabbit urine concentration (K) consisted of 4 levels, namely 0 mL L^{-1}, 100 mL L^{-1}, 150 mL L^{-1}, and 200 mL L^{-1}. The second factor is NPK inorganic fertilizer (16-16-16) dose (P) consisting of 3 levels, namely 0% (200 kg ha^{-1}), 25% (150 kg ha^{-1}), 50% (100 kg ha^{-1}). The two factors were combined to create 12 treatment combinations, each replicated three times, for a total of 36 experimental units. Each unit contained 45 plants, resulting in 1,620 plants in total. One experimental unit measured 2 x 1.2 m with a spacing of 20 x 20 cm. The observation variables included net assimilation rate (NAR) and crop growth rate (CGR) at 24, 38, and 52 days after planting (DAP). In addition, stomatal density, stomatal aperture, and chlorophyll content were also measured. Observation data were analyzed statistically by analysis of variance (ANOVA) at the 5% level. If the results show a significant effect, continue with Duncan's Multiple Range Test (DMRT) at the 5% level.

Net assimilation rate. Net assimilation rate (NAR) measures the average photosynthetic efficiency of leaves in a plant community. It is calculated using the formula by Gardner et al. (1991):

$$NAR = \frac{(w2-w1)}{(T2-T1)} \times \frac{(lnA2-lnA1)}{(A2-A1)}$$

Notes:
w_1: Plant dry weight at the first observation
w_2: Plant dry weight at the second observation
T_1: Plant age at the first observation
T_2: Plant age at the second observation
A_1: Leaf area at the first observation
A_2: Leaf area at the second observation

Crop growth rate. Crop growth rate (CGR) quantifies the increase in plant biomass over time and can be calculated using the formula by Rahman et al. (2021):

$$CGR = \frac{W2-W1}{A(T2-T1)}$$

Notes:
W_1: Total plant dry weight at time T_1
W_2: Total plant dry weight at time T_2
A: Land area
T_1: Initial observation time
T_2: Subsequent observation time.

Stomatal density. Stomatal density was measured using a microscope after determining the field of view area. With a magnification of 40 x 10, the field of view area was 0.1589 mm^2, calculated using the formula:

Density = number of stomata/field of view area (Izza, 2015).

Stomatal aperture. Stomatal aperture was measured at random points using a micrometer, and the average aperture was calculated from all measured points.

Chlorophyll content. A total of 0.1 g of shallot leaves was finely ground and extracted with 10 mL of 80% acetone. The extract was filtered using filter paper until clear, then transferred into a test tube and sealed with cotton. A 3 mL aliquot of the extract was placed in a cuvette for measurement using a UV-Vis spectrophotometer at wavelengths of 645 nm and 663 nm. Chlorophyll content was calculated using the equations from Arnon (1949):

$$\text{Chlorophyll a} = \{(12.7 \times A663) - (2.69 \times A645)\} \times 10^{-1}$$
$$\text{Chlorophyll b} = \{(22.9 \times A645) - (4.86 \times A663)\} \times 10^{-1}$$
$$\text{Total Chlorophyll} = \{(20.2 \times A645) - (8.02 \times A663)\} \times 10^{-1}$$

Notes:
A663: Absorbance at 663 nm
A645: Absorbance at 645 nm

Results and Discussion

Table 1 shows that the effect of rabbit urine LOF significantly affects the NAR at 38 DAP with the highest value of 0.0020 g cm^{-2} day^{-1} at a concentration of 200 mL L^{-1} greater 53.85% than the concentration of 0 mL L^{-1} and CGR at 38 DAP with the highest value at 200 mL L^{-1} (38.14 g cm^{-2} day^{-1}) and 150 mL L^{-1} (34.17 g cm^{-2} day^{-1}). Reduction of NPK fertilizer doses had a significant effect on NAR at 24 and 52 DAP with the highest values of 0.0009 g cm^{-2} day^{-1} and 0.0007 g cm^{-2} day^{-1} at 50% dose reduction, respectively, 28.57% and 250% greater than 0% dose reduction, while at CGR at 24 DAP, the highest value was 7.08 g cm^{-2} day^{-1} at 50% reduction, 39.92% greater than 0% dose reduction.

The combination of rabbit urine LOF and reduced doses of NPK fertilizer significantly increased NAR at 24, 38, and 52 DAP. The highest NAR values—0.0012, 0.0022, and 0.0009 g cm^{-2} day^{-1}—were achieved with LOF applications of 150, 200, and 150 mL L^{-1}, combined with NPK reductions of 50%, 0%, and 25%, respectively. These values were 100%, 120%, and 12.5% higher than the control (0 mL L^{-1} LOF and 0% NPK reduction). Rabbit urine LOF combined with reduced NPK doses significantly increased CGR at 24, 38, and 52 DAP. The highest CGR values—10.26, 47.14, and 34.57 g cm^{-2} day^{-1}—were achieved with LOF applications of 100, 200, and 150 mL L^{-1}, respectively, each combined with a 25% NPK reduction. These values were 161.07%, 152.36%, and 54.19% higher than the control (0 mL L^{-1} LOF and 0% NPK reduction).

Table 1. The effect of rabbit urine LOF and reduced dose of NPK fertilizer on NAR and CGR

Treatments	Net Assimilation Rate (NAR) (g cm^{-2} day^{-1})			Crop growth rate (CGR) (g cm^{-2} day^{-1})		
	24 DAP	38 DAP	52 DAP	24 DAP	38 DAP	52 DAP
Rabbit urine LOF concentration (mL L^{-1})						
0	0.0007a	0.0013b	0.0004a	5.48a	24.27b	19.57a
100	0.0009a	0.0013b	0.0002a	7.24a	24.70b	15.34a
150	0.0008a	0.0016b	0.0005a	7.13a	34.17a	20.34a
200	0.0008a	0.0020a	0.0006a	5.14a	38.14a	23.62a
Reduction of NPK fertilizer dosage (%)						
0%	0.0007b	0.0016a	0.0004ab	5.06b	27.75a	19.66a
25%	0.0007b	0.0018a	0.0002b	6.59ab	35.04a	16.09a
50%	0.0009a	0.0014a	0.0007a	7.08a	28.19a	23.41a
Combination of Rabbit urine LOF and Reduction of NPK fertilizer dosage						
0 + 0%	0.0006d	0.0010d	0.0008ab	3.93d	18.68f	22.42ab
0 + 25%	0.0007cd	0.0014bcd	-0.0002ef	6.22bcd	26.27de	8.62bc
0 + 50%	0.0007cd	0.0015bc	0.0007ab	6.29bcd	27.87d	27.65ab
100 + 0%	0.0007cd	0.0012cd	0.0001de	4.32cd	20.44ef	20.75ab
100 + 25%	0.0010ab	0.0015bc	-0.0004f	10.26a	36.62bc	-0.23c
100 + 50%	0.0008bcd	0.0012cd	0.0008ab	7.13bc	17.05f	25.49ab
150 + 0%	0.0008cd	0.0016b	0.0002cd	8.08ab	41.59ab	13.30abc
150 + 25%	0.0003e	0.0016b	0.0009a	5.03cd	30.11cd	34.57a
150 + 50%	0.0012a	0.0015bc	0.0004bcd	8.27ab	30.82cd	13.13abc
200 + 0%	0.0007cd	0.0022a	0.0004bcd	3.91d	30.29cd	22.14ab
200 + 25%	0.0007cd	0.0024a	0.0005abc	4.87cd	47.14a	21.38ab
200 + 50%	0.0009bc	0.0014bcd	0.0007ab	6.64bcd	37.00bc	27.34ab
CV. (%)	16.90	12.39	48.99	24.55	13.79	57.49

Note: The mean values followed by the same letter in the same column are not significantly different according to Duncan's Multiple Range Test at the 5% significance level. CV – coefficient of variance.

Table 2. The effect of rabbit urine LOF and inorganic fertilizer dose reduction on stomatal density (SD), stomatal aperture (SA), chlorophyll a content (CCa), chlorophyll b content (CCb), total chlorophyll content (CCt)

Treatments	Observed variables				
	SD (unit mm^{-2})	SA (µm)	CCa (mg g^{-1})	CCb (mg g^{-1})	CCt (mg g^{-1})
Rabbit urine LOF concentration (mL L^{-1})					
0	83.68a	7.01a	0.59b	0.22a	0.82b
100	80.16a	7.43a	0.67ab	0.24a	0.91ab
150	66.80b	8.89a	0.62ab	0.22a	0.85ab
200	65.40b	8.61a	0.69a	0.22a	0.92a
Reduction of NPK fertilizer dosage (%)					
0%	83.86a	8.07ab	0.55b	0.23a	0.79b
25%	66.45b	8.80a	0.76a	0.24a	1.01a
50%	71.73ab	7.08b	0.61b	0.21a	0.83b
Combination of Rabbit urine LOF and Reduction of NPK fertilizer dosage					
0 + 0%	101.27a	6.67a	0.59cd	0.23a	0.84a
0 + 25%	71.73a	7.71a	0.75abc	0.24a	1.01a
0 + 50%	78.06a	6.67a	0.42e	0.20a	0.63a
100 + 0%	88.61a	7.71a	0.52de	0.24a	0.78a
100 + 25%	78.06a	7.50a	0.80a	0.25a	1.06a
100 + 50%	73.84a	7.08a	0.68abc	0.22a	0.91a
150 + 0%	65.40a	8.75a	0.50de	0.23a	0.74a
150 + 25%	61.18a	10.42a	0.74abc	0.23a	0.97a
150 + 50%	73.84a	7.50a	0.63bcd	0.20a	0.84a
200 + 0%	80.17a	9.17a	0.60bcd	0.19a	0.81a
200 + 25%	54.85a	9.58a	0.75abc	0.24a	1.01a
200 + 50%	61.18a	7.08a	0.72abc	0.23a	0.96a
CV. (%)	17.06	20.04	12.96	25.85	13.24

Note: The mean values followed by the same letter in the same column are not significantly different according to Duncan's Multiple Range Test at the 5% significance level. CV – coefficient of variance.

Table 2. showed that the effect of rabbit urine LOF application had a significant effect on stomatal density with the highest value of 83.68 and 80.16 units mm^{-2} at a concentration of 0 and 100 mL L^{-1}; chlorophyll a content and total chlorophyll content with the highest values of 0.69 and 0.92 mg g^{-1}, respectively, at a concentration of 200 mL L^{-1}, 16.95% and 12.20% greater than the concentration of 0 mL L^{-1}. Reduction of NPK fertilizer dose significantly affected stomatal density with the highest value observed at 0% reduction (83.86 units mm^{-2}), which was statistically not different from 50% reduction (71.73 units mm^{-2}), but significantly higher than 25% NPK reduction (66.45 units mm^{-2}); the highest stomatal aperture was observed at a 25% NPK reduction (8.80 units mm^{-2}), which was statistically no different from a 0% NPK reduction (8.07 units mm^{-2}), but significantly higher than a 50% NPK reduction (7.08 units mm^{-2}), chlorophyll a content, and total chlorophyll content reached the highest values of 0.76 and 1.01 mg g^{-1}, which were 38.18% and 27.85% higher, respectively, than the 0% NPK reduction treatment. The combination of rabbit urine LOF and reduced NPK fertilizer dose significantly affected chlorophyll *a* content, with the highest value of 0.80 mg g^{-1} obtained from 100 mL L^{-1} LOF and a 25% reduced NPK dose—35.59% higher than the control (0 mL L^{-1} LOF and 0% NPK reduction).

NAR and CGR at 38 DAP were significant for the application of LOF rabbit urine as organic fertilizer. The use of organic fertilizer represents an effective technique for improving soil physical attributes through increased soil organic matter content, thereby significantly impacting soil quality improvement (Lasmini et al., 2022). Rabbit urine contains high nutrient values, including 2.72% N, 1.1% P, and 0.5% K. (Setyanto et al., 2014). High nitrogen levels in rabbit urine support shallot growth during the growth phase. The controlling factors of NAR and CGR are centered on photosynthetic performance. The availability of nutrients from rabbit urine LOF, particularly nitrogen as a component of

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chlorophyll, enhances carbon assimilation, promotes cell division, and drives biomass expansion, consistent with the source-sink model where photosynthates determine growth. Rabbit urine provides high levels of N, primarily as NH_4^+ and urea. Nitrogen is an essential structural component of chlorophyll for photosynthesis, the process of fixing CO_2 into organic compounds for plant growth. Plant roots absorb N in the form of NH_4^+, NO_3^-, amino acids, and urea, with NO_3^- needing to be reduced to NH_4^+ before assimilation (Marschner, 2012).

According to Irianto et al. (2017), the initial reduction in NAR during early growth stages was attributed to underdeveloped young leaves exhibiting suboptimal photosynthetic capacity. Both NAR and CGR subsequently increased until 35 – 42 DAP, driven by progressive leaf expansion and bulb development. This growth phase was characterized by a rising bulb-to-leaf dry weight ratio, reflecting resource allocation shifts toward reproductive structures. Mojaddam and Noori (2015) stated that CGR exhibited a gradual increase during initial vegetative development, followed by a significant acceleration in subsequent growth stages. The initial phase's attenuated CGR was attributed to high meristematic activity and incomplete leaf expansion, limiting photosynthetic capacity. A pronounced CGR surge occurred post-attainment of maximum leaf area index, driven by optimized solar radiation utilization efficiency.

The application of rabbit urine LOF affected the NAR at 38 DAP, but not at 52 DAP, due to the reduced effectiveness of plant light absorption due to higher leaf density and density at 52 DAP than at 38 DAP. NAR peaks under direct leaf sunlight exposure but declines during subsequent growth periods due to leaf area index expansion-induced mutual shading. Enhanced nitrogen availability promotes leaf area expansion and biomass accumulation, but mutual shading by older leaves with diminished photosynthetic capacity reduces NAR during plant aging (Islam et al., 2019). NAR in Table 1 at 24 and 52 DAP was significantly different at reduced NPK doses because the sunlight shaded by the leaves on the same plant was not as shaded during the vegetative peak towards the bulb formation phase. The leaves at 24 DAP were not yet shady, while at 52 DAP, they began to dry out because the plants focused on tuber formation. This is in accordance with the statement of Fauziah et al. (2016), that at 35 DAP to 56 DAP, shallot plants enter the phase of bulb formation and development, and the growth of shallot height and leaves begins to senescence because the energy of photosynthesis is used to form and fill the bulbs.

CGR at 38 DAP is significantly different, but at other ages it is not significantly different because at this age the plants are in the phase after the vegetative peak, which has the highest plant growth activity compared to other phases that have focused on tuber filling and plant yield. CGR at 52 DAP is also not significantly different from the application of rabbit urine LOF because at that age, the plants are already in the bulb enlargement phase. CGR at 24 DAP has a real effect, because the plants are in the vegetative phase, so they are more focused on the growth process by performing photosynthesis. Optimal plant growth depends critically on efficient photosynthetic light-to-chemical-energy conversion and precise photo assimilate management (Sonnewald & Fernie, 2018).

The main factor in reduced yields is due to leaves covering each other at certain phases. Sunlight is an important factor in the photosynthesis process and determines CGR, so that the intensity, duration of irradiation, and quality affect the photosynthesis process. Solar movement generates diurnal light redistribution within canopies, altering spectral composition and creating three radiation components: direct (85% under full sun), diffuse, and scattered/transmitted light (Durand et al., 2021). Geographic latitude critically influences plant performance by modulating solar position and day length, which interact with canopy architecture. Specifically, leaf inclination angle and leaf area determine radiation flux efficiency; erect leaves enhance light capture at low solar angles (e.g., high latitudes/dawn/dusk) but reduce interception at zenith positions (Ezcurra et al., 1991; Falster & Westoby, 2003; Murchie & Burgess, 2022).

Research conducted by Safrina et al. (2023) stated that the provision of biourin had a very significant effect on the relative growth rate and net assimilation rate of corn plants at the age of 4-6 weeks after planting, with the highest value of 1.819 at a concentration of 250 mL L^{-1}. Nitrogen application can increase the number of stomata, which affects the density of stomata, to increase the rate of transpiration and absorption of CO_2 for photosynthesis. Stomatal density shows the number of stomata per unit area of the observation field, where if the number of stomata on the leaves

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is large, the level of CO_2 absorption will be greater, to increase the rate of photosynthesis (Regazzoni et al., 2014). The results of research conducted by Nisa & Rahayu (2022), showed that the provision of LOF-Si on soybean plants was significantly different and resulted in a higher number of stomata in LOF Si 20 mL LOF mixed with 1 L of water with the addition of Si 1 g L^{-1} the value of stomatal density reached 111.77 while the lowest number of stomata was found in plants treated with control LOF 20 mL LOF mixed with 1 L of water with a value of 89.50.

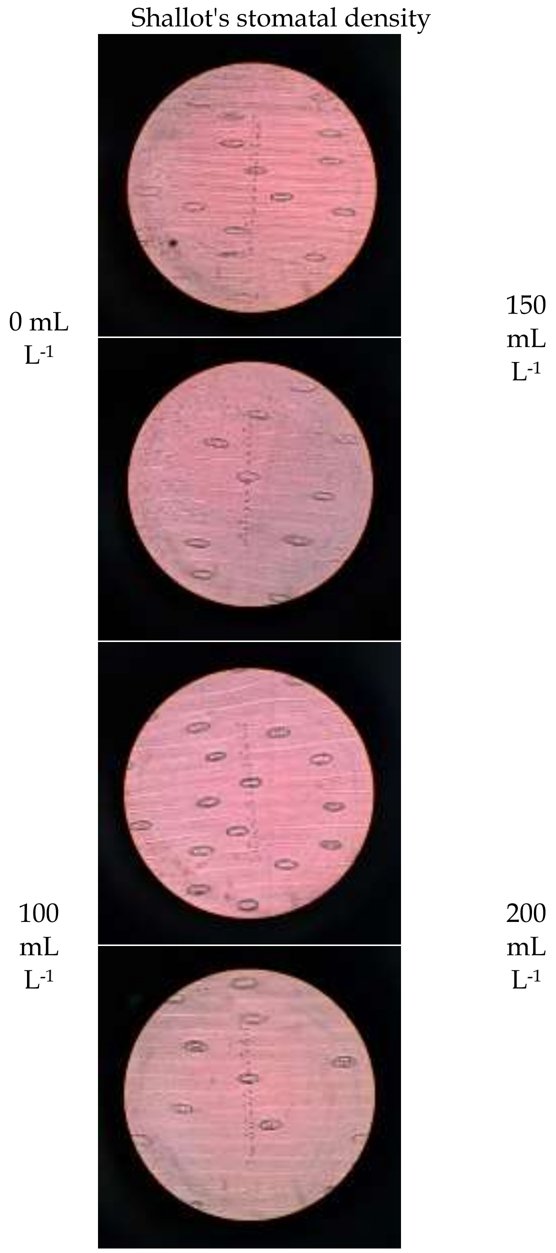

Figure 1. Shallot's stomatal density in the treatment of rabbit urine liquid organic fertilizer

Stomatal mechanism occurs due to changes in the volume of the guard cell controlled by ion exchange and cytoskeleton modifications (Golec & Szarejko, 2013). Regulation of stomatal opening is also influenced by physiological and environmental factors, including CO_2 concentration, the hormone abscisic acid (ABA), humidity levels, drought, pathogen attack, and ozone exposure (Shemer, 2015). Research by Zhou et al. (2010), showed that plants respond quickly to changes in temperature through physiological adjustments, one of which is by regulating the opening and closure of stomata. K^+ controls stomatal aperture to facilitate gas exchange and water flux (Andrés et al., 2014; Tränker et al.,2018), while adequate chloroplast K^+ concentrations maintain stroma lamella structure, supporting chloroplast integrity and photosynthetic efficiency (Jia et al., 2008; Sustr et al., 2019). Reducing the dose of NPK fertilizer has a significant effect on stomatal openings. Research by Rahayu et al. (2023), that the stomatal openings of barley plants are influenced by the dose of fertilizer; 100% fertilization of N, P, and K produces lower stomatal openings, while 50% doses show optimal openings. At 4-5 cm of waterlogging, 25% doses produce the best stomatal openings. Stomatal openings are influenced by leaf anatomy, whose growth is influenced by nutrients, where the application of NPK fertilizers provides nutrient supplies for plants; besides, potassium nutrients are known to affect stomatal openings. Stomatal opening is influenced by various factors, including potassium (K^+) and chloride (Cl^-) ions, humidity, temperature, sunlight intensity, pH, and CO_2 levels.

The application of rabbit urine LOF has a low effect, but it is suspected that other factors, affect chlorophyll levels in plants in the form of NPK fertilizer as a nitrogen source. Providing additional nitrogen to the leaves can produce wider leaves and a higher chlorophyll content. Leaf chlorophyll content increased with nitrogen application and irrigation compared to unfertilized and rainfed conditions (Wang et al., 2021). Reducing the dose of NPK fertilizer has no significant effect on chlorophyll b. This is in accordance with the research of Same (2019), that the provision of NPK fertilizer has no significant effect on the chlorophyll content of pepper seedlings, the highest average result is 42.62 at 3 g $polybag^{-1}$, and the lowest average is 37.31 at 0 g $polybag^{-1}$.

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Shallot's stomatal aperture

0%

25%

50%

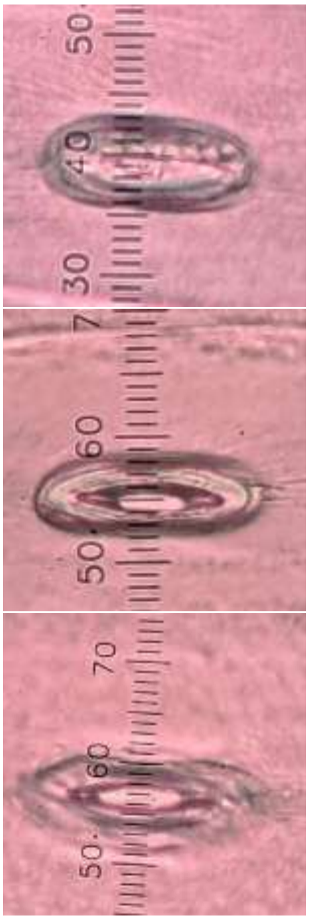

Figure 2. Shallot's stomatal aperture in the treatment of the reduction of NPK fertilizer dosage

Plant chlorophyll levels are influenced by the availability of nutrients in the soil, where NPK fertilizers are thought to be able to provide nutrient requirements for chlorophyll levels, but the provision of nutrients is needed in optimal amounts that are not excessive to saturation. Factors that limit soil fertility include N, P, K, and Mg (Marchesi, 2020). This shows that the provision of a certain amount of plant nutrients increases growth to an optimal limit, which then cannot increase again and even tends to decrease. Liebig's minimum law states "that the yield of a crop is limited by the nutrient that is present in the least quantity in that environment" (Liebig 1943; Tang & Riley 2021; Baio et al., 2024). The combination of rabbit urine LOF and reduced NPK fertilizer dose interacted with the chlorophyll a level. Rabbit urine LOF increases enzyme activity that plays a role in chlorophyll synthesis, while the reduction of NPK avoids excess nutrients that inhibit photosynthesis. N fertilization under low irrigation water elevated chlorophyll content and antioxidant enzyme activity, which preserved photosynthetic integrity and enzymatic function, optimizing the production of assimilates as organic compounds, increased plant yield (Muhammad et al., 2022).

Nutrient deficiencies critically impair plant physiology: Nitrogen (N) limitation induces anthocyanin accumulation (manifested as purple leaves) and reduces chlorophyll/carotenoid content by 34-36% in rice (Huang et al., 2004), while suppressing energy metabolism enzymes in photosynthesis and respiration (Lin et al., 2011). Phosphorus (P) deficiency decreases $NADP^+$ substrate for NADPH synthesis, diminishing ATP synthase activity and limiting proton export to the chloroplast stroma, thereby acidifying the thylakoid lumen (Goltsev et al., 2016; Karlsson et al., 2015). Concurrently, potassium (K) deficiency triggers necrotic lesions via chlorophyll degradation, disrupting carbon translocation and plant-water homeostasis (Abbas et al., 2021). Reducing the dose of NPK fertilizer can reduce the risk of stress due to chemical nutrient overload, while rabbit urine LOF helps plants to still get enough nutrients, so that the chlorophyll a level remains optimal.

Rabbit urine contains high levels of salt and ammonia, which trigger the accumulation of Na^+ and NH_4^+ ions in the root zone. The increase in pH and EC values after the addition of composted rabbit manure is due to its high salt content (Greco, 2021), which has the potential to cause salinity stress in plants (Ceccarini et al., 2019). Similar findings were reported in rabbit and goat manure compost with a pH of 9.41 and EC of 11.33 dS m^{-1} (Paredes et al., 2015; Li et al., 2022). In salt-sensitive shallots, this reduces soil water potential, causing osmotic stress. Plants under osmotic stress respond by increasing abscisic acid (ABA) synthesis. Stomatal responses to hydraulic/non-hydraulic signals confirm ABA's key role in plant signaling adaptation to environmental dynamics (Cai et al., 2017). In contrast, in the control without rabbit urine, no osmotic pressure occurred, so stomata remained optimally open.

The conversion of urea in rabbit urine produces excess ammonia (NH_3) in the soil. This compound inhibits the activity of the enzyme glutamine synthetase in guard cells, disrupting glutamate synthesis. As a result, there is an accumulation of reactive oxygen species (ROS) that damage the guard cell membrane, reducing the ability of the stomata to open. Guard cell plasma membranes contain anion channels activated by key stimuli, including ABA, NADPH metabolism, and voltage-gated K^+ channel-mediated anion fluxes (Murata et al., 2015; Roelfsema et al., 2012). ROS functions as an essential regulator of stomatal closure (Song et al., 2014). During stomatal movement regulation, ROS generated initially in the guard cell apoplast subsequently activate anion channels through sensing and signaling pathways (Singh et al., 2017). This toxic mechanism does not occur in controls (concentration 0 mL L^{-1}) at stomatal aperture parameter, so stomatal function remains normal.

Conclusion

Application of rabbit urine at a concentration of 200 mL L^{-1} increased the net assimilation rate, crop growth rate at 38 DAP, chlorophyll a content, and total chlorophyll content. A 0% reduction in NPK fertilizer dosage increased stomatal density. A 50% reduction in NPK fertilizer dosage increased net assimilation rate and growth rate at 24 DAP, and net assimilation rate at 52 DAP. The combination of rabbit urine LOF and reduced dose of NPK fertilizer on shallot plants was 100 mL L^{-1} and 25% highest in crop growth rate 24 DAP and chlorophyll a content; 150 mL L^{-1} and 25% highest in net assimilation rate 52 DAP and crop growth rate 52 DAP; 150 mL L^{-1} and 50% highest in net assimilation rate 24 DAP; 200 mL L^{-1} and 0% highest in net assimilation rate 38 DAP; 200 mL L^{-1} and 25% highest in net assimilation rate 38 DAP and crop growth rate 38 DAP.

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