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Optimizing Growth and Flavonoid Production in *Kaempferia angustifolia* Using Organic (Chicken and Cow Manure) and Inorganic Fertilizers

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Abstract

Kaempferia angustifolia originated in Southeast Asia and is widely used for its medicinal properties. One of which is from its flavonoids. The research aims to determine the optimal dosage of organic and inorganic fertilizers for promoting the growth and flavonoid production of *K. angustifolia*. The study was laid out in a split-plot design using organic fertilizers as the main plots (chicken and cow manure at 10 t.ha^{-1}) and inorganic fertilizers as the subplots, consisting of 19 combinations of urea, SP36, and KCl. Each treatment has three replications. The results showed that there is no interaction between organic and inorganic fertilizers in affecting the growth of *Kaempferia angustifolia*. Plants treated with chicken manure at 10 tons per hectare have a higher fresh weight than those treated with cow manure. Plants without inorganic fertilizer application showed suppressed growth and yielded more rhizomes three months after planting. Inorganic fertilizer promoted more shoots and tillers, with the highest value obtained from 100 kg.ha^{-1} urea + 200 kg.ha^{-1} SP36 + 200 kg.ha^{-1} KCl application. The range of total flavonoids in inorganic fertilizer applications is 39.30-131.51% higher than the control, with the highest value observed at 200 kg.ha^{-1} SP36 + 200 kg.ha^{-1} KCl application. The results of this study would be useful for producing *K. angustifolia* for the medicinal industry, using either organic or inorganic fertilizers.

Keywords: chicken manure, cow manure, KCl, medicinal plant, SP36, urea

Introduction

Kaempferia angustifolia originated in Southeast Asia and is distributed across the eastern Himalayas, Laos, Vietnam, Thailand, and Java, Indonesia (Hashiguchi et al., 2022). It belongs to the Zingiberaceae family and is widely used in traditional medicine in Indonesia, including in *jamu* (Java's traditional medicine) (Heyne, 1950; Husain et al., 2021), North Sumatra (Silalahi et al., 2021), and Madura (Yunita et al., 2020). Medicinal plants like *K. angustifolia* are valuable due to their bioactive compounds, particularly flavonoids, which possess antioxidants, antimicrobial, anticancer, and anti-obesity properties (Yenjai et al., 2004; Rafi et al., 2022; Hanif et al., 2022). Given its potential pharmaceutical applications, optimizing its cultivation is essential for ensuring a reliable supply for the medicinal industry (Singh et al., 2023).

Despite its medicinal significance, *K. angustifolia* remains underutilized and is not widely cultivated by farmers in Indonesia. It is commonly found growing wild in teak forests in West and Central Java (Hashiguchi et al., 2022), with propagation mainly occurring through rhizomes. While previous research on related Zingiberaceae species, such as turmeric, has shown positive effects of organic manure on growth and yield (Kamal and Yousuf, 2012; Ferdous et al., 2018), there is limited information on how different fertilizer combinations influence *K. angustifolia* growth and flavonoid production. Developing an optimal cultivation strategy by testing a wide range of organic and inorganic fertilizer combinations is important for increasing production. Testing multiple fertilizer treatments is justified for several reasons. First, different combinations have a unique impact on plant biomass, yield, and secondary metabolite production (Handravanshi et al., 2021). The optimal nutrient mix for enhancing plant growth and flavonoid accumulation can be determined by exploring various treatments. Second, *K. angustifolia* is typically

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grown in latosol soil, which is characterized by high acidity and low nutrient content (Adhi et al., 2017). Different fertilizer treatments allow us to assess which combination best improves soil fertility and plant health under such conditions. Third, organic fertilizers contribute to long-term soil health and microbial activity (Bhatt et al., 2019), while inorganic fertilizers provide immediate nutrient availability (Jabborova et al., 2021). Balancing these two inputs is crucial for sustainable cultivation. Finally, flavonoid production is influenced by nutrient availability, particularly nitrogen, phosphorus, and potassium (Xu et al., 2018; Wei et al., 2022), making it essential to test different fertilizer dosages to identify those that maximize secondary metabolite synthesis.

The results of this study will have significant implications beyond Indonesia. *Kaemphferia angustifolia* belongs to the same family as turmeric and ginger, two globally important medicinal and culinary crops. Understanding the optimal fertilization strategies for this species can contribute to best practices in the sustainable cultivation of medicinal Zingiberaceae species worldwide (Velmurugan et al., 2008; Ojikpong and Undie, 2019). Evaluating a wide range of organic and inorganic fertilizer combinations is necessary to develop an optimal cultivation strategy. A key aspect of this study is the comparison of chicken and cow manure, two widely used organic fertilizers with distinct characteristics. Chicken manure contains higher nitrogen, phosphorus, and potassium (NPK) levels, promoting faster plant growth and higher biomass accumulation (Al-Gaadi et al., 2019; Oyewole et al., 2020). However, it decomposes quickly and can lead to nutrient leaching if not adequately managed (Hlisnikovský et al., 2021). In contrast, cow manure has a higher organic matter content and can improve soil structure, water retention, and microbial activity, but its lower nutrient content may result in slower plant growth (Bhatt et al., 2019; Abbasi and Anwar, 2015). Additionally, the nutrient composition of organic fertilizers can influence the production of secondary metabolites. High nitrogen availability from chicken manure can enhance vegetative growth but may dilute flavonoid content due to increased shoot-to-root allocation (Guo et al., 2022). Cow manure's slower nutrient release may enhance plant stress responses, potentially leading to higher flavonoid accumulation (Agati et al., 2020). Understanding these differences is crucial for optimizing *K. angustifolia* cultivation for yield and medicinal compound production.

The study provides insights into how organic and inorganic fertilizers influence plant secondary metabolite production, which is valuable for researchers and agricultural practitioners working with medicinal plants in diverse climatic and soil conditions (Sun et al., 2022). Moreover, flavonoids are bioactive compounds widely studied for their pharmaceutical and nutraceutical applications. Optimizing their production through fertilization strategies aligns with the increasing global demand for natural antioxidants and functional food ingredients (Agati et al., 2020). The knowledge gained from this research can guide agronomic practices for medicinal plant cultivation in regions facing similar soil fertility challenges, such as tropical and subtropical countries in Asia, Africa, and Latin America (Alotaibi and Abd-Elgawad, 2023).

Finally, as global interest in sustainable agriculture grows, the study's findings contribute to ongoing discussions on balancing organic and inorganic fertilization to improve yield and phytochemical content. This research is relevant to scientists, farmers, and industry professionals seeking to enhance medicinal plant product quality and commercial viability while maintaining soil health and ecological sustainability (Hlisnikovský et al., 2021).

This study is the first to systematically investigate the effects of different organic (chicken and cow manure) and inorganic (urea, SP36, and KCl) fertilizer combinations on the growth and flavonoid content of *K. angustifolia*. The findings will contribute to developing a standard operating procedure (SOP) for cultivating this species on latosol soils in West Java, Indonesia, and supporting its potential for commercial and pharmaceutical applications.

Material and Methods

The study used *K. angustifolia* rhizomes from a teak plantation in Blora Central Java, Indonesia. No varieties have been developed from this species yet. Each rhizome was 1 cm x 2 cm in length, or 2-3 g per rhizome. Other materials are polybag, chicken manure, cow manure, urea, SP36, and KCl. The experiment was laid out in the split-plot design with three replications, totaling 114 experimental units. It was conducted from October to December 2021 at the IPB University Experimental Station, Indonesia (SL: -6.5467129, EL: 106.7158330) with latosol soil. The rhizomes were sown in a soil and manure mixture in the nursery. When they had 2-3 mature leaves, or about 1 month after planting (MAP), they were transplanted into polybags. The fertilizer was applied once at the beginning of the experiment. The main plot consisted of organic fertilizer (chicken and cow manure at 10 $t.ha^{-1}$), and the subplot included 19 inorganic fertilizers, as described in Table 1. The control plants were not fertilized; treatments A to F and K to O are without nitrogen (Urea).

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Every plant was measured monthly for height, leaf number, and tiller number. Harvest was conducted at 3 MAP. Other measurements include fresh and dry weight at 3 MAP, leaf NPK (N using the Kjehldahl method, P and K using the fresh ash method with a mixture of HNO_3 and $HClO_4$), relative growth rate (RGR), and net assimilation rate (NAR) 1-3 MAP, rhizome fresh and dry weight at 3 MAP, and total flavonoid at 3 MAP using spectrophotometer UV VIS using Chang et al. (2002) method. Data were analyzed using analysis of variance, followed by the Duncan Multiple Range Test, α = 0.05%.

The following formulas were used:

Relative growth rate [RGR = (ln W2 - ln W1)/ (t2 - t1)

Remarks: W1 and W2 = plant dry weights at times t1 and t2

Net assimilation rate [NAR = (W2 − W1)/ (t2 − t1) × (ln LA2 − ln LA1)/ (LA2 − LA1)

where W1 and W2 = plant dry weights at times t1 and t2

LA1 and LA2 = leaf area at times t1 and t2

Total flavonoid per plant = total flavonoid concentration x plant dry weight

Results and Discussion

When the experiment occurred, the average monthly temperatures were 26.7, 25.4, and 26.0 °C, with relative humidity levels of 76.8, 73.1, and 87.5%, and rainfall intensities of 381.9, 339.4, and 597.2 mm/month. The average rainfall intensity in Blora, Central Java, according to data from the Indonesian Central Statistical Bureau (2023), is 1470-2000 mm per year, which is lower than in Bogor, West Java, where the average is 3500-4000 mm per year. The differences in conditions are significantly pronounced, particularly in rainfall intensity. High rainfall intensity leads to high humidity, providing suitable conditions for the pathogenic bacterium *Ralstonia solanacearum* at 4 MAP.

The soil texture is clay with a pH of 3.37 (very acidic), an organic carbon content of 1.44% (low), a total nitrogen content of 0.15% (low), a P Bray 1 level of 2.42 ppm (very low), and a Ca level of 10.14 cmol.kg^{-1} (moderate), Mg 1.03 cmol.kg^{-1} (low), K 0.10 cmol.kg^{-1} (low), and a cation exchange capacity of 16.86 (low). The soil texture is clay with a very low pH and an overall low cation value, indicating that the soil requires fertilization with both organic and inorganic fertilizers. Solly et al. (2020) stated that a relationship exists between the adequate cation exchange capacity of soil and its organic content in both topsoil

Table 1. Description of inorganic fertilizer treatments

Codes	Urea ($kg.ha^{-1}$)	SP36 ($kg.ha^{-1}$)	KCl ($kg.ha^{-1}$)
A	0	0	0
B	0	200	0
C	0	200	100
D	0	200	200
E	0	200	300
F	0	200	400
G	100	200	100
H	100	200	200
I	100	200	300
J	100	200	400
K	0	400	0
L	0	400	100
M	0	400	200
N	0	400	300
O	0	400	400
P	100	400	100
Q	100	400	200
R	100	400	300
S	100	400	400

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and subsoil. This indicates that the soil has low organic carbon and should be stabilized by metal cations through coordination between soil minerals and organic matter. In acidic soil, exchangeable aluminum contributes more than 20% of the adequate cation exchange capacity. Velmurugan et al. (2008) found that using organic farm manure added with *Azospirillum*, phosphobacteria, and VAM (vesicular arbuscular Mycorhiza) containing fungus *Glomus fasciculatum, G. mossae,* and *Gigaspora* sp. on turmeric produced the highest cured rhizome yield and quality of turmeric than inorganic fertilizer or just farm manure.

The Effects of Organic Fertilizer

The experiment showed that organic fertilizer had a significant effect on plant fresh weight at 3 MAP ($P < 0.05$). Inorganic fertilizer affected plant height and tiller number at 2 and 3 MAP (Table 2), as well as the plant's fresh weight, total flavonoids (Table 3), and N leaf content (Table 4). There was no interaction between the treatments in the main plot (organic fertilizer) and the subplots (inorganic fertilizer). Plants without inorganic fertilizer exhibited suppressed growth, a darker green color, and produced rhizomes at 3 months after planting (MAP). In contrast, the application of inorganic fertilizer promoted the development of more shoots and tillers and initiated rhizome formation.

Organic fertilizer application did not affect plant height, primary tiller leaf number, or tiller number (Table 2). Plant height and tiller number increased to 3 MAP, but the primary tiller leaf number decreased from 2 to 3 MAP. The planting season affected the vegetative organs of *K. angustifolia* that we harvested, the rhizomes. The Zingiberaceae family exhibits a distinct phenology, with vegetative growth occurring during the rainy season and rhizome harvesting typically in the dry season (Souvannakhoummane, 2014). In this experiment, the high rainfall intensity stimulated increased shoot production, including tillers, and resulted in greater plant height. The primary tiller grew from the rhizome as propagules in this experiment. This sympodial type of plant develops from the primary tiller and then forms the tillers around it. These phenomena indicate that the primary tiller is the source of all subsequent tillers, which serve as the next sink in plant growth. As the source of photosynthate, the primary tiller will grow and reach its maximum growth at 2 MAP, after which it will decrease the number of primary tiller leaves to 3 MAP (Table 1). The subsequent tiller is increased from 2 to 3 MAP with more leaves and then produces rhizomes. The leaves in the subsequent tiller also became the source of photosynthesis in the clump for the next sink formed, i.e., the leaves and the rhizomes. Increasing the leaf number per plant, caused by an increase in the tiller number, resulted in more photosynthate production, as the leaf is the primary source (Gamalei, 2002).

Kaempferia angustifolia is a recently domesticated species that can be observed growing as an understory in teak forests in Indonesia, relying on its organic matter (Giweta, 2020). Latosol soil in Indonesia has low nutrients and organic matter (Adhi et al., 2017), which is also seen in the soil analysis in this experiment. The plant litter in the teak forests, where the *in-situ K. angustifolia*, resembles the natural forest conditions, where organic matters are available in large quantities. Plant litter in forest ecosystems is essential for the forest biogeochemical cycle as it accumulates soil organic matter (Giweta, 2020) and improves the soil's physical and biological activities, but the nutrient content of organic fertilizer is low, so a larger quantity is required for plant growth (Bhatt et al., 2019; Hwang et al., 2020; Hlisnikovský et al., 2021). With climate change, plants adapt their physiology and morphology (Aziz, 2022) to enable *K. angustifolia* to form rhizomes in the soil as they sink, and to decrease the number of primary tiller leaves to those on the tiller above ground. Initially, rapid growth was observed up to 2 MAP, as the nutrients in the organic fertilizer still met the plant's needs. However, growth declined from 2 to 3 MAP, likely because the low organic fertilizer content was insufficient for the plant's growth. The control plants have a low number of leaves and tillers, with reddish leaves, indicating that the plant lacks sufficient nutrients and is stressed. Figure 1 shows *K. angustifolia* relative growth rate and net assimilation rate. Organic fertilizer resulted in growth acceleration, as demonstrated by the relative growth rate (RGR) (Figure 1A) and an increase in net assimilation rate (NAR) (Figure 1C) from 1 to 2 MAP. However, from 2 to 3 MAP, RGR and NAR declined. Organic fertilizer applications showed the relative growth rate (Figure 1A) of plants with declining added biomass and net assimilation rate (Figure 1C) with declining added primary tiller leaf numbers from 2-1 to 3-2 MAP. Chicken manure has a higher primary tiller leaf number value than cow manure application ($P>0.05$). The RGR of *K.* angustifolia exhibits rapid growth from 1 to 2 MAP, supported by increasing plant height and tiller number, followed by a decline from 2 to 3 MAP. The formation of roots and shoots occurred simultaneously, and rhizomes formed later in the 3 MAP. In *K. angustifolia*, the vegetative morphological development involves the emergence of shoots and roots from the rhizome, followed by the increase in both roots and leaves. Subsequent tillers appear and increase at 60-90 days after planting (DAP), while simultaneously forming the rhizome. A study

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on white ginger, also from the Zingiberaceae family, demonstrated that active biomass accumulation occurred at 60-120 days after planting (DAP), coinciding with the accumulation of dry matter in the rhizome (Krishnamurthy and Kandiannan, 2020).

The type of organic fertilizer treatment affected both fresh and dry weight (Table 3); plants treated with chicken manure had a higher fresh weight than those treated with cow manure. A study on chili reported that the fresh and dry weight is significantly higher with chicken manure application (Oyewole et al., 2020; Putra et al., 2020). Chicken manure applications have increased the plant's fresh weight, dry weight, and total flavonoids by 20.75, 10.64, and 16.80%, respectively, compared to cow manure. These results are likely due to the higher nitrogen content of chicken manure compared to cow manure, as reported by Al-Gaadi et al. (2019) in cabbage, cauliflower, lettuce, and broccoli. Organic fertilizer application did not change leaf NPK content (Table 4). The variation in total flavonoid content is due to differences in flavonoid concentration rather than plant dry weight, as total flavonoid content is determined by multiplying flavonoid concentration by plant dry weight. Higher nitrogen resulted in more water in a plant, which caused the difference in fresh weight but did not affect the plant's dry weight.

The Effects of Inorganic Fertilizers

Primary tiller leaf number was not affected by inorganic fertilizer application (Table 2). The primary tiller has reached its maximum leaf number and weight at 2 MAP (Table 2 and Figure 1). Control treatment has a significantly lower plant height and tiller number at 2 and 3 MAP, i.e., 11.60 and 13.33 cm at 2 MAP and 3.37 and 5.87 at 3 MAP; these values

Table 2. *Kaempferia angustifolia* plant height and primary tiller leaf number at 2 and 3 MAP, and tiller number at 3 MAP

Treatments	Plant height (cm)		Primary tiller leaf number		Tiller number	
	2 MAP	3 MAP	2 MAT	3 MAT	2MAT	3MAT
<u>Main plot: Organic fertilizer</u>						
Chicken manure	12.86	15.04	5.91	5.58	3.51	7.53
Cow manure	12.19	14.69	5.88	5.58	3.49	7.00
<u>Subplot: Inorganic fertilizer</u>						
Control (without fertilizers)	11.60 cd	13.33 b	5.13	5.40	3.37bc	5.87 de
200 kg.ha^{-1} SP36	11.71a-d	13.31 b	5.77	5.70	3.17bc	5.63 e
200 kg.ha^{-1} SP36 + 100 kg.ha^{-1} KCl	12.91a-c	14.96ab	5.97	5.78	3.43bc	7.17b-e
200 kg.ha^{-1} SP36 + 200 kg.ha^{-1} KCl	12.50a-d	14.67ab	5.93	5.23	3.22bc	6.53c-e
200 kg.ha^{-1} SP36 + 300 kg.ha^{-1} KCl	13.62 ab	16.31 a	5.90	5.63	3.43bc	7.70a-d
200 kg.ha^{-1} SP36 + 400 kg.ha^{-1} KCl	12.04a-d	14.90ab	6.03	5.52	2.97bc	6.48c-e
100 kg.ha^{-1} urea + 200 kg.ha^{-1} SP36 + 100 kg.ha^{-1} KCl	13.69 a	16.27 a	6.07	5.53	3.43bc	7.80a-d
100 kg.ha^{-1} urea + 200 kg.ha^{-1} SP36 + 200 kg.ha^{-1} KCl	11.64b-d	13.90ab	5.97	5.43	3.27bc	6.60c-e
100 kg.ha^{-1} urea + 200 kg.ha^{-1} SP36 + 300 kg.ha^{-1} KCl	12.54a-d	15.11ab	6.29	5.68	2.88 c	6.47c-e
100 kg.ha^{-1} urea + 200 kg.ha^{-1} SP36 + 400 kg.ha^{-1} KCl	11.86a-d	14.34ab	5.88	5.55	2.97bc	6.58c-e
400 kg.ha^{-1} SP36	10.77 d	13.11 b	5.26	5.76	3.50bc	6.92b-e
400 kg.ha^{-1} SP36 + 100 kg.ha^{-1} KCl	12.24a-d	14.42ab	6.43	5.67	4.03ab	7.97a-c
400 kg.ha^{-1} SP36 + 200 kg.ha^{-1} KCl	12.85a-c	15.62ab	5.93	5.73	3.53bc	7.50a-d
400 kg.ha^{-1} SP36 + 300 kg.ha^{-1} KCl	12.62a-d	15.42ab	6.13	5.63	3.43bc	7.27a-e
400 kg.ha^{-1} SP36 + 400 kg.ha^{-1} KCl	13.64 ab	16.34 a	6.03	5.64	4.77 a	9.16 a
100 kg.ha^{-1} urea + 400 kg.ha^{-1} SP36 + 100 kg.ha^{-1} KCl	13.13a-c	15.59ab	5.80	5.40	3.84a-c	8.72 ab
100 kg.ha^{-1} urea + 400 kg.ha^{-1} SP36 + 200 kg.ha^{-1} KCl	13.65ab	15.97 a	6.27	5.30	4.00ab	8.33a-c
100 kg.ha^{-1} urea + 400 kg.ha^{-1} SP36 + 300 kg.ha^{-1} KCl	12.73a-d	14.37ab	5.55	5.89	3.55bc	8.38a-c
100 kg.ha^{-1} urea + 400 kg.ha^{-1} SP36 + 400 kg.ha^{-1} KCl	12.24a-d	14.62ab	5.13	5.40	3.59bc	6.93b-e
Organic x inorganic fertilizer	ns					

Note: Mean values within a column followed by different letters are not significantly different at P < 0.05 using Duncan's Multiple Range Test; ns = non-significant.

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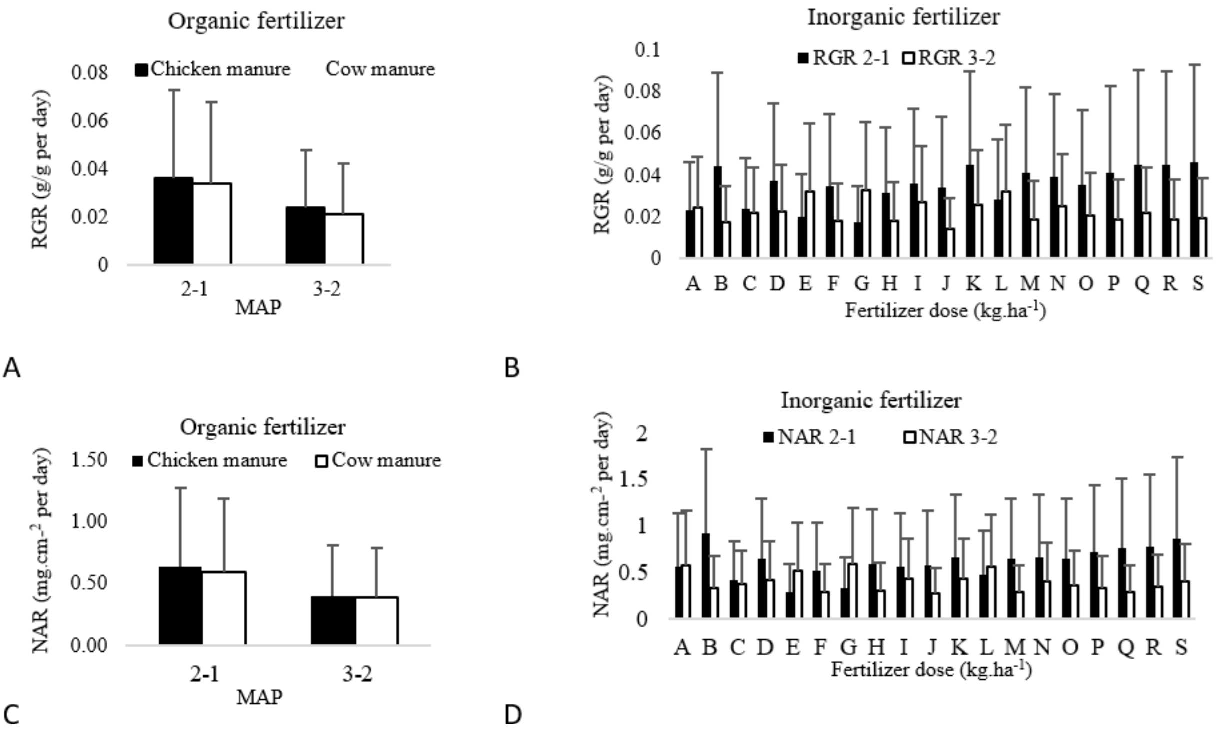

Figure 1. K*aempferia angustifolia* relative growth rate (RGR) with organic fertilizer (A), inorganic fertilizer (B), and net assimilation rate (NAR) with organic fertilizer (C), and inorganic fertilizer (D), 1 to 3 months after planting (MAP). Fertilizer dosages A to S are described in Table 1.

are not significantly different from fertilization with 200 kg.ha^{-1} SP36 only (Table 2). The control plants produced rhizomes earlier than other treatments, as observed by the rhizome formation. The stems look reddish with a significantly low tiller number. Without organic fertilizer application and in times of water deficit, the plant's investment in root tissue is more beneficial than in leaf tissue; in this case, the earlier rhizome formation reduces the area for water loss via transpiration (Gallery, 2016). Cultivated and wild plants differ in yield stability, nutrient acquisition strategies, and the success of ecological nutrient management (Isaac et al., 2021). Species-specific factors relating to plant development and physiological changes will also influence the yield (Li et al., 2020).

The roots and rhizomes of plants treated with inorganic fertilizers are more developed than those without fertilizer, resulting in green-colored leaves. In contrast, plants without fertilizer exhibit reddish leaves (Figure 2). Anthocyanin is a pigment and secondary metabolite that imparts a reddish color to leaves, often as a response to abiotic stresses, such as mineral nutrient deficiencies (Gould, 2004; Gould and Lister, 2006; Isah, 2019). The rhizome form is a push method, where the increasing leaf and the increasing tiller number serve as the source to discharge sucrose from the source organ, as observed in tuber formation in potatoes (Katoh et al., 2015).

Plants treated with 200 kg.ha^{-1} SP36 plus 100, 200, or 400 kg.ha^{-1} KCl, 100 kg.ha^{-1} urea + 200 kg.ha^{-1} SP36 plus 200, 300, or 400 kg.ha^{-1} KCl, 400 kg.ha^{-1} SP36, 400 kg.ha^{-1} SP36 plus 300 or 400 kg.ha^{-1} KCl, have the lowest tiller number, similar to the control, and those treated with 200 kg.ha^{-1} SP36 . Taller plants and higher numbers of primary tiller leaves do not always correlate with an increase in the number of tillers.

Inorganic fertilizer application increased biomass (Figure 1B) and photosynthetic rates (Figure 1D), as indicated by the RGR and NAR in plants treated with SP36 and KCl combinations (200 kg.ha^{-1} SP36, 200 kg.ha^{-1} SP36 + 300 kg.ha^{-1} KCl, and 400 kg.ha^{-1} SP36 + 100 kg.ha^{-1} KCl, between 2-1 and 3-2 MAP. Other inorganic treatments showed decreasing trends. It showed that sufficient nitrogen in the soil, combined with phosphorus in the inorganic fertilizer, promoted increased plant biomass earlier, i.e., at 3 MAP. Overall, the plant height and tiller number increased, but the primary tiller leaf number decreased from 2 to 3 MAP (Table 3).

Inorganic fertilizer did not affect the plant's dry weight. The fresh weight of the plants applied with 100 kg.ha^{-1} urea + 200 kg.ha^{-1} SP36 + 200 kg.ha^{-1} KCl was

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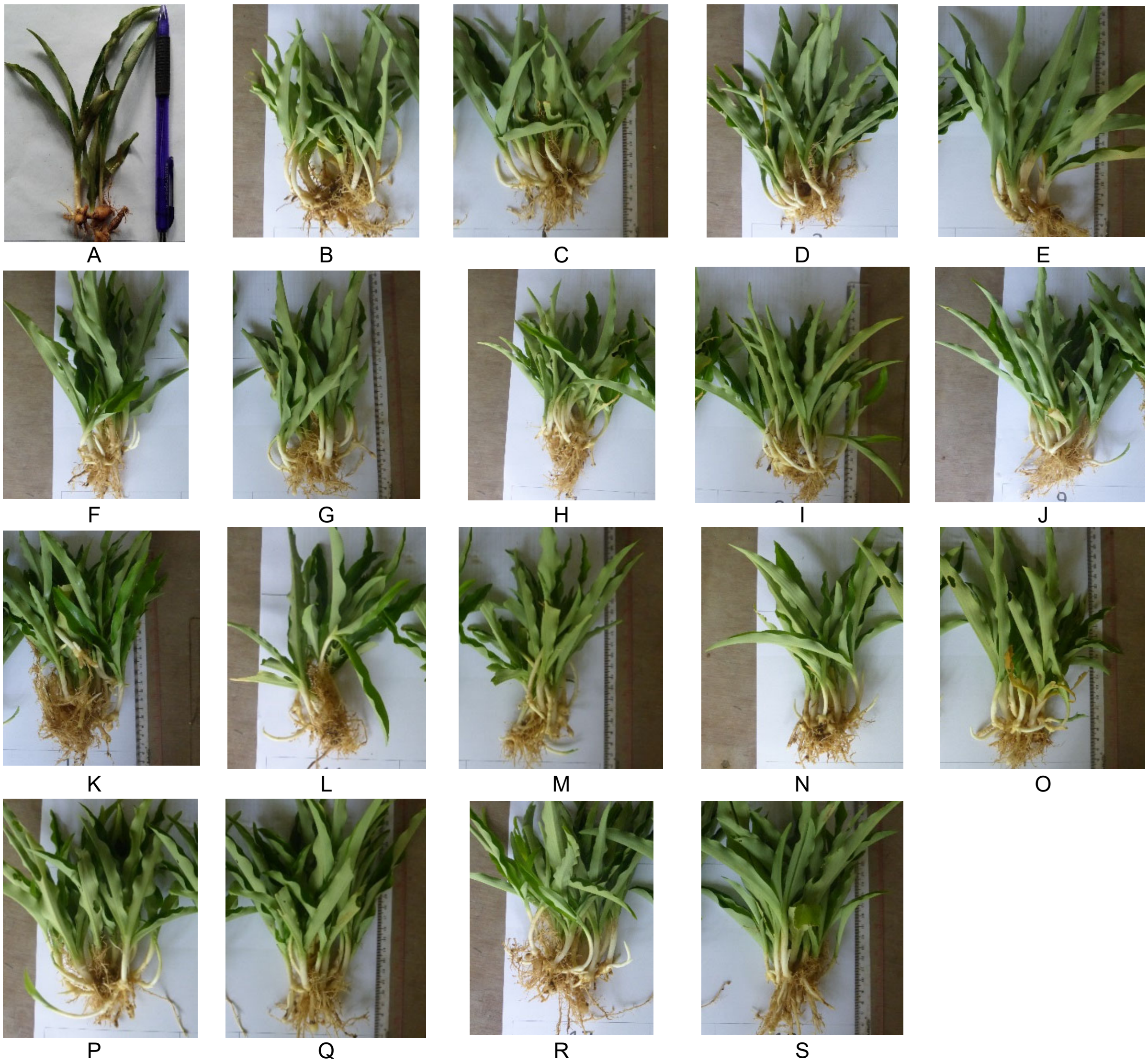

Figure 2. *Kaempferia angustifolia* at 3 months after planting: control plants (without inorganic fertilizers) that looks reddish (A), 200 kg.ha^{-1} SP36 (B) ; 200 kg.ha^{-1} SP36 + 100 kg.ha^{-1} KCl (C), 200 kg.ha^{-1} SP36 + 200 kg.ha^{-1} KCl (D), 200 kg.ha^{-1} SP36 + 300 kg.ha^{-1} KCl (E); 200 kg.ha^{-1} SP36 + 400 kg.ha^{-1} KCl (F); 100 kg.ha^{-1} urea + 200 kg.ha^{-1} SP36 + 100 kg.ha^{-1} KCl (G), 100 kg.ha^{-1} urea + 200 kg.ha^{-1} SP36 + 200 kg.ha^{-1} KCl (H); 100 kg.ha^{-1} urea + 200 kg.ha^{-1} SP36 + 300 kg.ha^{-1} KCl (I); 100 kg.ha^{-1} urea + 200 kg.ha^{-1} SP36 + 400 kg.ha^{-1} KCl (J); 400 kg.ha^{-1} SP36 ; 400 kg.ha^{-1} SP36 + 100 kg.ha^{-1} KCl (L); 400 kg.ha^{-1} SP36 + 200 kg.ha^{-1} KCl (M); 400 kg.ha^{-1} SP36 + 300 kg.ha^{-1} KCl (N); 400 kg.ha^{-1} SP36 + 400 kg.ha^{-1} KCl (O); 100 kg.ha^{-1} urea + 400 kg.ha^{-1} SP36 + 100 kg.ha^{-1} KCl (P), 100 kg.ha^{-1} urea + 400 kg.ha^{-1} SP36 + 200 kg.ha^{-1} KCl (Q), 100 kg.ha^{-1} urea + 400 kg.ha^{-1} SP36 + 300 kg.ha^{-1} KCl (R); 100 kg.ha^{-1} urea + 400 kg.ha^{-1} SP36 + 400 kg.ha^{-1} KCl (S).

significantly higher than other treatments but similar to the application of 200 kg.ha^{-1} SP36 + 200 kg.ha^{-1} KCl, 100 kg.ha^{-1} urea + 200 kg.ha^{-1} SP36 + 300 kg.ha^{-1} KCl, and 400 kg.ha^{-1} SP36 + 100 kg.ha^{-1} KCl. The lowest fresh weight is from those applied with 200 kg.ha^{-1} SP36 (P only), with values similar to those from the rest of the inorganic fertilizer treatments.

Application of 200 kg.ha^{-1} SP36 + 200 kg.ha^{-1} KCl has the highest total flavonoids (Table 3), but it is similar to those treated with 200 kg.ha^{-1} SP36 + 300 kg.ha^{-1} KCl, or 200 kg.ha^{-1} SP36 + 400 kg.ha^{-1} KCl, 100 kg.ha^{-1} urea + 200 kg.ha^{-1} SP36 + 200 kg.ha^{-1} KCl, 100 kg.ha^{-1} urea + 200 kg.ha^{-1} SP36 + 300 kg.ha^{-1} KCl, 100 kg.ha^{-1} urea + 200 kg.ha^{-1} SP36 + 400 kg.ha^{-1} KCl, and 400 kg.ha^{-1} SP36, or 131.51, 78.94, 52.34, 66.94, 79.12, 116.41, and 39.30% higher than control. The total flavonoids were significantly the lowest for those applied at 100 kg.ha^{-1} urea + 400 kg.ha^{-1} SP36 + 300 kg.ha^{-1} KCl, which is not significantly different from the control (Table 3).

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Table 3. *Kaempferia angustifolia* fresh and dry weight, and total flavonoids with different organic and inorganic fertilizer applications

Treatments	Fresh weight[1)] (g)	Dry weight (g)	Total flavonoids μmg QE eq per g of dry weight
Main plot: Organic fertilizer			
Chicken manure	2.91 a	0.52	5.403
Cow manure	2.41 b	0.47	4.626
Subplot: Inorganic Fertilizer			
Control (without fertilizers)	2.26 bc	0.36	3.875 cd
200 kg.ha^{-1} SP36	1.84 c	0.33	2.905 d
200 kg.ha^{-1} SP36 + 100 kg.ha^{-1} KCl	2.57 bc	0.46	4.110 cd
200 kg.ha^{-1} SP36 + 200 kg.ha^{-1} KCl	3.45 ab	0.78	8.971 a
200 kg.ha^{-1} SP36 + 300 kg.ha^{-1} KCl	3.08 bc	0.47	6.905 a-c
200 kg.ha^{-1} SP36 + 400 kg.ha^{-1} KCl	2.76 bc	0.63	5.903 a-d
100 kg.ha^{-1} urea + 200 kg.ha^{-1} SP36 + 100 kg.ha^{-1} KCl	2.26 bc	0.43	4.679 b-d
100 kg.ha^{-1} urea + 200 kg.ha^{-1} SP36 + 200 kg.ha^{-1} KCl	4.39 a	0.68	6.469 a-d
100 kg.ha^{-1} urea + 200 kg.ha^{-1} SP36 + 300 kg.ha^{-1} KCl	3.41 ab	0.65	6.941 a-c
100 kg.ha^{-1} urea + 200 kg.ha^{-1} SP36 + 400 kg.ha^{-1} KCl	2.86 bc	0.62	8.386 ab
400 kg.ha^{-1} SP36	2.27 bc	0.50	5.398 a-d
400 kg.ha^{-1} SP36 + 100 kg.ha^{-1} KCl	3.15 a-c	0.37	3.553 cd
400 kg.ha^{-1} SP36 + 200 kg.ha^{-1} KCl	2.22 bc	0.42	4.101 cd
400 kg.ha^{-1} SP36 + 300 kg.ha^{-1} KCl	2.18 bc	0.40	4.206 cd
400 kg.ha^{-1} SP36 + 400 kg.ha^{-1} KCl	2.20 bc	0.40	3.673 cd
100 kg.ha^{-1} urea + 400 kg.ha^{-1} SP36 + 100 kg.ha^{-1} KCl	2.60 bc	0.50	4.429 cd
100 kg.ha^{-1} urea + 400 kg.ha^{-1} SP36 + 200 kg.ha^{-1} KCl	2.83 bc	0.62	4.873 b-d
100 kg.ha^{-1} urea + 400 kg.ha^{-1} SP36 + 300 kg.ha^{-1} KCl	2.03 bc	0.33	2.859 d
100 kg.ha^{-1} urea + 400 kg.ha^{-1} SP36 + 400 kg.ha^{-1} KCl	2.22 bc	0.43	3.047 cd
Interaction of organic vs inorganic fertilizer	ns	ns	ns

Notes: Mean values within a column followed by different letters are not significantly different at P< 0.05 according to Duncan's Multiple Range Test. [1)] =Data are transformed with √x+0.5; ns = non-significant.

Leaf P and K showed no differences in inorganic fertilizer application compared to the control. The highest significant leaf nitrogen was found in the control, and the lowest was found at 200 kg.ha^{-1} SP36 + 300 kg.ha^{-1} KCl is 18.72% lower than the control (Table 4). As reported in rice, leaf nitrogen in inorganic fertilizer applications experiences concentration dilution due to the higher shoot weight and increased leaf and tiller numbers (data not shown) (Guo et al., 2022). Inorganic fertilizers usually contain all necessary nutrients directly accessible to plants immediately and quickly (Bhatt et al., 2019; Jabborova et al., 2021). The dilution effect occurs because as the plant grows and produces more leaves and tillers, the total amount of nitrogen in the plant is distributed among a greater number of leaves, resulting in a lower nitrogen content per leaf (Gastal and Lemaire, 2002).

Carbohydrates are a product of photosynthesis in plants (Taiz et al., 2018). Carbohydrates significantly affect phase-specific leaf traits, and recent research suggests that sugars may be the leaf signals that promote vegetative phase change (Poethig, 2013). A mature plant will move on to the next phase of its life cycle, the rhizome forming in *K. angustifolia* (Tomlinson, 1956). The carbon-to-nitrogen (C/N) ratio is an important parameter in plant physiology that can influence photosynthesis. The plant growth phase is affected by the C/N ratio and can be used to predict plant growth and development (Elser et al., 2010). Lower N in organic or inorganic fertilizers and higher P or K fertilizers applied will shift the C/N ratio and promote morphological changes (Coruzzi and Bush, 2001; Coruzzi and Zhou, 2001; Zheng, 2009), as well as total flavonoids production (Xu et al., 2018).

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Table 4. N, P, and K of *Kaempferia angustifolia* leaves with different organic and inorganic fertilizer applications

Treatments	Leaf N (%)	Leaf P (%)	Leaf K (%)
Main plot: Organic fertilizer			
Chicken manure	2.44	0.61	4.75
Cow manure	2.51	0.59	3.96
Subplot: Inorganic Fertilizer			
Control (without fertilizers)	2.83 a	0.48	3.28
200 kg.ha^{-1} SP36	2.47b-d	0.65	3.96
200 kg.ha^{-1} SP36 + 100 kg.ha^{-1} KCl	2.50b-d	0.60	3.77
200 kg.ha^{-1} SP36 + 200 kg.ha^{-1} KCl	2.45b-d	0.60	4.40
200 kg.ha^{-1} SP36 + 300 kg.ha^{-1} KCl	2.30 d	0.94	4.80
200 kg.ha^{-1} SP36 + 400 kg.ha^{-1} KCl	2.43b-d	0.57	5.02
100 kg.ha^{-1} urea + 200 kg.ha^{-1} SP36 + 100 kg.ha^{-1} KCl	2.58 bc	0.63	4.24
100 kg.ha^{-1} urea + 200 kg.ha^{-1} SP36 + 200 kg.ha^{-1} KCl	2.41b-d	0.57	4.67
100 kg.ha^{-1} urea + 200 kg.ha^{-1} SP36 + 300 kg.ha^{-1} KCl	2.52b-d	0.51	4.15
100 kg.ha^{-1} urea + 200 kg.ha^{-1} SP36 + 400 kg.ha^{-1} KCl	2.46b-d	0.57	4.87
400 kg.ha^{-1} SP36	2.62 b	0.65	3.82
400 kg.ha^{-1} SP36 + 100 kg.ha^{-1} KCl	2.39 cd	0.61	4.32
400 kg.ha^{-1} SP36 + 200 kg.ha^{-1} KCl	2.53b-d	0.60	4.46
400 kg.ha^{-1} SP36 + 300 kg.ha^{-1} KCl	2.44b-d	0.61	4.59
400 kg.ha^{-1} SP36 + 400 kg.ha^{-1} KCl	2.36 cd	0.58	4.68
100 kg.ha^{-1} urea + 400 kg.ha^{-1} SP36 + 100 kg.ha^{-1} KCl	2.52b-d	0.61	4.17
100 kg.ha^{-1} urea + 400 kg.ha^{-1} SP36 + 200 kg.ha^{-1} KCl	2.39 cd	0.57	4.29
100 kg.ha^{-1} urea + 400 kg.ha^{-1} SP36 + 300 kg.ha^{-1} KCl	2.38 cd	0.53	4.31
100 kg.ha^{-1} urea + 400 kg.ha^{-1} SP36 + 400 kg.ha^{-1} KCl	2.41b-d	0.53	4.57
Interaction of organic vs inorganic fertilizer		ns	

Notes: Mean values within a column followed by different letters are not significantly different at P < 0.05 using Duncan's Multiple Range Test. [1)] =Data are transformed with √x+0.5; ns = non-significant.

The optimal NPK fertilizer ratio for rhizome production in *K. angustifolia* may vary depending on several factors, such as soil fertility, environmental conditions, and cultivation practices. Balanced fertilization with appropriate ratios of NPK can enhance the growth and yield of *K. angustifolia* rhizomes (Tables 1 and 2). The balance of NPK fertilizer application affected the plant products such as shallot (Mato et al., 2022), *Atractylodes chinensis* (Sun et al., 2022), *Kaempferia galanga* (Pal, 2002; Subaryanti et al., 2020).

Total flavonoids in plants without inorganic fertilizer application are significantly higher than those with inorganic fertilizer application, as shown in Table 3. The plants appeared stressed and exhibited less shoot growth than those receiving the inorganic fertilizer application at 3 MAP. In contrast, better shoot and plant growth were achieved with inorganic fertilizer application, which promoted more shoots and tillers, and showed lower total flavonoid concentrations. In this case, the increasing total flavonoid functions as a plant defense system during stress of no inorganic fertilizer input (Agati et al., 2020; Moradzadeh et al., 2021; Wahyuni et al., 2021). The results of this study can be helpful for researchers worldwide, particularly those working in the fields of medicinal plants, sustainable agriculture, and soil fertility management. The findings may also be applicable to other agroecological regions with similar conditions, such as those in tropical and subtropical climates in Asia, Africa, and Latin America, where soil fertility limitations often necessitate the use of organic amendments. Regions with acidic, low-fertility soils, like latosols in Indonesia, Amazonian oxisols, and lateritic soils in India and West Africa, could benefit from similar organic and inorganic fertilizer applications (Hlisnikovský et al., 2021; Alotaibi and Abd-Elgawad, 2023). The study provides valuable guidance on balancing organic and inorganic fertilizers for medicinal plant production, ensuring sustainable

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yield improvement while maintaining soil health. Given the increasing demand for natural antioxidants and functional foods, optimizing flavonoid production through fertilization strategies aligns with global trends in nutraceutical and pharmaceutical research (Sun et al., 2022).

These findings suggest that alternative organic fertilizers with properties similar to those of chicken manure could be evaluated for medicinal plant cultivation worldwide, such as poultry litter with a comparable nutrient profile, which can serve as a slow-release organic fertilizer (Bhatt et al., 2019). Guano (bat or bird manure) has high NPK, particularly phosphorus, which is beneficial for flavonoid accumulation (Anwar et al., 2015). In contrast, vermicompost (worm castings) enhances soil microbial diversity and improves nutrient availability, similar to cow manure (Hlisnikovský et al., 2021). Another study reported that composted food waste is high in organic matter and nutrients, comparable to cow manure, but decomposes faster (Alotaibi and Abd-Elgawad, 2023).

Conclusions

Kaempferia angustifolia treated with 10 kg ha^{-1} chicken manure has significantly higher fresh weight than those treated with cow manure. Fertilization with 400 kg.ha^{-1} SP36 + 400 kg.ha^{-1} KCl promoted the highest number of shoots and tillers. The total flavonoids from inorganic fertilizer applications are 39.30-131.51% higher; the highest was those treated with 200 kg.ha^{-1} SP36 + 200 kg.ha^{-1} KCl, i.e., 8.971 µg QE eq. per g of plant dry weight, compared to the control of 3.875 µg QUE eq per g of plant dry weight.

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