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Effects of fermented cow urine on the growth and quality of *katuk* (*Sauropus androgynus*) accessions from West Java, Indonesia

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Abstract. *Rahayu A, Rochman N, Nahraeni W, Yuliawati. 2025. Effects of fermented cow urine on the growth and quality of* katuk *(*Sauropus androgynus*) accessions from West Java, Indonesia. Asian J Agric 9: 831-843.* This study examined the growth, yield, and nutritional quality of *katuk* using local germplasm and natural nitrogen sources. The objective was to evaluate the performance of ten *katuk* accessions from West Java, Indonesia, and to assess the potential of fermented cow urine as a supplementary nitrogen source relative to urea. A factorial completely randomized design with three replications was employed, consisting of ten accessions (Sarampad, Maleber1, Maleber2, Kadudampit1, Kadudampit2, Gegerbitung, Dramaga, Cinangneng1, Cinangneng2, and Katulampa) and six fertilizer compositions (0% cow urine + 100% Urea; 100% cow urine + 0% Urea; 75% cow urine + 25% Urea; 50% cow urine + 50% Urea; 25% cow urine +75% Urea; 0% cow urine + 0% Urea). Sarampad, Maleber1, and Maleber2 exhibited superior fresh and dry harvest weight, vitamin C, chlorophyll a and b content, while maintaining low nitrate accumulation. Plant height under 100% cow urine, shoot number and vitamin C under 25% cow urine + 75% urea, and chlorophyll a, b, and total chlorophyll under 50% cow urine + 50% Urea were higher than under 100% Urea, with superiority values of 14.7%, 19.0%, 20.2%, 14.6%, 18.2%, and 15.9%, respectively. These findings indicate that fermented cow urine may serve as a low-cost supplementary nitrogen source for *katuk*; however, its use as a full replacement for synthetic nitrogen fertilizer requires further validation under field conditions.

Keywords: Chlorophyll, harvest weight, nitrate, urea, vitamin C

INTRODUCTION

Katuk (*Sauropus androgynus* L.) is a multifunctional plant used as a vegetable, medicinal herb, food ingredient, animal feed, and for phytoextraction. Its leaves contain high levels of vitamin A, vitamin C, carbohydrates, protein, and calcium (Naveena et al. 2020) and provide essential oils, tannins, cinnamic acid, and flavonoids that support antioxidant, antidiabetic, and antihemolytic activities (Purba and Paengkoum 2022). Katuk also shows strong DPPH scavenging activity (886.64±15.89 µmol L^{-1}) and high chlorophyll content (14.43±0.16 µg mL^{-1}), making it a potential natural dye source (Nguyen et al. 2020). Among lactation-promoting plants, it contains the highest flavonoid and antioxidant levels (Iwansyah et al. 2016), and it retains 98% of its vitamin C during cold storage (Umaramani and Sivakanesan 2015). These qualities have contributed to its classification as a superfood (Mulyati et al. 2024). Katuk is also effective in phytoextraction, capable of accumulating heavy metals such as Cd, Cu, Pb, and Zn (Xia et al. 2021).

The rich nutritional and phytochemical properties of *katuk* indicate strong potential for further improvement and utilization through local germplasm exploration. West Java, Indonesia, hosts diverse *katuk* accessions with broad morphological and biochemical variation, including differences in leaf shape, flower color, fruit pigmentation, and chlorophyll and vitamin C levels (Santana et al. 2021). FTIR and PCA analyses further confirmed their genetic and metabolic diversity, grouping five accessions into three clusters (Winasih et al. 2023).

Rising consumer concern over food safety has increased demand for organically grown indigenous vegetables, as buyers prioritize produce free from harmful residues (Nahraeni et al. 2016). This supports the use of eco-friendly nutrient sources such as organic fertilizers from agricultural waste for katuk cultivation. Fermented cow urine is a promising option because it is affordable, easy to prepare, and nutrient rich. Adding biofertilizer and molasses increases its nitrogen, organic matter, and microbial populations (Nuraini and Asgianingrum 2017). It contains higher levels of organic C, N, P_2O_5, K_2O, and micronutrients (Setiyo et al. 2021) than unfermented urine, which has much lower N, P, and K contents (Rahayu et al. 2019). Its nitrogen mineralization efficiency reaches about 46% with minimal volatilization loss (Ramírez-Sandoval et al. 2023), and its released N has been shown to support higher dry matter production in Timothy grass (Bélanger et al. 2015).

Recent studies show that fermented cow urine can improve soil fertility, nutrient uptake, and crop yield (Devasena and Sangeetha 2022). Surachman et al. (2025) reported that applying biofertilizer (4 mL L^{-1}) with liquid cow urine (300 mL L^{-1}) increased N, P, and K absorption, root volume, biomass, and leaf area in caisim. Cow urine combined with phosphorus fertilizer also boosted chlorophyll content in Vanilla planifolia (Haryuni et al. 2018). Using urine as fertilizer provides key nutrients and helps reduce nitrate and phosphate pollution. In Malmö (Sweden), recycling just 15-30% of human urine could supply up to 50% of fertilizer needs while lowering nitrate levels in surface waters. Urine-diversion systems can also recover up to 75% of nitrogen and over 40% of phosphorus from wastewater (Gunnarsson et al. 2023).

Previous studies found that applying different doses of cow urine to five *katuk* accessions from West Java produced wet and dry harvest weights similar to those obtained with the recommended dose of urea fertilizer (Kurniawan et al. 2019). Different urea–cow urine substitution ratios in four katuk accessions from Bogor produced similar leaflet traits, harvest weights, TSS, and chlorophyll levels to those given either 100% urea or 100% cow urine (Rahayu et al. 2023). However, studies that assess growth, yield, and key quality traits (nitrate, chlorophyll, vitamin C) across multiple *katuk* accessions in West Java are still limited. Such information is needed to determine whether fermented cow urine is a practical, safe, and economical biofertilizer.

Therefore, this study aimed to (i) evaluate the growth performance; (ii) assess yield attributes through fresh and dry harvest weights; and (iii) analyze the quality variables including nitrate, chlorophyll a, b, total chlorophyll, and vitamin C contents of various *katuk* accessions from West Java under different fermented cow urine compositions. This research is expected to provide scientific evidence supporting the feasibility of using fermented cow urine as an eco-friendly and economically viable nitrogen source to substitute urea fertilizer for sustainable *katuk* production.

MATERIALS AND METHODS

Study area

This research was conducted at the Experimental Field and Laboratory of the Agrotechnology Study Program, Faculty of Agriculture, Universitas Djuanda, Bogor, West Java, Indonesia. The plant materials used in this study were *katuk* (*S. androgynus*) accessions from West Java, including those from Cianjur (Sarampad, Maleber1, and Maleber2), Sukabumi (Kadudampit1, Kadudampit2, and Gegerbitung), and Bogor (Dramaga, Cinangneng1, Cinangneng2, and Katulampa).

Research design and data analysis

This study employed a factorial Completely Randomized Design (CRD), incorporating *katuk* accessions and the composition of fermented cow urine. This study used ten *katuk* accessions included Sarampad, Maleber1, Maleber2, Kadudampit1, Kadudampit2, Gegerbitung, Dramaga, Cinangneng1, Cinangneng2, and Katulampa and six level of cow urine and urea compositions, that are 0% R (recommended dose) cow urine + 100% R urea, 100% R cow urine + 0% R urea, 75% R cow urine + 25% R urea, 50% R cow urine + 50% R urea, 25% R cow urine + 75% R urea, and 0% R cow urine + 0% R urea (without nitrogen fertilizer). The recommended nitrogen (N) fertilizer dose applied was 250 kg N ha^{-1}. This experiment comprised 60 treatment combinations with three replications. One experimental unit consisted of a single cutting grown in one polybag, with each experimental unit consisting of four polybags as observation units (subsamples). The four subsamples per treatment–replicate were averaged prior to analysis, yielding three replicate mean values per treatment.

Data were analyzed using Analysis of Variance (ANOVA) through the F-test at a significance level of $p \leq 0.05$ (α=5%) and $p \leq 0.01$ (α=1%). When significant differences among treatments were detected, mean separation was carried out using Duncan's Multiple Range Test (DMRT) at $p \leq 0.05$ (α=5%). All statistical analyses were performed using the Statistical Analysis System (SAS) software (SAS Institute Inc., Cary, NC, USA). DMRT was selected because the experiment involved a large number of treatment combinations (10 accessions × 6 fertilizer compositions) and a relatively small number of replications (n=3), conditions under which a more sensitive and statistically powerful post-hoc procedure is required. DMRT provides greater power than the conservative Tukey's HSD and offers better control of Type I error than the highly liberal LSD, making it more appropriate for agricultural experiments with limited replication and complex factorial structures (Calinski et al. 1981; Gomez and Gomez 1984). Its stepwise comparison approach allows finer discrimination among treatment means while maintaining acceptable experimental error rates, thus providing a balanced and justified method for mean separation in this study (Hoshmand 2018).

Procedures

Cow urine fermentation and applications

Cow urine was naturally fermented for two weeks under ambient conditions. Ambient conditions refer to uncontrolled, naturally occurring environmental conditions under which the experiment is conducted. Measurements of pH, temperature, and total solids were not recorded, which may introduce variability in the final product and is acknowledged as a limitation of the procedure. Fresh urine (approximately one day after excretion) was collected from local dairy farmers using clean plastic buckets, then transferred into 20 L plastic containers with tightly sealed lids. During fermentation, the gas produced was released every three days by briefly opening the lid to prevent excessive pressure buildup. The fermented cow urine was then stored in a closed container until use.

Cow urine was applied according to the designated treatment levels. Urea fertilizer and cow urine were applied gradually. At transplanting, 50% of the urea dose was applied, followed by 25% at 3 and 6 weeks after planting (WAP). Cow urine was applied weekly from 2 to 7 WAP at a rate of 208 mL plant^{-1} week^{-1}. Before each application, cow urine was diluted in water to a total volume of 1 L and applied directly to the planting medium by watering.

SP-36 and KCl fertilizers were applied at 100% of the recommended rate as basal fertilizers. The dosage of synthetic fertilizer and cow urine per polybag was calculated based on fertilizer requirements per plant, converted from the field planting distance of 50 cm × 30 cm (Table 1).

Table 1. Doses of synthetic fertilizer and fermented cow urine applied to *katuk* accessions

Fertilizer	Dosage	
	Hectare	Plant/polybag
Urea	543.48 kg	10.87 g
SP-36	416.67 kg	8.34 g
KCl	175.00 kg	3.50 g
Cow urine	62,500 L	1,250 mL

Field condition

The research was conducted in an open field located in Bogor, West Java, at an altitude of 650 m above sea level. The experimental site had an average temperature ranging from 23 to 33.7°C, relative humidity of 77.2%, and average monthly rainfall of 247.9 mm.

The soil type was latosol with a pH of 7.53 (slightly alkaline), 1.55% organic carbon (low), 0.12% total nitrogen (low), and a C/N ratio of 13 (medium). The soil contained 58.35 ppm available P_2O_5, 159.23 mg 100 g^{-1} potential P_2O_5 (very high), and 33.73 mg 100 g^{-1} potential K_2O (medium). Soil chemical properties were determined prior to planting according to standard procedures using the methods of Walkley and Black (for organic C), Kjeldahl (for total N), Bray I (for available P), and flame photometry (for K).

Planting procedure

The seedlings and planting media used in this study consisted of a mixture of soil and rice husk charcoal in a 1:1 volume ratio. The planting material comprised stem cuttings approximately 20 cm in length, which were initially sown in polybags measuring 12×20 cm. These seedlings were then transplanted at 4 weeks after sowing into polybags measuring 30×40 cm.

Observation variables

In this study, plant growth, yield, and quality variables were systematically measured to evaluate the physiological and biochemical responses of *katuk* under different fertilizer treatments. The observations were conducted following standardized procedures between 2 and 17 WAP, with clearly defined variables, instruments, and measurement intervals as follows.

Plant growth performance was evaluated at 2-16 WAP using the following variables: (i) plant height (cm), measured from the soil surface to the shoot apex using a length gauge. (ii) Number of shoots (shoot $plant^{-1}$), determined by counting all primary shoots emerging from the main stem. (iii) Stem diameter (mm), recorded at 5 cm above the soil surface using a digital caliper. (iv) Number of leaflets (leaflets $plant^{-1}$), recorded by counting all fully expanded leaflets per plant. (v) Number of leaves (leaves $plant^{-1}$), determined by counting each compound leaf as a single unit across all primary shoots per plant. (vi) Leaf area (cm^2), measured on a single leaflet taken from the fifth fully expanded leaf, following a standardized gravimetric procedure, where leaf area = (leaf disc area × total leaf weight)/leaf disc weight.

Yield attributes were measured at 17 WAP to assess productive performance: (i) Fresh harvest weight (g $plant^{-1}$), determined by weighing the upper 25 cm shoot tips immediately after harvest using a digital balance. (ii) Dry harvest weight (g $plant^{-1}$), measured after oven-drying samples at 60°C to a constant weight using a forced-air oven, representing the plant's dry biomass accumulation.

Leaf quality variables were analyzed at 15 WAP, focusing on biochemical and nutritional aspects including nitrate content, chlorophyll a, chlorophyll b, total chlorophyll, and vitamin C content. These variables indicate photosynthetic efficiency, antioxidant capacity, and nutritional value. (i) Nitrate Content (mg kg^{-1} FW), quantified using a Horiba LAQUAtwin nitrate meter, following calibration standards recommended by the manufacturer. (ii) Vitamin C (Ascorbic Acid) content (mg 100 g^{-1} FW), determined using the titrimetric method described by the Association of Official Analytical Chemists (AOAC 1995), employing 2,6-dichlorophenol indophenol as the titrant. (iii) Chlorophyll a, chlorophyll b, and total chlorophyll (mg g^{-1} FW) were quantified following the Lichtenthaler and Wellburn (1983) protocol using absorbance readings at 663 nm, 645 nm, and 470 nm.

RESULTS AND DISCUSSION

The results of the ANOVA revealed that both *katuk* accession and cow urine composition significantly affected all measured growth, yield, and quality variables. However, their interaction significantly influenced only the number of leaflets, leaf area, and nitrate content (Table 2). This finding indicates a distinct variability among *katuk* accessions and highlights their differential physiological responses to variations in natural nitrogen sources.

Table 3 presents plant height, number of shoots, stem diameter, number of leaflets, fresh harvest weight, and dry harvest weight variables, all of which reflect the variation attributable to *katuk* accession. As shown in table, plant height and stem diameter varied markedly among the ten *katuk* accessions. The accession Kadudampit1 exhibited the greatest plant height (115.1±5.11 cm) and stem diameter (0.90±0.02 cm), while Maleber2 produced the shortest plants (62.7±4.26 cm) and Dramaga thinnest stems (0.59±0.02 cm). The number of shoots was highest in Maleber2 (17.4±1.40 shoots) and lowest in Cinangneng1, Cinangneng2, and Katulampa, indicating variation in branching potential among local germplasms.

The number of leaflets was significantly greater in Maleber1, although it did not differ statistically from Kadudampit1 and Cinangneng1, suggesting comparable leaf production efficiency among these accessions. In terms of yield, Sarampad showed the highest fresh (57.0±4.62 g $plant^{-1}$) and dry harvest weights (17.3±17.3 g $plant^{-1}$), followed by Maleber2 and Maleber1. These accessions performed significantly better than the remaining seven, demonstrating superior adaptability and biomass accumulation under the experimental conditions.

Table 2. ANOVA for growth, yield, and quality variables of *katuk* as affected by accession (A), cow-urine/urea composition (B), and their interaction (A×B)

Variables	F value			CV (%)
	A	B	A×B	
Plant height (cm)	26.91**	15.00**	1.38	17.11
Number of shoots	26.53**	8.18**	1.13	30.22
Stem diameter (cm)	23.00**	3.17*	0.74	11.55
Number of leaflets	8.83**	10.69**	0.78	16.74
Number of leaves	9.26**	20.55**	1.87*	23.11
Leaf area (cm^2)	183.12**	6.64**	1.48*	18.32
Fresh harvest weight (g $plant^{-1}$)	12.03**	23.50**	1.23	25.48
Dry harvest weight (g $plant^{-1}$)	10.28**	21.84**	1.04	22.62
Nitrate (mg kg^{-1} FW)	2.39**	37.71**	3.89**	23.77
Chlorophyll a (mg g^{-1} FW)	7.12**	16.41**	1.20	22.67
Chlorophyll b (mg g^{-1} FW)	4.68**	11.26**	1.09	30.31
Total chlorophyll (mg g^{-1} FW)	6.21**	14.83**	1.15	24.71
Vitamin C (mg 100 g^{-1} FW)	4.78**	3.55*	1.16	23.77

Note: F-values are shown for: A. The main effects of accession, B. Cow urine–urea composition, and A×B: Their interaction. Asterisks indicate significance levels: *=p≤0.05; **=p≤0.01. CV: Coefficient of Variation

Table 3. Growth and yield variables of *katuk* accessions from West Java, Indonesia

Accessions	Plant height (cm)	Number of shoots	Stem diameter (cm)	Number of leaflets	Fresh harvest weight (g $plant^{-1}$)	Dry harvest weight (g $plant^{-1}$)
Sarampad	68.3±3.50^{cd}	14.9±1.43^{b}	0.61±0.01^{e}	11.2±0.60^{de}	57.0±4.62^{a}	17.3±1.37^{a}
Maleber1	69.4±3.48^{cd}	9.8±0.70c^{d}	0.77±0.02^{bc}	13.8±0.67^{a}	44.5±3.54^{bc}	14.6±0.97^{b}
Maleber2	62.7±4.26^{d}	17.4±1.40^{a}	0.63±0.01^{e}	10.3±0.52^{ef}	49.7±4.41^{b}	16.6±1.30^{a}
Kadudampit1	115.1±5.11^{a}	10.0±0.39^{cd}	0.90±0.02^{a}	12.8±0.32^{abc}	45.3±2.68^{bc}	14.1±0.80^{bc}
Kadudampit2	94.5±4.88^{b}	9.6±0.49^{cd}	0.81±0.02^{b}	11.5±0.33^{cde}	48.3±3.45^{b}	14.2±0.80^{bc}
Gegerbitung	67.4±4.44^{cd}	9.3±0.69^{d}	0.77±0.03^{bc}	11.2±0.53^{de}	30.2±3.01^{e}	10.0±0.75^{e}
Dramaga	69.4±2.78^{cd}	11.7±0.69^{c}	0.59±0.02^{e}	9.1±0.32^{f}	33.4±1.91^{de}	10.8±0.47^{de}
Cinangneng1	72.2±2.87^{cd}	6.1±0.43^{e}	0.70±0.02^{d}	13.0±0.46^{ab}	35.4±2.75^{de}	12.4±0.70^{bcd}
Cinangneng2	76.0±2.50^{c}	6.1±0.52^{e}	0.74±0.02^{cd}	12.0±0.54^{bcd}	33.1±2.76^{de}	12.0±0.89^{cde}
Katulampa	70.5±3.11^{cd}	6.5±0.46^{e}	0.71±0.02^{cd}	11.2±0.64^{de}	40.0±2.78^{cd}	13.7±0.80^{bc}

Note: Values followed by the same letter in the same column are not significantly different according to DMRT at p≤0.05

The influence of cow urine and urea combinations on *katuk* growth and yield variables is summarized in Table 4. However, stem diameter, number of leaflets, and both fresh and dry weights of *katuk* harvested from different cow urine compositions showed no significant differences, suggesting that these traits are less sensitive to nitrogen source ratios. The lowest values across all observed variables were recorded in plants that received no cow urine or urea, emphasizing the importance of nitrogen supplementation in supporting *katuk* growth.

The height of *katuk* accessions treated with 100% cow urine (84.9±3.72 cm) was significantly higher than that of accessions treated with 100% urea (74.0±3.17 cm) (Figure 1.A), showing a 14.7% superiority. The results indicate that the application of 100% cow urine markedly promoted plant height compared to 100% urea, suggesting that cow urine serves as a more effective nitrogen source for vegetative growth in *katuk* plants.

The number of shoots of *katuk* accessions treated with a 25% cow urine + 75% Urea (11.9±1.21 shoots $palnt^{-1}$) mixture were significantly higher than those treated with 100% urea (10.0±0.77 shoots $plant^{-1}$) and showing a 19.0% superiority, indicating a synergistic effect of combining organic and inorganic nitrogen sources (Figure 1.B).

A significant interaction was observed between *katuk* accessions and fertilizer composition for the number of leaves (Table 5). The lowest leaf number (13.4±0.9 leaves $plant^{-1}$) was recorded in the Cinangneng1 accession under the control treatment (0% cow urine + 0% urea), while the highest value (50.8±6.3 leaves $plant^{-1}$) was observed in Katulampa accession treated with 25% cow urine + 75% urea. In general, *katuk* accessions that did not receive nitrogen fertilization produced markedly fewer leaves than those treated with either cow urine or urea. Among accessions, Dramaga and Katulampa consistently exhibited higher leaf numbers across treatments, while Maleber1 had the lowest values under all nitrogen compositions. The Maleber2 accession, however, showed no significant differences between fertilizer compositions, indicating a relatively stable but lower response to nitrogen input (Figure 2).

The interaction between accessions and fertilizer composition significantly influenced the leaf area of *katuk* (p≤0.05). The largest leaf areas were consistently produced by the Sukabumi accessions Kadudampit1 and Kadudampit2, particularly under mixed nitrogen treatments. Kadudampit1 achieved its highest leaf area under the 50% cow urine + 50% urea treatment (31.9±1.76

cm²) and 25% cow urine + 75% (31.9±3.23 cm²), whereas Kadudampit2 reached similarly high values under 25% cow urine + 75% urea (30.6±2.73 cm²) and 100% cow urine (29.8±1.96 cm²). These accessions displayed strong responsiveness to both organic and inorganic nitrogen sources, suggesting higher nitrogen-use efficiency or greater inherent leaf expansion capacity. In contrast, accessions originating from Cianjur (Sarampad, Maleber1, and Maleber2) consistently exhibited smaller leaf areas across all treatments, with values ranging mostly between 5.6±0.53 and 9.8±0.69 cm². Their minimal increases under nitrogen fertilization indicate lower leaf-expansion potential compared with the Sukabumi accessions. For most accessions, mixed-source nitrogen treatments (25-50% cow urine combined with 50-75% urea) tended to promote equal or greater leaf area than single-source applications. This pattern suggests a synergistic effect, where the combination of fast-release inorganic N and slower-release organic N supports more sustained leaf development. The absence of nitrogen fertilization (0% cow urine + 0% urea) consistently produced the smallest leaf areas across all accessions, confirming the essential role of nitrogen in leaf growth (Table 6).

The graphical trend further highlights that Kadudampit1 and Kadudampit2 maintained superior leaf area under nearly all nitrogen combinations, whereas Cianjur accessions remained low and relatively flat across treatments. This reinforces the genotype-specific nature of leaf expansion responses to nitrogen sources (Figure 3).

Table 4. Growth and yield variables of *katuk* accessions from West Jawa, Indonesia, under different cow urine compositions

Cow urine composition	Stem diameter (cm)	Number of leaflets	Fresh harvest weight (g plant^{-1})	Dry harvest weight (g plant^{-1})
100% R Urea	0.71±0.02^{ab}	11.6±0.40^{a}	44.0±1.94^{a}	14.4±0.58^{a}
100% R cow urine	0.74±0.02^{a}	12.3±0.43^{a}	44.9±2.26^{a}	13.8±0.57^{a}
75% R cow urine + 25% R Urea	0.73±0.02^{a}	12.1±0.44^{a}	45.2±3.41^{a}	15.0±0.91^{a}
50% R cow urine + 50% R Urea	0.75±0.03^{a}	12.1±0.40^{a}	46.7±2.41^{a}	15.0±0.71^{a}
25% R cow urine + 75% R Urea	0.73±0.02^{a}	12.3±0.45^{a}	46.8±3.00^{a}	14.9±0.86^{a}
0% R cow urine + 0% R Urea	0.68±0.02^{b}	9.3±0.34^{b}	22.6±1.25^{b}	8.3±0.27^{b}

Note: Values followed by the same letter in the same column are not significantly different according to DMRT at $p \leq 0.05$

Table 5. Interaction between *katuk* accessions and cow urine compositions on number of leaves variable

Treatments	Number of leaves					
	100% Urea	100% cow urine	75% cow urine + 25% Urea	50% cow urine + 50% Urea	25% cow urine + 75% Urea	0% cow urine + 0% Urea
Sarampad	33.6±3.4^{c-o}	45.2±6.2^{abc}	26.8±0.8^{g-u}	44.7±8.4^{abc}	43.4±5.5^{a-e}	27.5±2.4^{f-u}
Maleber1	20.2±2.6^{n-u}	24.2±3.5^{i-u}	19.0±1.7^{o-u}	22.7±3.1^{k-u}	20.7±2.0^{m-u}	18.1±2.8^{p-u}
Maleber2	23.3±4.6^{j-u}	33.2±10.7^{c-o}	22.7±4.4^{k-u}	30.8±1.8^{c-s}	43.6±0.5^{a-d}	27.4±1.4^{f-u}
Kadudampit1	28.5±2.4^{e-t}	42.2±2.3^{a-f}	37.8±0.6^{a-j}	35.3±0.5^{b-m}	32.8±4.1^{c-s}	17.7±1.4^{r-u}
Kadudampit2	29.1±5.5^{d-t}	36.7±1.6^{a-l}	38.0±2.0^{a-j}	40.2±3.1^{a-h}	35.5±0.0^{b-m}	16.8±1.0^{stu}
Gegerbitung	36.8±7.3^{a-k}	43.3±1.3^{a-e}	31.9±4.4^{c-r}	33.5±3.3^{c-o}	31.4±3.2^{c-s}	14.5±2.3^{tu}
Dramaga	45.7±10.8^{abc}	41.5±3.8^{a-g}	32.9±8.4^{c-p}	45.2±7.4^{abc}	39.2±1.7^{a-i}	26.3±3.9^{h-u}
Cinangneng1	29.3±4.3^{d-t}	33.2±0.9^{c-o}	26.0±4.5^{h-u}	28.0±3.2^{f-u}	35.6±6.3^{b-m}	13.4±0.9^{u}
Cinangneng2	34.3±2.9^{b-n}	27.5±2.7^{f-u}	29.0±4.5^{d-t}	38.2±2.2^{a-j}	26.9±3.2^{g-u}	21.7±1.3^{l-u}
Katulampa	38.0±5.9^{a-j}	33.7±3.1^{c-o}	49.1±0.2^{ab}	36.6±5.9^{b-l}	50.8±6.3^{a}	17.9±2.5 $^{q-u}$

Note: Values followed by the same letter are not significantly different according to DMRT at $p \leq 0.05$

Table 6. Interaction between *katuk* accessions and cow urine compositions on leaf area variable

Treatments	Leaf area (cm^2)					
	100% Urea	100% cow urine	75% cow urine + 25% Urea	50% cow urine + 50% Urea	25% cow urine + 75% Urea	0% cow urine + 0% Urea
Sarampad	5.9±0.3^{j}	7.1±0.5^{hij}	5.6±0.53^{j}	7.5±0.83^{hij}	7.2±0.42^{hij}	7.0±0.64^{hij}
Maleber1	8.2±0.3^{g-j}	9.5±0.79^{e-j}	8.3±1.51^{g-j}	9.4±0.66^{e-j}	9.8±0.69^{e-j}	8.4±0.77^{g-j}
Maleber2	6.0±0.2^{ij}	8.8±0.60^{f-j}	7.3±1.19^{hij}	7.1±0.65^{hij}	7.1±0.41^{hij}	7.6±0.48^{hij}
Kadudampit1	27.6±1.9^{abc}	31.8±1.56^{a}	31.8±2.74^{a}	31.9±1.76^{a}	31.9±3.23^{a}	25.5±2.14^{bc}
Kadudampit2	27.4±2.5^{abc}	29.8±1.96^{ab}	24.0±2.46^{c}	29.6±2.44^{ab}	30.6±2.73^{a}	18.1±3.72^{d}
Gegerbitung	11.9±1.1^{e-h}	13.1±1.52^{efg}	11.8±0.63^{e-h}	11.1±1.66^{e-i}	14.4±1.04^{de}	11.6±1.32^{e-h}
Dramaga	9.4±0.6^{e-j}	8.4±0.84^{f-j}	10.0±0.66^{e-j}	9.9±0.77^{e-j}	8.8±0.08^{f-j}	8.2±1.34^{g-j}
Cinangneng1	12.8±0.5^{efg}	13.3±1.24^{efg}	14.1±0.63^{de}	14.1±0.52^{de}	13.5±1.42^{def}	10.2±0.86^{e-j}
Cinangneng2	10.4±1.3^{e-j}	13.0±0.89^{efg}	13.0±1.87^{efg}	13.0±0.85^{efg}	13.1±1.24^{efg}	9.4±0.45^{e-j}
Katulampa	13.7±1.7^{def}	11.9±1.25^{e-h}	11.5±1.45^{e-h}	9.9±2.30^{e-j}	10.6±1.07^{e-j}	9.8±1.59^{e-j}

Note: Values followed by the same letter are not significantly different according to DMRT at $p \leq 0.05$

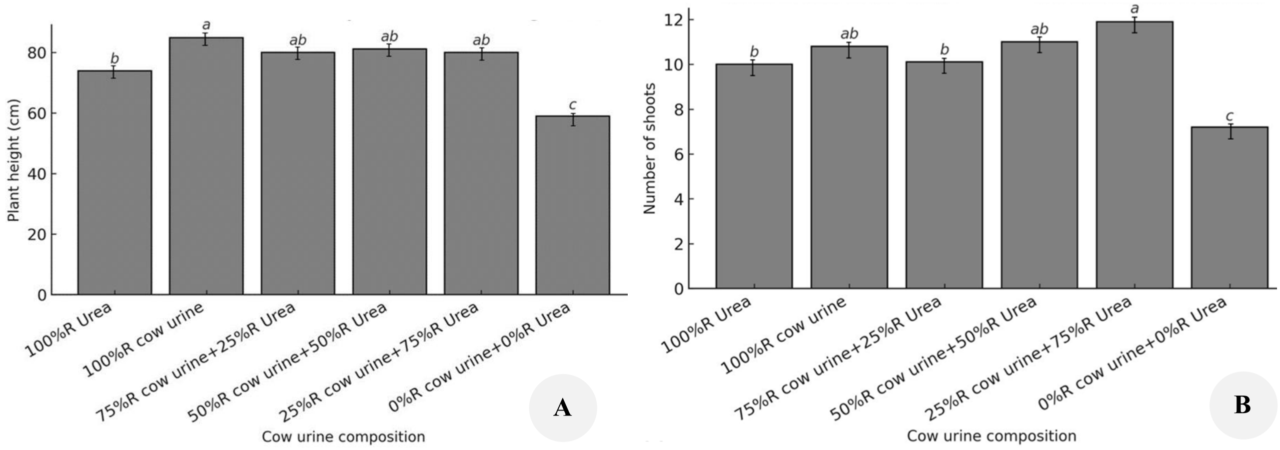

Figure 1. Growth variables of *katuk* accessions from West Java, Indonesia, under different cow urine compositions. A. Plant height, B. Number of shoots

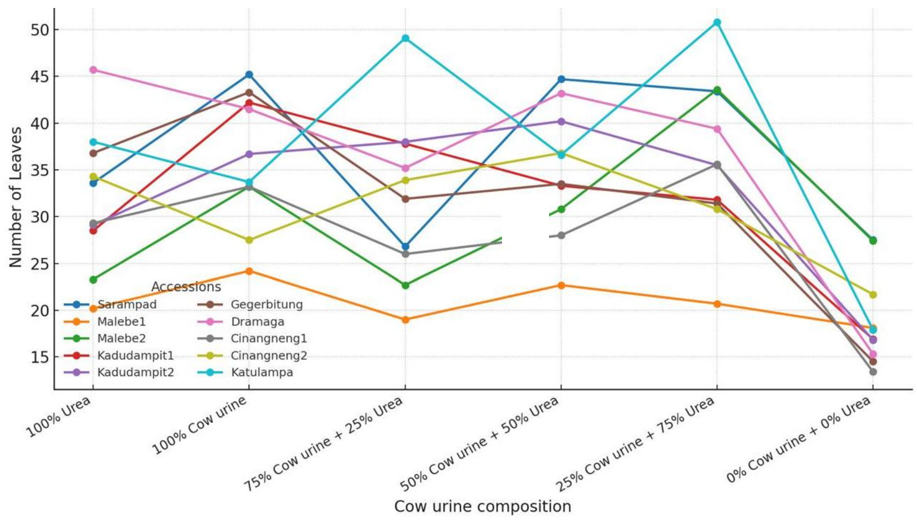

Figure 2. Interaction between *katuk* accessions and cow urine composition on number of leaves variable

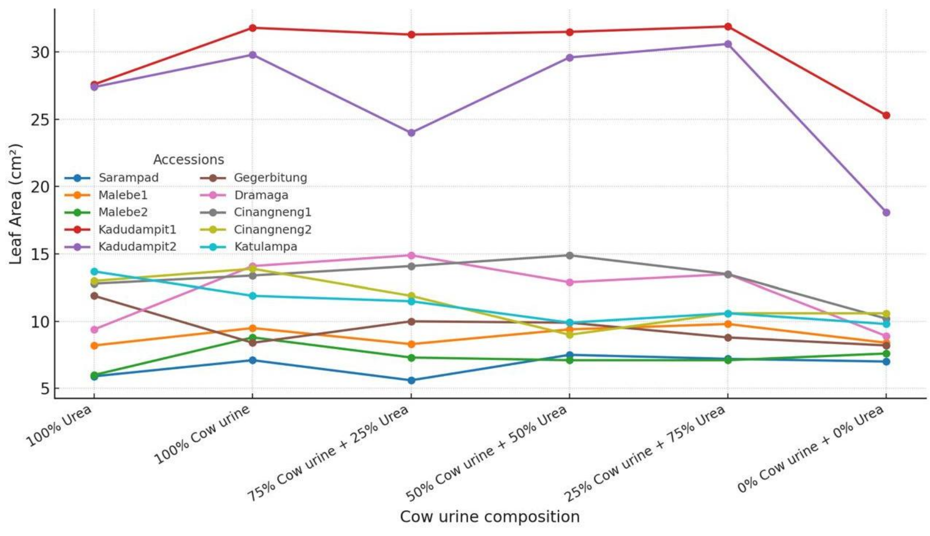

Figure 3. Interaction between *katuk* accessions and cow urine composition on leaf area variable

A significant interaction was observed between *katuk* accessions and cow urine–urea compositions for nitrate content, indicating that nitrate accumulation varies strongly across accessions and nitrogen sources. Across all accessions, nitrate concentrations were lowest in plants receiving no nitrogen (0% cow urine + 0% urea), confirming the direct dependence of nitrate accumulation on external nitrogen input. Among the accessions, Katulampa, Dramaga, and Kadudampit2 tended to accumulate the highest nitrate levels under most nitrogen-supplied treatments. Katulampa recorded the highest nitrate concentration (57.7±6.1 mg kg^{-1} FW) under the 75% cow urine + 25% urea treatment, followed by elevated levels under 100% urea and 100% cow urine. Dramaga exhibited markedly high nitrate accumulation under 100% cow urine (55.3±6.4 mg kg^{-1} FW), while Kadudampit2 maintained consistently high nitrate values across many fertilized treatments (29.0-44.3 mg kg^{-1} FW). These patterns indicate that certain accessions are more prone to nitrate storage regardless of the nitrogen source. In contrast, Maleber1 consistently exhibited the lowest nitrate concentrations across nearly all treatments, including nitrogen-supplied ones, with values generally below 20 mg kg^{-1} FW. This suggests that Maleber1 has inherently low nitrate-uptake or nitrate-storage capacity, making it a potential low-nitrate cultivar. Across fertilizer compositions, nitrate accumulation did not follow a uniform trend. In several accessions (Katulampa, Kadudampit1, Cinangneng2), mixed cow urine and urea treatments, especially 75% cow urine + 25% urea and 50% cow urine + 50% urea, produced higher nitrate concentrations than single-source nitrogen input (Table 7; Figure 4).

Table 8 presents the content of chlorophyll a, chlorophyll b, total chlorophyll, and vitamin C as influenced by *katuk* accession. The chlorophyll content among *katuk* accessions from West Java varied significantly. *Katuk* accessions from Cianjur, particularly Sarampad and Maleber2 exhibited the highest levels of chlorophyll a (4.7±0.36 and 5.0±0.32 mg g^{-1} FW, respectively), chlorophyll b (2.7±0.37 and 2.6±0.16 mg g^{-1} FW), and total chlorophyll (7.4±0.72 and 7.7±0.47 mg g^{-1} FW). These values were significantly higher than those observed in other accessions such from Sukabumi and Bogor. The results indicate that *katuk* plants from higher elevation regions in Cianjur possess greater photosynthetic pigment concentration.

Vitamin C levels also varied among the *katuk* accessions. The Cinangneng1 accession exhibited the highest vitamin C concentration (148.89±5.52 mg 100 g^{-1} FW), followed by Sarampad (132.53±11.92 mg 100 g^{-1} FW) and Katulampa (128.81±7.34 mg 100 g^{-1} FW). Cinangneng1 and Sarampad did not differ significantly ($p>0.05$) in vitamin C content. In contrast, both accessions displayed significantly higher vitamin C levels than Gegebitung (98.7±3.77 mg 100 g^{-1} FW) and Kadudampit2 (106.81±3.91 mg 100 g^{-1} FW). These findings suggest that *katuk* from Cianjur and Bogor Districts exhibit superior antioxidant capacity, likely due to higher metabolic activity and efficient chloroplast function associated with elevated chlorophyll content.

The different proportions of cow urine and urea fertilizers significantly affected the chlorophyll a, chlorophyll b, and total chlorophyll contents of *katuk* leaves. Chlorophyll a content on *katuk* accessions ranged from 2.7±0.15 to 4.7±0.26 mg g^{-1} FW, with the maximum value at 50% cow urine + 50% urea and the minimum at 0% cow urine + 0% urea (Figure 5.A). Chlorophyll b followed the same pattern, ranging from 1.4±0.08 to 2.6±0.23 mg g^{-1} FW, again with the 50% cow urine + 50% urea treatment showing the highest concentration (Figure 5.B). The chlorophyll a and b content of *katuk* accessions treated with 50% cow urine + 50% urea was significantly higher than those treated with 100% urea (4.1±0.18 mg g^{-1} FW for chlorophyll a and 2.2±0.10 mg g^{-1} FW for chlorophyll b) with a superiority percentage of 14.63% and 18.18%, respectively.

Total chlorophyll content varied between 4.1±0.24 and 7.3±0.48 mg g^{-1} FW, with the highest level recorded in plants treated with 50% cow urine + 50% urea. This treatment was significantly superior ($p<0.05$) to all others, highlighting the synergistic effect of combining both nitrogen sources. The lowest chlorophyll content was found in the control plants (no fertilizer), emphasizing the essential role of nitrogen in chlorophyll formation (Figure 6.A). The total chlorophyll content of *katuk* accessions treated with 50% cow urine + 50% urea (7.34±0.48 mg g^{-1} FW) was significantly higher than those treated with 100% urea (6.35±0.28 mg g^{-1} FW) with a superiority percentage of 15.59%.

Table 7. Interaction between *katuk* accessions and cow urine compositions on nitrate content variable

Treatments	Nitrate content (mg kg^{-1} FW)					
	100% Urea	100% cow urine	75% cow urine + 25% Urea	50% cow urine + 50% Urea	25% cow urine + 75% Urea	0% cow urine + 0% Urea
Sarampad	28.3±4.4^{h-r}	17.0±3.8^{q-x}	24.7±0.9^{i-s}	35.3±3.3^{c-l}	24.3±1.5^{j-t}	10.9±1.1^{t-x}
Maleber1	16.7±2.0^{q-x}	9.0±1.5^{vwx}	11.3±0.9^{s-x}	14.7±0.9^{s-x}	15.3±2.3^{r-x}	6.1±1.5^{wx}
Maleber2	23.7±2.3^{k-u}	13.4±4.4^{s-x}	24.3±6.3^{j-t}	21.0±4.7^{m-v}	14.7±0.3^{s-x}	5.4±0.5^{x}
Kadudampit1	31.0±2.5^{e-p}	37.0±4.6^{c-k}	33.3±2.0^{d-n}	34.3±2.4^{c-m}	19.0±0.6^{o-w}	10.3±0.8^{u-x}
Kadudampit2	40.0±4.9^{c-h}	29.0±6.8^{g-q}	42.3±3.5^{c-g}	44.3±9.0^{b-e}	38.0±1.5^{c-i}	11.7±0.3^{s-x}
Gegerbitung	40.7±2.7^{c-h}	37.7±4.8^{c-j}	31.3±4.7^{e-o}	20.7±0.9^{n-v}	36.0±2.1^{c-l}	17.7±4.9^{p-x}
Dramaga	29.3±2.4^{g-q}	55.3±6.4^{ab}	39.3±9.6^{c-h}	12.0±1.5^{s-x}	30.3±3.7^{f-p}	20.7±2.8^{n-v}
Cinangneng1	38.0±1.5^{c-i}	30.3±1.8^{f-p}	43.7±1.8^{b-f}	38.7±1.5^{c-h}	45.0±3.0^{bcd}	22.7±5.0^{l-u}
Cinangneng2	32.7±2.7^{d-n}	41.7±1.9^{c-h}	36.0±1.1^{c-l}	28.3±4.3^{h-r}	36.0±4.5^{c-l}	16.0±4.5^{q-x}
Katulampa	47.0±3.8^{abc}	34.7±9.0^{c-l}	57.7±6.1^{a}	43.3±6.5^{b-f}	36.0±3.5^{c-l}	13.0±1.2^{s-x}

Note: Values followed by the same letter are not significantly different according to DMRT at $p \leq 0.05$

Vitamin C concentration ranged from 109.69±4.30 to 134.71±7.00 mg 100 g^{-1} FW across treatments. The highest Vitamin C content was observed in plants fertilized with 25% cow urine + 75% urea (134.71±7.00 mg 100 g^{-1} FW), which was not significantly different (p>0.05) from those treated with 50% cow urine + 50% urea (126.22±8.23 mg 100 g^{-1} FW) (Figure 6.B). Both treatments produced higher Vitamin C levels compared to plants receiving only urea or cow urine. This suggests that a balanced combination of organic and inorganic fertilizers optimizes the availability of nutrients and promotes secondary metabolite synthesis such as ascorbic acid. In contrast, plants treated with 100% cow urine had the lowest Vitamin C content, indicating that excessive organic input without urea may limit nitrogen mineralization required for ascorbate biosynthesis. The vitamin C content of *katuk* accessions treated with 25% cow urine + 75% urea was significantly higher than those treated with 100% urea (112.05±3.64 mg 100 g^{-1} FW) with a percentage of 20.22%.

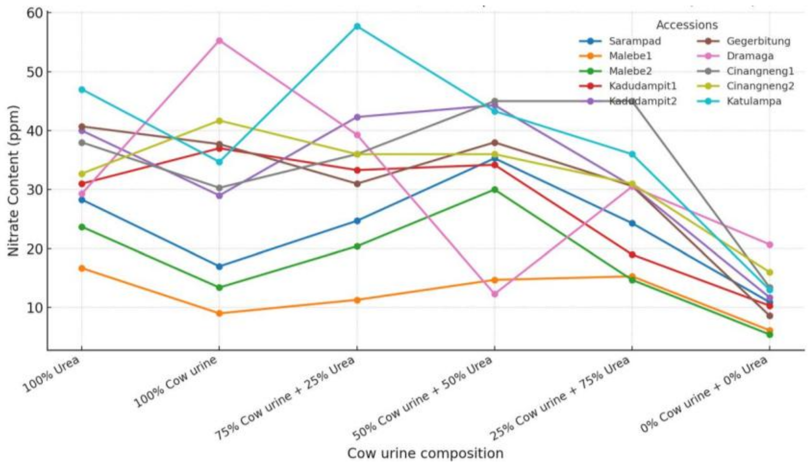

Figure 4. Interaction between *katuk* accessions and cow urine composition on nitrate content variable

Table 8. Quality variables of *katuk* accessions from West Java, Indonesia

Accessions	Chlorophyll a (mg g^{-1} FW)	Chlorophyll b (mg g^{-1} FW)	Total Chlorophyll (mg g^{-1} FW)	Vitamin C (mg 100 g^{-1} FW)
Sarampad	4.7±0.36^{a}	2.7±0.37^{a}	7.4±0.72^{ab}	132.5±11.92^{ab}
Maleber1	4.1±0.20^{b}	2.3±0.13^{ab}	6.4±0.33^{bc}	110.8±7.52^{cd}
Maleber2	5.0±0.32^{a}	2.6±0.16^{a}	7.7±0.47^{a}	118.1±11.01^{bcd}
Kadudampit1	3.5±0.35^{bc}	1.9±0.20^{bc}	5.4±0.54^{cd}	110.8±3.08^{cd}
Kadudampit2	3.6±0.22^{bc}	1.9±0.12^{bc}	5.5±0.35^{cd}	106.8±3.91^{d}
Gegerbitung	3.9±0.15^{b}	2.1±0.08^{bc}	6.0±0.23^{cd}	98.7±3.77^{d}
Dramaga	3.7±0.18^{bc}	1.8±0.09^{c}	5.5±0.27^{cd}	114.2±6.26^{bcd}
Cinangneng1	3.2±0.14^{c}	1.8±0.09^{c}	4.9±0.24^{d}	148.9±5.52^{a}
Cinangneng2	3.9±0.28^{b}	2.0±0.17^{bc}	5.9±0.44^{cd}	118.7±3.61^{bcd}
Katulampa	3.9±0.26^{b}	2.1±0.16^{bc}	6.0±0.42^{cd}	128.8±7.34^{bc}

Note: Values followed by the same letter in the same column are not significantly different according to DMRT at p≤0.05

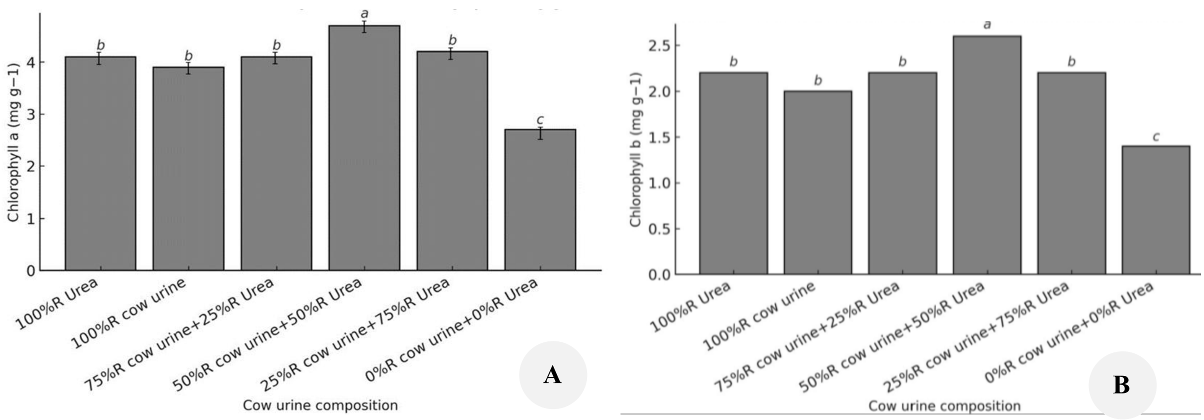

Figure 5. Chlorophyll content on *katuk* accessions. A. Chlorophyll a, B. Chlorophyll b

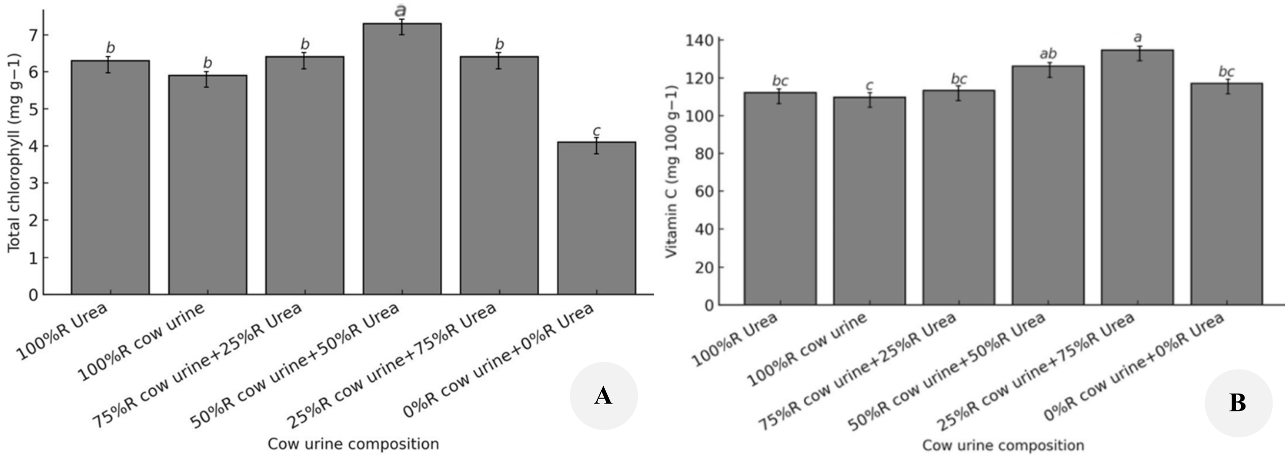

Figure 6. Total chlorophyll and vitamin C content on *katuk* accessions. A. Total chlorophyll, B. Vitamin C

Discussion

The soil used in this experiment was classified as slightly alkaline. Slightly alkaline conditions (pH 7.0-7.8) are known to reduce the solubility and plant availability of several micronutrients (particularly Fe, Mn, Zn, and Cu) which serve as essential cofactors in chlorophyll biosynthesis and photosynthetic electron transport (Marschner 2012). At elevated pH, these micronutrients tend to form insoluble hydroxides and carbonates, thereby limiting their uptake by plant roots (Fageria 2016; Speight 2017). Accordingly, the slightly alkaline soil pH of 7.53 may affect chlorophyll content and overall nutrient acquisition in *katuk*, given the central role of Fe and Mn in chlorophyll formation and the functioning of photosystem complexes.

The *katuk* accessions from Cianjur (Sarampad, Maleber1, and Maleber2) produced the highest fresh and dry harvest weights, with the highest number of leaves and shoots. The number of leaves and shoots plays a critical role in biomass production and directly influences the plant's photosynthetic capacity. Leaves are the primary organs responsible for photosynthesis, converting sunlight into chemical energy, which is essential for growth and the accumulation of biomass (Bazmi and Panichayupakaranant 2023). In *katuk*, the higher the number of leaves, the greater the surface area available for photosynthesis, thus enhancing the plant's ability to produce energy. This increased energy production supports the growth of both the vegetative parts and the storage of nutrients in the form of dry matter, leading to a higher fresh and dry harvest weight (Liu et al. 2020). The *katuk* accessions from Cianjur demonstrated superior quality compared to those from Bogor and Sukabumi, as reflected by their lower leaf nitrate concentrations. Similar genotype-dependent variations in nitrate accumulation have been reported in lettuce cultivars (Kappel et al. 2021). Nitrate accumulation in leafy vegetables is largely influenced by genotype, nutrient availability, and environmental stress factors, with some accessions exhibiting intrinsic efficiency in nitrate reduction and assimilation (Mazahar et al. 2025). Although nitrate itself is relatively non-toxic, its conversion into nitrite within the body may lead to the formation of N-nitroso compounds, which are strongly associated with an increased risk of gastrointestinal cancers, congenital abnormalities, impaired immune function (Gupta et al. 2017; Seyyedsalehi et al. 2023; Bowles et al. 2025). Recent toxicological reviews have emphasized the genotoxic and carcinogenic potential of nitrosamines formed via nitrite–amine reactions in food and biological systems (Schrenk et al. 2023), further underscoring the nutritional and health advantage of Cianjur accessions with lower nitrate content.

The Cianjur accessions (Sarampad and Maleber2) exhibited the highest chlorophyll a, chlorophyll b, and total chlorophyll content, indicating strong photosynthetic potential. High chlorophyll levels are directly correlated with improved photosynthetic efficiency and crop productivity, as observed in maize and rice where chlorophyll content reliably predicts biomass accumulation and yield (Ahmed et al. 2020). The chlorophyll content index, when applied in mathematical functions, can effectively provide accurate statistical diagnosis between simulated and observed biomass in rice plants (Liu et al. 2019). From a nutritional perspective, chlorophylls and their derivatives act as potent antioxidants and exhibit antimutagenic and anti-obesity properties (Martins et al. 2023). They can reduce lipid oxidation and regulate fat metabolism by decreasing free fatty acid release during digestion (Wang et al. 2023). Chlorophyll's multifunctional bioactivity, including metabolic regulation and free-radical scavenging, has made it a promising phytonutrient in functional foods (Ebrahimi et al. 2023). Thus, the concurrent presence of high chlorophyll and low nitrate levels in Cianjur accessions highlights their dual advantage (enhanced photosynthetic capacity and improved nutritional safety) making them ideal candidates for future breeding and sustainable cultivation of *katuk*.

Katuk accessions that were not fertilized with nitrogen (N) demonstrated the lowest values for most observed variables, although vitamin C content did not differ significantly from accessions receiving N fertilization. Similar findings have been observed in leafy vegetables where nitrogen deprivation reduces biomass and photosynthetic efficiency but does not markedly alter antioxidant metabolites such as ascorbic acid (Ansar et al.

2022). This outcome can be attributed to the soil's inherent nutrient composition, characterized by low organic carbon (1.55%) and total nitrogen (0.12%), which restricts microbial activity, nitrogen mineralization, and overall soil fertility (Aumtong et al. 2024). The medium C/N ratio (~13) indicates limited N mineralization potential, as organic matter decomposition and nutrient release are reduced when the carbon-to-nitrogen balance is suboptimal (Li et al. 2022). Although the soil exhibited very high available and potential phosphorus (P_2O_5 = 58.35 ppm; 159.23 mg $100g^{-1}$) and medium potassium levels (33.73 mg $100g^{-1}$), these macronutrients cannot compensate for nitrogen scarcity, as N governs the synthesis of core biomolecules and photosynthetic pigments essential for plant metabolism (de Bang et al. 2021). Nitrogen deficiency disrupts the biosynthesis of chlorophyll, amino acids, proteins, nucleic acids, coenzymes, and phytohormones, which collectively underpin vegetative growth and productivity (Javed et al. 2022).

The low nitrogen availability in soil not only reduces photosynthetic pigment concentration but also limits enzyme activation and energy transfer processes, thereby constraining the formation of structural and functional biomolecules (Fathi 2022). Nitrogen also regulates the production of key secondary metabolites such as alkaloids, phenolics, and flavonoids, which are critical for plant defense and nutritional quality (Wang et al. 2023). Consequently, plants grown under N-deficient conditions exhibit stunted growth, reduced chlorophyll concentration, and lower photosynthetic rates, yet may maintain relatively stable vitamin C levels due to the activation of antioxidant mechanisms under stress (Chen et al. 2024). The resilience of ascorbate metabolism under low-N stress aligns with the broader concept that reactive oxygen species scavenging mechanisms are upregulated to mitigate oxidative damage when nitrogen assimilation is limited (Zhao et al. 2021). Some studies report that vitamin C content is relatively stable under low N, possibly because antioxidant pathways may be maintained or upregulated as part of stress responses (Chen et al. 2024).

The application of 100% cow urine resulted in growth (stem diameter, number of leaflets, number of leaves, and leaf area), and yield (fresh and dry weight of the harvest) outcomes that were not significantly different from those treated with 100% urea or other cow urine compositions. Similar findings were reported by de Oliveira et al. (2009), who demonstrated that increasing cow urine concentration enhanced the fresh and dry weight of leaves in lettuce plants. Khanal et al. (2011) also observed that applying cow urine equivalent to 125 kg Nha^{-1} significantly improved vegetative growth in flowering cabbage. The production of rice seeds and straw treated with 120 kg Nha^{-1} plus cow urine significantly surpassed that of those treated with 150 kg Nha^{-1} (Singh et al. 2011). Lestari et al. (2023) demonstrated that cow urine application significantly influenced leaf number at 40 and 45 days after transplanting, with the 20 mL L^{-1} concentration producing the highest leaf count in Tosakan caisim. Cow urine contains 8.1-8.7 gL^{-1} nitrogen, with 70-90% of it being urea, and other compounds such as allantoin, creatinine, uric acid, and hypuric acid (Cardenas et al. 2016). In addition to its role as a nutrient source, cow urine contributes to pest and disease control. It exhibits antifungal properties, with a 15% concentration capable of suppressing the growth of *Fusarium oxysporum*, *Rhizoctonia solani*, and *Sclerotium rolfsii* by up to 78.57% in fenugreek and okra plants (Jandaik et al. 2015). Moreover, among the 16 compounds identified in cow urine, six were antifungal, three were antibacterial, and two promoted plant growth (Gottimukkala et al. 2019).

The superiority of cow-urine–based fertilizer over 100% urea across multiple traits in *katuk* accessions aligns with growing evidence that biostimulant and organo-mineral fertilizers improve nutrient-use efficiency and photosynthetic performance beyond mineral nitrogen alone (Ruzzi et al. 2024; Chojnacka and Baltrusaitis 2025). The 14.7% increase in plant height under 100% cow urine confirms findings that fermented urine-based inputs stimulate vegetative vigor due to their content of readily available nitrogen, potassium, and plant growth–promoting substances (Jin et al. 2022; Hata et al. 2024).

Likewise, the significantly higher shoot number and vitamin C concentration under the 25% cow urine + 75% urea treatment indicate a positive organo-mineral synergy, where urea supplies fast-release N while cow urine contributes organic matter, enzymes, and hormones that enhance shoot differentiation and antioxidant metabolism (Hata et al. 2024; Pan et al. 2024). These results are consistent with evidence from leafy vegetables showing that integrated organic–inorganic nutrition improves vitamin C accumulation by balancing nitrogen assimilation and oxidative metabolism (Nguyen et al. 2025). Similarly, the 14.6-18.2% increase in chlorophyll a, b, and total chlorophyll in plants treated with 50% cow urine + 50% urea aligns with studies reporting enhanced chlorophyll stability and chloroplast integrity under organo-mineral and biostimulant-based fertilization regimes (Hata et al. 2024). Spraying fenugreek (*Trigonella foenum-graecum*) and okra (*Abelmoschus esculentus*) plants with 1-5% cow urine concentrations increased the protein, carbohydrate, chlorophyll a, chlorophyll b, and total chlorophyll content of their leaves (Jandaik et al. 2015). These benefits derive from the presence of amino acids, cytokinin-like compounds, and micronutrients in cow urine, which enhance nitrogen metabolism, photosynthetic enzyme activity, and chlorophyll biosynthesis (Ruzzi et al. 2024). In *S. androgynus*, a species naturally rich in antioxidants such as ascorbic acid, the 20.2% increase in vitamin C reflects an improved redox balance and metabolic activation triggered by bio-organic inputs (Anju et al. 2022). However, the use of cow urine also presents potential negative impacts if not properly managed. High concentrations of urea and salts (mainly sodium and chloride) in raw urine can lead to soil salinization, increased electrical conductivity, and reduced crop productivity due to osmotic stress on plant roots (Alemayehu et al. 2020; Yu et al. 2025). Moreover, excessive ammonia and urea levels can induce nitrogen toxicity and ammonia volatilization, resulting in nitrogen losses and potential air pollution (Pathy et al. 2021). Fresh urine may also contain pathogenic microorganisms or pharmaceutical residues that persist in soil ecosystems, posing potential health and environmental risks (Woldeyohannis and Desta 2024). Therefore, proper fermentation or stabilization is essential to minimize these negative impacts and ensure safe, sustainable use of urine-based fertilizers.

The cow urine used in this study has been fermented, thus minimizing various negative impacts that may arise. Fermentation changes the chemical and biological properties of cow urine, making it more effective as an organic fertilizer. Fresh (non-fermented) cow urine usually has a neutral pH (around 7.0-7.2) and contains nutrients such as nitrogen (N), phosphorus (P), potassium (K), and calcium (Ca) in moderate amounts. However, some of these nutrients are easily lost, especially nitrogen through ammonia evaporation. After fermentation, the pH increases (around 8.7), and the nutrient content becomes higher because microorganisms help break down organic compounds into forms that are easier for plants to absorb (Samah and Candra 2022). The fermentation process also reduces the strong odor of fresh urine and makes the fertilizer more stable and easier to store (Polakitan et al. 2024). When used on plants, fermented cow urine has been reported to improve growth, yield, and nutrient uptake better than non-fermented urine (Sitinjak 2023; Polakitan et al. 2024). Therefore, fermentation is an important step to improve the quality and effectiveness of cow urine as a liquid organic fertilizer.

The use of cow urine as fertilizer is highly applicable and affordable for smallholder farmers because it is locally available, easy to produce, and compatible with sustainable and low-input agricultural systems. Fermented or diluted cow urine can effectively supplement or replace synthetic fertilizers under smallholder conditions, significantly improving crop growth, yield, and soil fertility while reducing dependence on chemical inputs (Nurhapsa et al. 2024; Yemata and Mengistu 2024). Compared with commercial urea or NPK fertilizers that are frequently affected by global price fluctuations, fermented cow urine can be produced on-farm at negligible cost using simple collection, storage, and fermentation methods (Chojnacka 2023). Some studies report that Indonesian farmers recognize cow urine as a low-cost and environmentally friendly alternative to chemical fertilizers, particularly in regions with mixed crop–livestock systems (Ansar et al. 2022). A study in Netpala Village, Indonesia showed that 88.89% of farmers had a positive perception of using liquid organic fertilizer (cow urine bio-fertilizer) on mustard plants. This reflects high acceptance of the use of this fertilizer (Manu et al. 2019).

In conclusion, this study demonstrated that *katuk* (*S. androgynus*) accessions from Cianjur, particularly Sarampad, Maleber1, and Maleber2 showed superior agronomic and nutritional performance, with the highest fresh (57.0 g plant^{-1}) and dry (17.3 g plant^{-1}) weights, elevated chlorophyll (up to 7.7 mg g^{-1}) and vitamin C (up to 148.9 mg 100 g^{-1}) contents, and low nitrate levels (<30 ppm). In contrast, the accessions from Sukabumi (Kadudampit1, Kadudampit2) exhibited the largest plant height and stem diameter. Fertilization with 100% fermented cow urine increased plant height by 14.7% compared with urea, while 25% cow urine + 75% urea enhanced shoot number and vitamin C by 19% and 20.2%, respectively. The 50% cow urine + 50% urea treatment yielded the highest chlorophyll content, exceeding urea-only treatment by 14.6-18.2%. In this pot-scale experiment, fermented cow urine demonstrated potential as a low-cost supplemental nitrogen source that improved several growth, yield, and nutritional traits of katuk. These findings should be interpreted within the constraints of a short-term controlled environment, and they do not yet confirm its capacity to fully replace synthetic nitrogen fertilizers under field conditions. Nevertheless, the use of fermented cow urine is consistent with principles of low-input and sustainable agriculture, and further field-based evaluations are required to assess its agronomic reliability, environmental performance, and suitability for integration into local cropping systems in West Java.

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