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Enhancing growth and flavonoid content of *Eleutherine palmifolia* using chitosan and NPK fertilizer under greenhouse conditions

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Abstract. *Rochma FA, Mudyantini W, Solichatun. 2025. Enhancing growth and flavonoid content of* Eleutherine palmifolia *using chitosan and NPK fertilizer under greenhouse conditions. Cell Biol Dev 9: 26-36.* This study evaluated the effects of chitosan and NPK fertilizer on the growth performance, physiological traits, and flavonoid content of *Eleutherine palmifolia*, a medicinal plant widely used in Indonesia. A factorial experiment was conducted under greenhouse conditions using three chitosan concentrations (0, 0.3%, and 0.6%) and three levels of NPK fertilizer (0, 100, and 200 kg/ha). The combination of 0.6% chitosan and 200 kg/ha NPK significantly enhanced leaf number, leaf size, shoot biomass, chlorophyll and carotenoid contents, as well as flavonoid concentration in the tubers. While flowering occurred in selected treatments, overall reproductive development remained limited during the 11-week cultivation period. Notably, the highest total flavonoid content in tuber extracts (6.30 mg QE/g) was recorded under the same treatment, highlighting the potential role of chitosan as a biostimulant and elicitor of secondary metabolites. Although some parameters showed non-significant interaction effects, consistent positive trends support the hypothesis that chitosan improves nutrient uptake and physiological vigor, particularly when combined with adequate macronutrients. This study emphasizes the potential of integrating biostimulant and fertilizer treatments to enhance both agronomic performance and phytochemical accumulation in *E. palmifolia*. These findings provide a foundation for sustainable cultivation practices aimed at increasing the functional quality of this underutilized medicinal plant for future pharmaceutical or nutraceutical applications.

Keywords: Biostimulant, chitosan, *Eleutherine palmifolia*, flavonoids, NPK fertilizer

INTRODUCTION

Eleutherine palmifolia (L.) Merr., commonly known as Dayak onion, is a traditional medicinal plant in the Iridaceae family, extensively utilized by the Dayak ethnic group in Kalimantan, Indonesia. This species is widely applied in folk medicine for treating various ailments, including cardiovascular diseases, tumors, and inflammatory conditions (Kuntorini and Nugroho 2010; Paramita and Nuryanto 2018). Its pharmacological value is attributed to a diverse phytochemical profile, comprising flavonoids, alkaloids, saponins, tannins, and other secondary metabolites (Tamal and Aryanto 2020). Notably, its tubers and leaves exhibit high flavonoid levels, with values reported up to 116.56 mg QE/g extract (Yuswi 2017), which contribute to antioxidant, anti-inflammatory, antihypertensive, and antimicrobia effects (Ferreyra et al. 2012; Ibrahim 2012; Prayitno and Murtini 2018).

Despite this therapeutic potential and rising public interest, large-scale cultivation of *E. palmifolia* remains underdeveloped in Indonesia. The mismatch between demand and supply is mainly due to the absence of standardized cultivation protocols that optimize both biomass production and the accumulation of bioactive compounds (Sari et al. 2020; Atikah et al. 2021). Hence, targeted agronomic interventions are urgently needed to enhance both yield and quality. One promising strategy is the application of biostimulants, such as chitosan, in conjunction with essential macronutrient fertilizers like NPK.

Biostimulants are non-nutritional substances that promote plant growth, improve physiological responses, and enhance stress tolerance. Among them, chitosan, a biopolymer derived from the deacetylation of chitin has garnered significant attention due to its multifunctional properties. It can enhance nutrient uptake, induce systemic resistance, and stimulate secondary metabolite production (Saharan and Pal 2016; Lalla 2022). Chitosan has also been shown to promote the biosynthesis of endogenous hormones such as auxins and gibberellins, which are crucial for vegetative growth and metabolic coordination (Moza et al. 2017; Ingle et al. 2022). Furthermore, it acts as a metabolic elicitor by activating defense-related pathways and increasing flavonoid and phenolic compound accumulation (Chadchawan et al. 2015; Singh and Singla 2020). Supporting this, Suci (2020) demonstrated increased flavonoid synthesis in *Schleichera oleosa* callus cultures, while Nuraini et al. (2017) reported higher tuber weights in potatoes following chitosan treatment at 0.3-0.6%.

In parallel, macronutrient fertilization is a cornerstone of plant productivity. NPK fertilizers, which supply nitrogen (N), phosphorus (P), and potassium (K), are essential for protein synthesis, energy metabolism, and osmotic regulation, respectively (Manurund and Zahrah 2018; Mutua et al. 2021). Beyond vegetative growth, these nutrients also modulate key secondary metabolic pathways. Specifically,

phosphorus and potassium have been linked to enhanced flavonoid and carotenoid biosynthesis (Fanciullino et al. 2014; Cocozza et al. 2020). In *E. palmifolia*, Rosmawaty et al. (2019) reported improved tuber yield and flavonoid concentration following NPK application, while Sumarni et al. (2012) found similar effects in *Allium ascalonicum*, a crop with comparable morphology and organ use.

The interaction between chitosan and NPK has recently emerged as an important area of research in plant physiology. Chitosan is known to facilitate nutrient assimilation by reducing leaching, enhancing root absorption, and serving as a slow-release carrier when formulated in nano-size particles (Duhan et al. 2017; Perez-de-Luque 2017). Studies on crops like cucumber and wheat have shown that chitosan-NPK combinations improve nutrient use efficiency, biomass production, and metabolic output (Abdel-Aziz et al. 2016; Modi et al. 2021). These findings suggest that an integrated chitosan-NPK strategy may be highly beneficial for medicinal plants, including *E. palmifolia*.

However, research on the agronomic responses of *E. palmifolia* to chitosan and NPK particularly in combination —remains limited. Although the phytochemistry and medicinal benefits of the plant are well documented, there is a lack of experimental data evaluating cultivation strategies that simultaneously enhance both vegetative traits and secondary metabolite accumulation.

This study was therefore conducted to investigate the separate and combined effects of chitosan and NPK fertilizer on the growth performance and flavonoid content of *E. palmifolia*. The central hypothesis is that both treatments will enhance vegetative growth and secondary metabolism, and that their interaction will yield synergistic effects. The outcomes of this research are expected to contribute to the development of sustainable and efficient cultivation practices for this underutilized yet valuable medicinal crop.

MATERIALS AND METHODS

Study site and duration

The experiment was conducted at the Integrated Greenhouse Facility of Universitas Sebelas Maret, Surakarta, Central Java, Indonesia (7°33′20″S, 110°50′30″E; ~95 m above sea level). The greenhouse provides a semi-controlled environment with partial exposure to natural sunlight, suitable for experimental cultivation of medicinal plants under tropical monsoon conditions. The site experiences an average daily temperature of 27-32°C and relative humidity ranging from 60% to 85%.

The study was carried out over an 11-week period, from May to July 2023, covering the vegetative and early generative stages of *E. palmifolia*. Daily maintenance activities, including irrigation, random repositioning of plant bags, and basic environmental monitoring, were consistently implemented.

Treatment applications including chitosan and NPK fertilizer were administered during the early vegetative phase, specifically at 2 and 6 weeks after planting (WAP), as described in Section 2.4. Harvest and final observations were conducted at the end of the 11th week.

Plant material and growth medium

Uniform tubers of *E. palmifolia* were used as planting material in this experiment. The tubers were sourced from Pasir Besar Village, South Pontianak District, West Kalimantan, Indonesia. To minimize initial growth variation, only healthy, disease-free tubers of uniform physiological age and weighing between 7 and 12 g were selected.

The planting medium consisted of a homogeneous mixture of topsoil, manure, and rice husk in a 2:1:1 (v/v/v) ratio. This substrate was selected to provide adequate drainage, sufficient organic matter, and balanced nutrient availability. The mixture was thoroughly blended and packed into polyethylene planting bags (25 × 25 cm), each containing approximately 3.5 kg of growing media.

Prior to transplanting, the field capacity of the planting medium was determined using the gravimetric method described by Patoni (2000). The calculation was based on the difference in weight before and after full saturation:

$$\text{Field Capacity} = (W_{\text{wet}} - W_{\text{dry}})$$

Where:

Wwet : the weight of the polybag at saturation

Wdry : the weight before watering.

All tubers were directly transplanted into polybags and arranged randomly within the greenhouse. Plants were irrigated every two days using a volume of water adjusted to maintain field capacity. To reduce microclimatic variability, the position of each polybag was rotated weekly throughout the 11-week cultivation period.

Chitosan and NPK fertilizer preparation

Chitosan used in this study was obtained from E. Merck (Germany) with a degree of deacetylation of 95%. Two concentrations were prepared: 0.3% and 0.6% (w/v), by dissolving 0.3 g or 0.6 g of chitosan powder in 100 mL of 1% (v/v) acetic acid. The mixtures were stirred continuously at room temperature until fully dissolved, yielding homogeneous solutions. Prepared chitosan solutions were stored at room temperature and used within 24 hours to preserve their physicochemical stability and bioactivity.

NPK compound fertilizer with a nutrient composition of 16:16:16 (N:P:K) was commercially obtained under the brand name *Mutiara*. Fertilizer application rates were based on field-equivalent doses of 0 kg/ha (control), 100 kg/ha, and 200 kg/ha, which were converted to 0.104 g and 0.208 g per polybag, respectively. The appropriate amount of fertilizer was dissolved in distilled water immediately before application.

For combined treatments, chitosan and NPK solutions were mixed just before use to ensure physical compatibility and uniform delivery. All treatments including chitosan-only, NPK-only, and chitosan + NPK combinations—were applied twice during the vegetative stage, at 2 and 6 weeks after planting (WAP). Each plant received 100 mL of the respective solution per application, applied directly to the soil surface near the root zone to optimize nutrient and elicitor uptake. Control plants were treated with 100 mL of distilled water on the same schedule.

Experimental design and treatment application

The experiment was conducted using a Completely Randomized Design (CRD) with a factorial arrangement involving two factors: chitosan concentration and NPK fertilizer dose. Each factor consisted of three levels chitosan at 0% (control), 0.3%, and 0.6%; and NPK fertilizer at 0, 100, and 200 kg/ha (equivalent to 0, 0.104, and 0.208 g per polybag, respectively). The combination of these factors resulted in nine treatment groups, each replicated five times, yielding a total of 45 experimental units.

Uniform tubers of *E. palmifolia* were transplanted into polybags containing the pre-prepared soil medium. The polybags were arranged randomly in the greenhouse and re-randomized weekly to reduce the effect of microclimatic heterogeneity.

Treatment solutions were applied via root-zone drenching using 100 mL per plant. Applications were conducted twice, at 2 and 6 weeks after planting (WAP), during the vegetative growth phase. For combined treatments, chitosan and NPK solutions were mixed immediately prior to application to ensure homogeneity. Control plants received the same volume (100 mL) of distilled water on the same schedule.

Irrigation was provided every two days, with water volumes adjusted to maintain substrate moisture near field capacity. All other cultivation practices, including weeding and environmental maintenance, were applied uniformly across treatments. At harvest (week 11), data collection was performed on morphological, physiological, and biochemical parameters, as detailed in subsequent sections.

Growth and morphological measurements

The growth performance of *E. palmifolia* was evaluated using a set of morphological parameters measured at the end of the 11-week cultivation period. The assessed variables included number of leaves, leaf length, leaf width, and number of flowers per plant, serving as indicators of vegetative vigor and transition toward the generative phase. Leaf and flower counts were recorded manually, while leaf length and width were measured using a ruler, focusing on the longest and widest fully expanded leaves on each plant.

To complement aboveground measurements, fresh and dry biomass of both leaves and tubers were also recorded. Fresh weights were determined immediately after harvest using a calibrated analytical balance. For dry weight measurement, samples were oven-dried at 50°C for 72 hours or until constant weight was achieved. The difference between fresh and dry weights was used to calculate relative water content and to evaluate biomass allocation between shoot and root organs.

All measurements were conducted on individual plants within each replicate, and mean values were calculated per treatment group. These morphological assessments provided a comprehensive understanding of vegetative growth, early flowering tendencies, and biomass accumulation of *E. palmifolia* under different combinations of chitosan and NPK fertilizer treatments.

Chlorophyll and carotenoid analysis

Leaf pigment analysis was performed to quantify total chlorophyll and carotenoid contents in *E. palmifolia*. Chlorophyll extraction followed the acetone-based spectrophotometric method described by Hendry and Grime (1993). A total of 0.1 g of fresh leaf tissue was homogenized in 10 mL of 80% acetone using a mortar and pestle. The resulting homogenate was filtered through Whatman No. 42 filter paper, and the filtrate was collected in clean test tubes for analysis.

Spectrophotometric absorbance was measured at 645 nm, 663 nm, and 480 nm using a UV-Vis spectrophotometer (Perkin Elmer Lambda 25 series). An 80% acetone solution was used as the blank. Chlorophyll a, chlorophyll b, and total chlorophyll contents (expressed in mg/g fresh weight) were calculated using standard equations:

$$\text{Chlorophyll a} = [12.7 \times A_{663} - 2.69 \times A_{645}] \times \frac{V}{1000 \times W}$$

$$\text{Chlorophyll b} = [22.9 \times A_{645} - 4.68 \times A_{663}] \times \frac{V}{1000 \times W}$$

$$\text{Total chlorophyll} = [8.02 \times A_{663} + 20.2 \times A_{645}] \times \frac{V}{1000 \times W}$$

$$\text{Carotenoids } (\mu\text{mol/g}) = \frac{(A_{480} + A_{645} - A_{663}) \times V}{1000 \times W}$$

Where:

A_λ : absorbance at wavelength λ

V : volume of extract (mL)

W : fresh weight of sample (g)

These analyses provided quantitative estimates of photosynthetic pigment concentrations, offering insights into the physiological status of plants in response to different chitosan and NPK fertilizer treatments.

Flavonoid content determination

Total flavonoid content in tubers of *E. palmifolia* was quantified using a colorimetric method based on aluminum chloride ($AlCl_3$) complexation, following the protocol of Stankovic (2011). Approximately 1.0 g of oven-dried tuber powder was extracted by maceration in 10 mL of 96% ethanol (p.a. grade) for 24 hours at room temperature. The resulting extract was filtered through Whatman No. 42 filter paper, and the residue was subjected to a second maceration with another 10 mL of ethanol for 2 hours, until the filtrate became colorless. Both filtrates were pooled and evaporated at room temperature to yield the crude ethanol extract.

For analysis, a 1 mL aliquot of the extract was mixed with 1 mL of 2% (w/v) $AlCl_3$ and 1 mL of 120 mM potassium acetate. The mixture was incubated at room temperature for 60 minutes, after which the absorbance was measured at 435 nm using a UV-Vis spectrophotometer. Flavonoid concentration was determined from a quercetin standard calibration curve prepared at 6, 8, 10, 12, and 14 ppm, with all standards undergoing the same treatment as the sample.

The total flavonoid content was expressed as milligrams of quercetin equivalent per gram of dry tuber (mg QE/g), using the following formula:

$$\text{Flavonoid content} = \frac{C \times V \times fp}{m}$$

Where:

C : concentration of quercetin (mg/mL) based on absorbance

V : total volume of extract (mL)

fp : dilution factor

m : dry weight of the sample (g)

This method enabled reliable estimation of flavonoid accumulation as influenced by the chitosan and NPK fertilizer treatments.

Data analysis

All quantitative data derived from morphological, physiological, and biochemical observations were processed and analyzed using IBM SPSS Statistics version 26. A Two-Way Analysis of Variance (ANOVA) was conducted to assess the main effects and interaction between chitosan concentration and NPK fertilizer dosage for each measured parameter. When the ANOVA indicated statistically significant differences ($p<0.05$), Duncan's Multiple Range Test (DMRT) was applied at a 5% significance level to determine pairwise differences among treatment means.

Prior to analysis, data were tested for normality and homogeneity of variance to meet the assumptions of parametric tests. Results are expressed as mean ± Standard Deviation (SD), and statistically significant differences among treatments are denoted by different superscript letters in tables or figure legends, where appropriate.

RESULTS AND DISCUSSION

Effect of chitosan and NPK fertilizer on leaf number

The application of chitosan and NPK fertilizer influenced the number of leaves produced by *E. palmifolia* at harvest. As shown in Table 1, plants treated with higher levels of NPK generally produced more leaves compared to the untreated control. The highest leaf number was observed in the treatment combination of 0% chitosan and 200 kg/ha NPK (K0N2), averaging 18.60 ± 5.90 leaves per plant. Conversely, the lowest value was recorded in the 0.3% chitosan and 0 kg/ha NPK treatment (K1N0), with only 7.80 ± 2.28 leaves per plant.

Although the interaction between chitosan and NPK was statistically significant ($p<0.05$), differences in leaf number across treatment combinations were not consistently significant, as indicated by overlapping superscript letters. While chitosan at 0.3% tended to reduce leaf number in the absence of fertilizer, the application of NPK improved leaf production across all chitosan levels. Treatment with 0.6% chitosan generally resulted in higher leaf numbers under increased NPK levels, particularly in K2N2 (16.40 ± 5.88), although this was slightly lower than the value observed in K0N2.

These trends suggest a modest but observable dose-dependent interaction between biostimulant and fertilizer application. Chitosan is known to enhance nitrogen metabolism by activating enzymes such as nitrate reductase and glutamine synthetase, which play critical roles in vegetative development (Gornik et al. 2008). Simultaneously, NPK fertilizer supplies essential macronutrients that promote meristematic activity and cell expansion in developing leaves (Parvin et al. 2019). Together, these results indicate that the combined use of chitosan and NPK fertilizer can support improved vegetative growth in *E. palmifolia*, although the magnitude of response is influenced by the specific treatment combination.

Leaf length response to treatment combinations

The leaf length of *E. palmifolia* varied in response to different combinations of chitosan and NPK fertilizer. As shown in Table 2, the longest average leaf length (51.04 ± 2.05 cm) was recorded in the K1N2 treatment (0.3% chitosan with 200 kg/ha NPK), whereas the shortest leaves were observed in K0N1 (0% chitosan with 100 kg/ha NPK), with a mean of 33.50 ± 8.12 cm.

Statistical analysis indicated a significant interaction between chitosan and NPK treatments ($p<0.05$), suggesting that the impact of chitosan on leaf elongation is dependent on nutrient availability. Interestingly, all treatments with 200 kg/ha NPK yielded mean leaf lengths exceeding 50 cm, regardless of chitosan concentration, implying the presence of a nutrient threshold that supports maximum elongation.

The elongation observed may be due to enhanced nutrient uptake and hormone signaling triggered by chitosan, especially in pathways involving auxins and gibberellins (Ingle et al. 2022). Meanwhile, nitrogen supplied through NPK plays a fundamental role in promoting cell elongation and division, thereby facilitating leaf blade development (Sun et al. 2022). These results highlight that under optimal fertilization, the presence of chitosan can further amplify vegetative parameters such as leaf length, although further studies are needed to verify synergism.

Table 1. Number of leaves (mean ± SD) of *Eleutherine palmifolia* after treatment with chitosan and NPK fertilizer

Chitosan (%)	NPK 0 kg/ha	NPK 100 kg/ha	NPK 200 kg/ha
0.0	14.20 ± 6.22^{b}	15.40 ± 6.95^{a}	18.60 ± 5.90^{a}
0.3	7.80 ± 2.28^{a}	11.00 ± 5.00^{a}	12.80 ± 3.35^{a}
0.6	11.80 ± 3.70^{ab}	13.00 ± 4.80^{a}	16.40 ± 5.88^{a}

Note: Different superscript letters in the same column indicate significant differences at $p<0.05$ (DMRT)

Table 2. Leaf length (mean ± SD, in cm) of *Eleutherine palmifolia* after chitosan and NPK fertilizer treatments

Chitosan (%)	NPK 0 kg/ha	NPK 100 kg/ha	NPK 200 kg/ha
0.0	38.40 ± 8.39^{a}	33.50 ± 8.12^{a}	50.60 ± 4.38^{a}
0.3	49.58 ± 2.58^{b}	47.04 ± 4.45^{b}	51.04 ± 2.05^{a}
0.6	44.16 ± 7.15^{a}	45.74 ± 3.86^{b}	50.24 ± 4.88^{a}

Note: Different superscript letters in the same column indicate significant differences at $p<0.05$ (DMRT)

Variation in leaf width under chitosan and NPK application

Leaf width in *E. palmifolia* was also influenced by the interaction between chitosan and NPK fertilizer. As shown in Table 3, the widest leaves were observed in the treatment combination of 0.3% chitosan and 200 kg/ha NPK (K1N2), with a mean width of 2.94 ± 0.42 cm, followed closely by the 0.6% chitosan and 200 kg/ha NPK (K2N2) treatment (2.78 ± 0.32 cm). The narrowest leaves were recorded in the group receiving 0% chitosan and 100 kg/ha NPK (K0N1), which averaged only 1.82 ± 0.30 cm.

Statistical analysis confirmed a significant interaction between chitosan and NPK fertilizer ($p<0.05$), indicating that leaf width expansion was influenced by their combined application. The application of chitosan at both 0.3% and 0.6% concentrations enhanced leaf width more effectively when accompanied by higher NPK doses.

The increased width may be due to chitosan-induced expression of genes associated with photosynthesis and hormonal regulation (Landi et al. 2017). At the same time, the presence of essential macronutrients particularly potassium and phosphorus likely supported turgor maintenance and enhanced cell expansion, contributing to broader leaf blades (Yamika et al. 2021).

Flower emergence and flowering speed in response to treatment

The transition of *E. palmifolia* from the vegetative to the generative phase was assessed through the observation of flower emergence and flower count per plant. As shown in Figure 1, flowering occurred in only four out of the nine treatment combinations during the 11-week cultivation period. The treatment K2N2 (0.6% chitosan and 200 kg/ha NPK) recorded the highest average number of flowers (2.6 per plant), followed by K2N0, K1N2, and K0N2. In contrast, the other five treatments showed no flowering response by the end of the study.

The onset of flowering ranged from day 74 to 77 after sowing. Although these findings suggest that higher chitosan and NPK levels might promote earlier or more frequent flowering, statistical analysis revealed no significant differences among treatments ($p>0.05$). Therefore, the flowering pattern should be interpreted with caution and considered a supplementary outcome rather than a primary indicator of treatment effectiveness.

Since the tuber is the main organ of economic interest in *E. palmifolia*, floral traits such as flower number and timing hold limited agronomic relevance. Furthermore, floral emergence did not exhibit strong correlations with vegetative traits (e.g., leaf number) or final tuber yield. Thus, while biologically notable, further studies under extended cultivation periods would be needed to confirm any consistent flowering response.

Fresh leaf biomass accumulation across treatments

The accumulation of fresh leaf biomass in *E. palmifolia* was influenced by the combined application of chitosan and NPK fertilizer. As presented in Table 4, the highest fresh leaf weight was recorded in the K2N2 treatment (0.6% chitosan and 200 kg/ha NPK), averaging 9.07 ± 2.73 g per plant. In contrast, the lowest value was observed in the K1N0 treatment (0.3% chitosan without NPK), which averaged only 3.95 ± 2.37 g.

Table 3. Leaf width (mean ± SD, in cm) of *E. palmifolia* following chitosan and NPK fertilizer treatments

Chitosan (%)	NPK 0 kg/ha	NPK 100 kg/ha	NPK 200 kg/ha
0.0	2.20 ± 0.65[a]	1.82 ± 0.30[a]	2.31 ± 0.51[a]
0.3	2.52 ± 0.13[a]	2.68 ± 0.26[b]	2.94 ± 0.42[b]
0.6	2.28 ± 0.45[a]	2.60 ± 0.29[b]	2.78 ± 0.32[ab]

Note: Different superscript letters in the same column indicate significant differences at $p<0.05$ (DMRT)

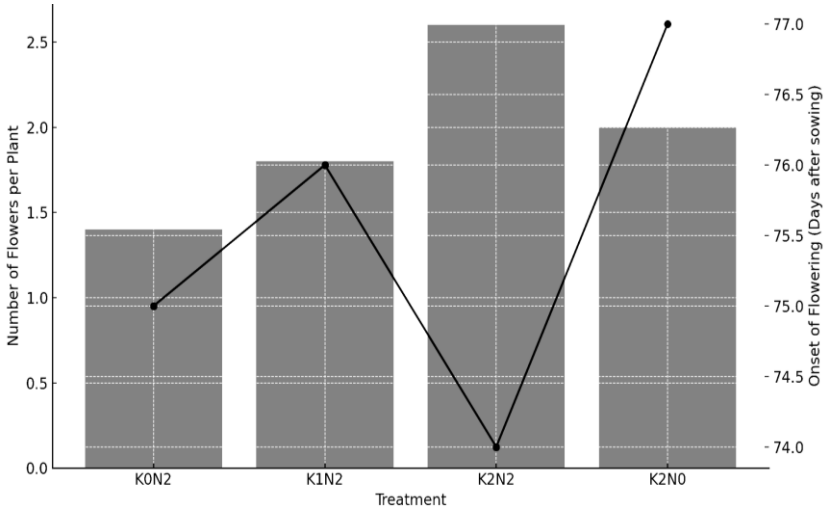

Figure 1. Number of flowers per plant and onset of flowering in *E. palmifolia* under various chitosan and NPK fertilizer treatments

Although statistical analysis did not reveal a significant interaction effect between chitosan and NPK fertilizer ($p>0.05$), a general trend was observed wherein higher doses of both inputs were associated with increased fresh leaf biomass. This pattern aligns with the established role of NPK macronutrients in supporting vegetative growth particularly nitrogen, which is crucial for chlorophyll synthesis, protein production, and cell expansion (Haryadi et al. 2015; Huang et al. 2019).

Chitosan may also contribute by enhancing nutrient absorption and water retention through improved membrane permeability and root function (Perez-de-Luque 2017). Although the differences were not statistically significant, the observed trends highlight the potential benefits of integrating biostimulants with conventional fertilizers. Additional studies with longer cultivation durations or increased replication may help clarify the extent of these effects.

Dry leaf biomass response to chitosan and NPK levels

Dry leaf weight is a key parameter indicating the accumulation of structural biomass and net photosynthate allocation in *E. palmifolia*. Based on the results in Table 5, the highest dry leaf weight was recorded in the K2N2 treatment (0.6% chitosan and 200 kg/ha NPK), with a mean of 2.04 ± 0.95 g per plant, while the lowest value was found in K1N0 (0.3% chitosan and 0 kg/ha NPK), averaging 1.02 ± 0.27 g.

Despite these differences, the statistical analysis did not show a significant interaction effect between chitosan and NPK fertilizer ($p>0.05$). The variation in dry biomass might be influenced by inconsistent rates of moisture loss during oven drying or by the diversion of photosynthates toward tuber development during later growth stages.

Nevertheless, a general increasing trend was observed with higher chitosan and NPK levels, particularly at 0.6% chitosan. Chitosan has been associated with enhanced photosynthetic enzyme activity and chloroplast integrity, which may contribute to greater dry matter accumulation (El-Miniawy et al. 2014; Acemi et al. 2021). Meanwhile, NPK fertilization supports cell wall thickening, structural protein synthesis, and other metabolic processes linked to dry biomass formation (Nuryani et al. 2019).

Fresh tuber weight as affected by treatment combinations

Fresh tuber weight is an important indicator of economic yield in *E. palmifolia*. As shown in Table 6, the highest average fresh tuber weight was obtained from the K2N2 treatment (0.6% chitosan and 200 kg/ha NPK), reaching 2.46 ± 0.95 g per plant. In contrast, the lowest value was recorded in the K1N0 treatment (0.3% chitosan and no NPK fertilizer), with a mean of 0.84 ± 0.37 g.

Statistical analysis revealed that only chitosan had a significant effect on fresh tuber weight ($p<0.05$), while the effects of NPK and the interaction between the two factors were not significant. This indicates that the observed increase in fresh tuber yield was mainly influenced by chitosan application. Notably, even in the absence of NPK, the 0.6% chitosan treatment (K2N0) still showed better results than several combinations that included fertilizer.

The stimulatory effect of chitosan on fresh tuber weight may be related to its role in improving root membrane permeability, enhancing nutrient absorption, and facilitating water retention, which collectively promote assimilate translocation to underground storage tissues (Duhan et al. 2017; Nuraini et al. 2019). Although NPK application alone did not produce significant effects, a general upward trend in fresh tuber weight with increasing fertilizer dose suggests potential additive benefits in longer-term or field-scale studies.

Dry tuber biomass and its variability among treatments

Dry tuber weight is considered a stable metric for evaluating storage biomass in *E. palmifolia*, as it reflects net assimilate accumulation after moisture removal. As shown in Table 7, the highest dry tuber weight was recorded in the K2N2 treatment (0.6% chitosan and 200 kg/ha NPK), averaging 2.46 ± 0.87 g per plant, followed by K2N1 and K0N1. The lowest value was found in the K0N0 group, with a mean of 2.04 ± 0.54 g.

Despite numerical variation, statistical analysis indicated no significant differences ($p>0.05$) among treatment combinations. This suggests that neither chitosan nor NPK fertilizer significantly influenced dry tuber weight under the current experimental conditions. Nonetheless, a consistent trend of increasing dry weight with higher chitosan concentrations especially when combined with moderate to high NPK doses was apparent.

Table 4. Fresh leaf weight (mean ± SD, in grams) of *E. palmifolia* after chitosan and NPK fertilizer treatments

Chitosan (%)	NPK 0 kg/ha	NPK 100 kg/ha	NPK 200 kg/ha
0.0	5.27 ± 1.30	6.57 ± 2.94	8.28 ± 2.37
0.3	3.95 ± 2.37	6.72 ± 3.04	7.26 ± 2.48
0.6	7.18 ± 1.26	5.95 ± 1.84	9.07 ± 2.73

Table 5. Dry leaf weight (mean ± SD, in grams) of *E. palmifolia* after chitosan and NPK fertilizer treatments

Chitosan (%)	NPK 0 kg/ha	NPK 100 kg/ha	NPK 200 kg/ha
0.0	1.15 ± 0.57	1.34 ± 0.47	1.60 ± 0.60
0.3	1.02 ± 0.27	1.23 ± 0.41	1.52 ± 0.64
0.6	1.71 ± 0.83	1.22 ± 0.48	2.04 ± 0.95

Table 6. Fresh tuber weight (mean ± SD, in grams) of *E. palmifolia* after chitosan and NPK fertilizer treatments

Chitosan (%)	NPK 0 kg/ha	NPK 100 kg/ha	NPK 200 kg/ha
00.00	1.21 ± 0.53^{ab}	1.47 ± 0.72^{a}	2.01 ± 0.84^{a}
00.03	0.84 ± 0.37^{a}	1.08 ± 0.39^{a}	1.15 ± 0.99^{a}
00.06	1.72 ± 0.54^{b}	2.16 ± 1.58^{a}	2.46 ± 0.95^{a}

Note: Different superscript letters indicate significant differences at $p<0.05$ (DMRT)

The absence of statistical significance may be attributed to several factors, including variability in drying rates, limited experimental duration, or diversion of assimilates to reproductive or foliar tissues. While the fresh weight showed clearer distinctions, the dry weight data suggest that chitosan primarily enhanced water retention and physiological activity rather than biomass structure alone.

Total chlorophyll content in leaves under chitosan and NPK treatment

Total chlorophyll content in the leaves of *E. palmifolia* exhibited notable variation in response to chitosan and NPK fertilizer treatments. As shown in Table 8, the highest chlorophyll level was observed in the K2N2 treatment (0.6% chitosan and 200 kg/ha NPK), reaching 1.79 ± 0.98 mg/g, while the lowest was recorded in K0N1 (0% chitosan and 100 kg/ha NPK), with a mean of 0.64 ± 0.06 mg/g.

Statistical analysis confirmed a significant interaction effect ($p<0.05$) between chitosan and NPK fertilizer, indicating that chlorophyll content was influenced by the combined action of biostimulant and macronutrient inputs. Treatments with 0.3% and 0.6% chitosan generally showed elevated chlorophyll levels, particularly when combined with 100-200 kg/ha NPK.

This enhancement can be attributed to the positive effect of chitosan on chloroplast development and nutrient absorption, including nitrogen and magnesium, which are critical for chlorophyll biosynthesis (Landi et al. 2017). Additionally, NPK fertilizer contributes essential building blocks for pigment formation and metabolic activity (Nur and Thohari 2007; Ebadi et al. 2024). These findings support the conclusion that combining chitosan and NPK enhances photosynthetic potential and overall plant vigor.

Carotenoid levels in leaves under different treatment combinations

Carotenoids are essential pigments involved in light harvesting and photoprotection in plant leaves. In *E. palmifolia*, carotenoid levels showed significant variation across the treatment groups. As presented in Table 9, the highest carotenoid content was observed in the K2N2 treatment (0.6% chitosan and 200 kg/ha NPK), reaching 21.04 ± 3.13 µmol/g, while the lowest was recorded in K0N1 (0% chitosan and 100 kg/ha NPK) with a mean of 4.47 ± 0.65 µmol/g.

A two-way ANOVA confirmed a significant interaction ($p<0.05$) between chitosan and NPK fertilizer, indicating that carotenoid accumulation was influenced by both factors. Across all NPK levels, carotenoid content increased consistently with rising chitosan concentration, demonstrating a clear dose-dependent trend. This trend was most pronounced at the highest fertilizer level (200 kg/ha NPK), where chitosan application resulted in sharp increases in carotenoid production.

The enhanced carotenoid synthesis can be attributed to the elicitor properties of chitosan, which are known to activate the isoprenoid and phenylpropanoid pathways, including the upregulation of key biosynthetic enzymes such as phytoene synthase (Fanciullino et al. 2014; Rahman et al. 2018). Meanwhile, NPK fertilizer provides essential precursors for energy metabolism and pigment synthesis, such as nitrogen and phosphorus. These findings emphasize the synergistic potential of combining biostimulants and macronutrients to enhance secondary metabolite accumulation in medicinal plants.

Total flavonoid content of tuber extracts

Flavonoids are important bioactive compounds with antioxidant, anti-inflammatory, and therapeutic properties, making their quantification a key component in medicinal plant research. In *E. palmifolia*, total flavonoid content in tuber ethanol extracts varied significantly across treatment combinations, as illustrated in Figure 2.

The highest flavonoid level was recorded in the K2N2 treatment (0.6% chitosan and 200 kg/ha NPK), reaching 6.30 mg quercetin equivalent (QE)/g dry tuber, followed by K2N1 (5.90 mg QE/g) and K2N0 (5.34 mg QE/g). The lowest flavonoid content was found in the control treatment (K0N0), with 3.45 mg QE/g.

Table 7. Dry tuber weight (mean ± SD, in grams) of *E. palmifolia* after chitosan and NPK fertilizer treatments

Chitosan (%)	NPK 0 kg/ha	NPK 100 kg/ha	NPK 200 kg/ha
0.0	2.04 ± 0.54	2.56 ± 0.26	2.07 ± 0.44
0.3	2.13 ± 0.55	2.15 ± 0.16	2.16 ± 0.53
0.6	2.09 ± 0.27	2.36 ± 0.48	2.46 ± 0.87

Note: No significant differences were detected among treatments (DMRT, $p>0.05$

Table 8. Total chlorophyll content (mean ± SD, in mg/g) of *E. palmifolia* leaves after chitosan and NPK fertilizer treatments

Chitosan (%)	NPK 0 kg/ha	NPK 100 kg/ha	NPK 200 kg/ha
0.0	0.74 ± 0.07^{a}	0.64 ± 0.06^{a}	0.64 ± 0.07^{a}
0.3	0.75 ± 0.14^{a}	1.52 ± 0.37^{b}	0.76 ± 0.27^{a}
0.6	0.89 ± 0.27^{a}	0.96 ± 0.35^{a}	1.79 ± 0.98^{b}

Note: Different superscript letters in the same column indicate significant differences at $p<0.05$ (DMRT)

Table 9. Carotenoid content (mean ± SD, in µmol/g) of *E. palmifolia* leaves after chitosan and NPK fertilizer treatments

Chitosan (%)	NPK 0 kg/ha	NPK 100 kg/ha	NPK 200 kg/ha
0.0	7.48 ± 1.00^{a}	4.47 ± 0.65^{a}	10.54 ± 0.85^{a}
0.3	10.60 ± 2.16^{ab}	12.54 ± 0.72^{b}	8.34 ± 2.61^{a}
0.6	13.56 ± 3.75^{b}	14.48 ± 5.81^{b}	21.04 ± 3.13^{b}

Note: Different superscript letters in the same column indicate significant differences at $p<0.05$ (DMRT)

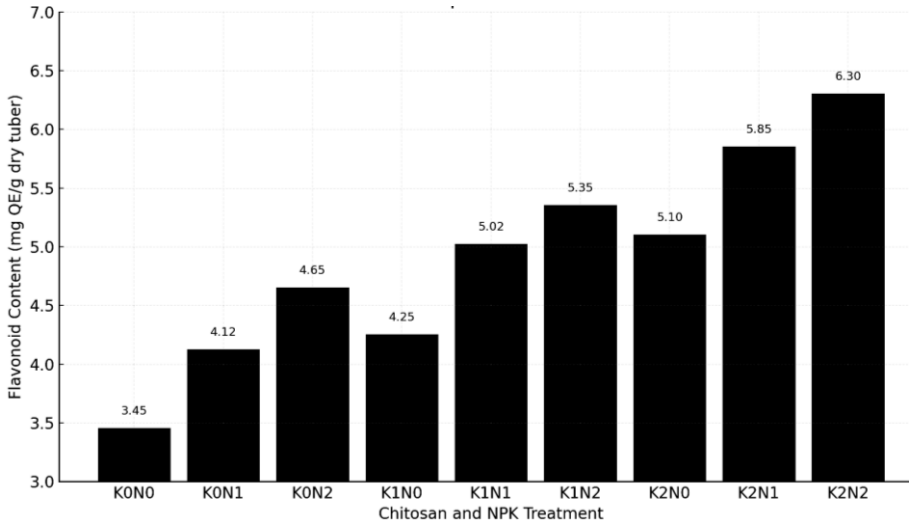

Figure 2. Total flavonoid content (mg quercetin equivalent/g dry tuber) of *E. palmifolia* under different combinations of chitosan and NPK fertilizer treatments

Statistical analysis revealed that chitosan concentration had a significant effect ($p<0.05$), while the effect of NPK and the interaction term were not statistically significant ($p>0.05$). Nevertheless, a consistent upward trend in flavonoid accumulation was observed with increasing levels of both chitosan and NPK, suggesting a possible additive response.

The increase in flavonoid content is likely linked to the elicitor activity of chitosan, which activates the phenylpropanoid pathway and stimulates the expression of biosynthetic enzymes such as Phenylalanine Ammonia-Lyase (PAL), Chalcone Synthase (CHS), and Flavonol Synthase (FLS) (Xing et al. 2015; Sathiyabama and Indhumathi 2022). The presence of sufficient NPK may further facilitate these biosynthetic processes by ensuring nutrient availability required for energy production and enzymatic activity. These findings suggest that chitosan-based biostimulant strategies, particularly when complemented by adequate fertilization, may enhance the accumulation of valuable secondary metabolites in underground organs.

Discussion

Morphological response to chitosan and NPK treatments

This study demonstrated that the morphological performance of *E. palmifolia* was moderately influenced by the interaction between chitosan and NPK fertilizer. Significant treatment effects were observed on key traits such as leaf number, length, and width ($p<0.05$). In particular, the K2N2 combination (0.6% chitosan with 200 kg/ha NPK) produced the highest average number of leaves (18.60 ± 5.90), indicating a possible additive effect of both treatments. However, the statistical differentiation among some treatment combinations remained limited, as reflected by overlapping superscript letters in Table 1. This suggests that although trends were visible, the effects were not uniformly strong across all levels.

Leaf length and width were also significantly affected, with the longest (51.04 ± 2.05 cm) and widest leaves (2.94 ± 0.42 cm) recorded under the K1N2 treatment (0.3% chitosan and 200 kg/ha NPK). These results support previous findings that chitosan can enhance vegetative traits by influencing hormonal activity such as auxin and gibberellin-mediated elongation (Ingle et al. 2022), while nitrogen and potassium contribute to cell expansion and division (Yamika et al. 2021; Sun et al. 2022). The significant interaction between chitosan and NPK for these traits suggests that their combined application may enhance morphological outcomes, especially under sufficient nutrient supply. Nonetheless, the magnitude of improvement was moderate and context-dependent, highlighting the need for further validation under field conditions.

Flowering and biomass accumulation

Although flowering frequency and timing did not show statistically significant differences ($p>0.05$), a trend was observed in which plants treated with both chitosan and higher doses of NPK tended to flower earlier and more frequently. The K2N2 treatment initiated flowering at day 74 and produced the highest flower count (2.6 per plant), followed by K2N0 and K1N2. While biologically suggestive, this trend should be interpreted cautiously due to the limited number of flowering individuals and the short duration of the experiment. A possible influence of chitosan on reproductive transition may involve enhanced sugar translocation and hormonal modulation, such as abscisic acid and carbohydrate signaling (Sharif et al. 2018; Alsanam et al. 2021), but further investigation under controlled flowering studies is needed.

For biomass parameters, both fresh and dry weights of leaves and tubers showed positive trends across increasing chitosan and NPK levels, with the highest values generally observed in the K2N2 group. Statistical significance was confirmed only for certain traits (e.g., fresh tuber weight and total chlorophyll content), while others displayed non-significant trends. These improvements may reflect enhanced nutrient absorption and water retention associated with

chitosan application (Gornik et al. 2008), along with improved leaf morphometry and photosynthetic capacity contributing to increased assimilate production. Although NPK fertilization alone contributed to growth, its combination with chitosan appeared more effective in optimizing biomass accumulation in several parameters. Nitrogen and potassium likely supported protein synthesis and osmotic adjustment, respectively, contributing to the observed physiological responses.

Pigment content and photosynthetic capacity

Chlorophyll and carotenoid contents responded significantly to the chitosan and NPK treatments ($p<0.05$), with the highest values observed in the K2N2 group. The increase in total chlorophyll, particularly chlorophyll a, was also accompanied by visibly greener foliage, suggesting improved photosynthetic efficiency. These results are consistent with previous findings that link biostimulant use with enhanced physiological traits. Chitosan may enhance nitrogen assimilation by stimulating nitrate reductase activity, thus promoting chlorophyll biosynthesis (Limpanavech et al. 2008), while nitrogen and magnesium from NPK fertilizer contribute structurally to the chlorophyll molecule.

Carotenoid accumulation also increased under combined treatments, potentially improving photoprotection and oxidative stress tolerance. The observed pigment responses help explain the improved vegetative growth under these treatments, as they indicate more robust photosynthetic machinery. These findings underline that the interaction between chitosan and NPK not only influences structural traits but also improves physiological function, particularly in photosynthetic pigment accumulation.

Flavonoid accumulation and chitosan as a metabolic elicitor

Total flavonoid content in *E. palmifolia* tubers was significantly enhanced by chitosan-NPK combinations ($p<0.05$), with the highest level (6.30 mg QE/g) recorded under K2N2. This represents a substantial increase compared to the control, suggesting that the treatments acted synergistically to stimulate secondary metabolism. This effect may be explained by the elicitor role of chitosan in activating the phenylpropanoid pathway, including key enzymes such as Phenylalanine Ammonia-Lyase (PAL) and Chalcone Synthase (CHS), which are central to flavonoid biosynthesis (Sharif et al. 2018). Chitosan likely initiates signaling pathways, such as MAPK cascades, which lead to transcriptional activation of secondary metabolite biosynthetic genes.

Meanwhile, the contribution of NPK especially phosphorus may support flavonoid synthesis by enhancing carbohydrate availability and energy metabolism (Foyer and Noctor 2011), both of which are necessary for the biosynthesis of carbon-based secondary metabolites. These results suggest that chitosan not only functions as a growth promoter but also as a modulator of secondary metabolism, with potential applications in enhancing the phytochemical value of medicinal plants such as *E. palmifolia*.

Reproductive traits and their relation to secondary metabolism

Although the number of flowers and timing of floral initiation did not show statistically significant differences ($p>0.05$), the observed patterns suggest a potential influence of chitosan and NPK on the reproductive behavior of *E. palmifolia*. Flowering occurred in only four of the nine treatment groups, with the earliest onset and highest flower count recorded in the K2N2 treatment (2.6 flowers/plant, day 74), followed by K2N0, K1N2, and K0N2. This trend may reflect enhanced photosynthetic performance and assimilate availability in treatments with greater vegetative growth and pigment content.

Nonetheless, the low reproductive output and absence of flowering in more than half of the treatments—despite favorable vegetative development indicate that flowering in this species may require longer cultivation periods or specific environmental cues beyond nutrient and biostimulant inputs. Chitosan has been reported to influence flowering through hormonal and sugar-related signaling pathways (Alsanam et al. 2021), while phosphorus, as part of NPK, regulates meristem transition to reproductive development (Campbell et al. 2008). However, the present study's timeframe (11 weeks) might have limited the full expression of these responses.

It is also important to note that *E. palmifolia* is cultivated primarily for its tubers, not its flowers. The lack of correlation between flower number and tuber flavonoid content, as observed in K2N2 versus K2N0 treatments, supports the hypothesis that reproductive output does not directly determine secondary metabolite accumulation in storage organs. In fact, the highest flavonoid concentration was recorded under K2N2, not K2N0, despite K2N0 yielding the highest tuber biomass suggesting that flavonoid biosynthesis may be more tightly linked to biostimulant-induced elicitation than to reproductive development per se.

Future studies should consider extending the observation period beyond 11 weeks and employing a phenology-focused design to better characterize the generative potential of this species and its relation to phytochemical accumulation.

Integrative interpretation and practical implications

The integration of chitosan and NPK fertilizer yielded synergistic improvements across multiple parameters, notably in leaf development, chlorophyll content, and tuber flavonoid accumulation. The most consistent gains were observed in the K2N2 treatment, suggesting that chitosan functions optimally when nutrient availability is sufficient, likely through enhanced nutrient uptake and metabolic activation. This combination supports both vegetative growth and phytochemical enhancement, positioning it as a practical strategy for improving both yield and functional quality of *E. palmifolia* in greenhouse settings. While some effects were not statistically significant, observable trends suggest potential for optimized biostimulant-fertilizer regimes. These findings offer a baseline for the sustainable cultivation of medicinal plants, especially in systems targeting biomass and bioactive compound productivity.

Future studies should explore longer growth periods and molecular mechanisms to validate and expand these results.

In conclusion, the combined application of 0.6% chitosan and 200 kg/ha NPK fertilizer significantly enhanced vegetative growth, pigment accumulation, and flavonoid content in *E. palmifolia* under greenhouse conditions. Notably, improvements in leaf number, size, chlorophyll content, and tuber flavonoids suggest a synergistic interaction between biostimulant and nutrient inputs. Although flowering induction was limited within the cultivation period, observed trends point to chitosan's potential role in supporting reproductive transition when adequate macronutrients are present. These findings provide practical insights for optimizing agronomic yield and phytochemical quality in *E. palmifolia*, supporting its sustainable cultivation as a medicinal crop. Further research is needed to explore long-term effects and underlying molecular mechanisms.

REFERENCES

Abdel-Aziz HM, Hasaneen MN, Omer AM. 2016. Nano chitosan-NPK fertilizer enhances the growth and productivity of wheat plants grown in sandy soil. Spanish J Agric Res 14 (1): e0902-e0902. DOI: 10.5424/sjar/2016141-8205

Acemi A, Polat EG, Cakir M, Demiryürek E, Yavuz B, Özen F. 2021. Molecular weight and concentration of chitosan affect plant development and phenolic substance pattern in arugula. Notulae Botanicae Horti Agrobotanici Cluj-Napoca 49 (2): 12296-12296. DOI: 10.15835/nbha49212296.

Alsanam R, Alsahli A, Ibrahim M, Ahmad P. 2021. Chitosan-mediated plant growth and salt stress tolerance in *Vigna radiata*: Modulation of ABA signaling and antioxidant defense. Plant Physiol Biochem 159: 336-345. DOI: 10.1016/j.plaphy.2020.11.037

Atikah TA, Wardiyati T, Nihayati E, Saputera S, Nendissa DR. 2021. Inovasi teknologi budidaya bawang dayak (*Eleutherine palmifolia* Merr) untuk meningkatkan produktivitas dan analisis kelayakan ekonomi. AGROMIX 12 (1): 39-46. DOI: 10.35891/agx.v12i1.2331. [Indonesian]

Campbell CS, Watanabe N, Yamamoto M, Hakomori S, Kariyone S. 2008. Phosphorus regulation of flowering and floral organ development: Insights from gene expression studies. Plant Cell Environ 31 (10): 1416-1426.

Chadchawan S. Chamnanmanoontham N, Pongprayoon W, Pichayangkura R, Roytrakul S. 2015. Chitosan enhances rice seedling growth via gene expression network between nucleus and chloroplast Plant Growth Regul 75: 101-114. DOI: 10.1007/s10725-014-9935-7.

Cocozza C, Brilli F, Pignattelli S et al. 2020. The excess of phosphorus in soil reduces physiological performances over time but enhances prompt recovery of salt-stressed *Arundo donax* plants. Plant Physiol Biochem 151: 556-565. DOI: 10.1016/j.plaphy.2020.04.011.

Duhan JS, Kumar R, Kumar N, Kaur P, Nehra K, Duhan S. 2017. Nanotechnology: The new perspective in precision agriculture. Biotechnol Rep 15: 11-23. DOI: 10.1016/j.btre.2017.03.002

Ebaid M, El-Hady MA, El-Temsah ME et al. 2024. Combined vinasse and mineral NPK fertilizer affect physio-biochemical, root, and yield characters of faba bean (*Vicia faba* L.) genotypes grown on saline soil. J Soil Sci Plant Nutr 24 (2): 3178-3194. DOI: 10.1007/s42729-024-01743-8.

El-Miniawy SM, Ragab ME, Youssef SM, Metwally AA. 2014. Influence of foliar spraying of seaweed extract on growth, yield and quality of strawberry plants. J Appl Sci Res 10: 88-94.

Fanciullino R, Mollard S, Correard F, Giacometti S, Serdjebi C, Iliadis A, Ciccolini J. 2014. Biodistribution, tumor uptake and efficacy of 5-FU-loaded liposomes: Why size matters. Pharm Res 31: 2677-2684. DOI: 10.1007/s11095-014-1364-9.

Ferreyra SNE, Inzaugarat ME, Baz P, et al. 2012. The role of innate cells is coupled to a Th1-polarized immune response in pediatric nonalcoholic steatohepatitis. J Clin Immunol 32: 611-621. DOI: 10.1007/s10875-011-9635-2.

Foyer CH, Noctor G. 2011. Ascorbate and glutathione: The heart of the redox hub. Plant Physiol 155 (1): 2-18. DOI: 10.1104/pp.110.167569.

Gornik K, Grzesik M, Duda BR. 2008. The effect of chitosan on rooting of grapevine cuttings and on subsequent plant growth under drought and pathogen stress. Sci Hortic 117 (3): 274-280.

Haryadi D, Siregar FA, Sasmita P. 2015. Effect of NPK fertilizer on the growth and yield of shallots in dry climates. J Agric Sci 20 (1): 45-51.

Hendry GAF, Grime JP. 1993. Methods in Comparative Plant Ecology: A Laboratory Manual. Chapman & Hall, London. DOI: 10.1007/978-94-011-1494-3.

Huang M, Jiang L, Zou Y, Xu S, Deng Y. 2019. Effects of different potassium fertilizer levels on physiological traits and growth of medicinal plants. J Plant Nutr 42 (2): 201-214.

Ibrahim M. 2012. Design, synthesis, molecular docking and biological evaluation of some novel quinazolin-4 (3h)-one derivatives as anti-inflammatory agents. Al-Azhar J Pharm Sci 46 (2): 185-203. DOI: 10.21608/ajps.2012.7145.

Ingle N, Giri P, Joshi K. 2022. Role of chitosan in regulation of phytohormones and secondary metabolites in horticultural crops. Front Plant Sci 13: 927643. DOI: 10.3389/fpls.2022.927643

Kuntorini EM, Nugroho LH. 2010. Structural development and bioactive content of red bulb plant (*Eleutherine americana*); a traditional medicines for local Kalimantan people. Biodiversitas 11 (2): 102-106. DOI: 10.13057/biodiv/d110210.

Lalla MSP. 2022. Biostimulan Untuk Tanah Dan Tanaman. Penerbit Qiara Media, Pasuruan. [Indonesian]

Landi M, Esposito S, Nali C, Giordano C. 2017. Chitosan promotes antioxidative and photoprotective mechanisms under moderate UV-B radiation in lettuce plants. Sci Hortic 225: 295-302.

Limpanavech P, Chieochai S, Phornvillay S, Kumla S, Cha-um S. 2008. Chitosan effects on growth and postharvest quality of *Dendrobium* orchid. Sci Hortic 116: 65-72. DOI: 10.1016/j.scienta.2007.10.034.

Manurung B, Zahrah S. 2018. Pemberian Hormax dan NPK Mutiara 16: 16: 16 pada tanaman ubi jalar (*Ipomoea batatas* L.). Dinamika Pertanian 34 (2): 139-150. DOI: 10.25299/dp.2018.vol34(2).5423. [Indonesian]

Modi S, Kumar S, Dubey PK. 2021. Dynamics of chitosan based NPK-nanofertilizers in greenhouse cucumber production system. J Environ Biol 42 (1): 162-168. DOI: 10.22438/jeb/42/1/MRN-1251.

Moza A, Thomas T, Prasad ST. 2024. Local drug delivery using chitosan microspheres–A review of literature. Intl J Recenr Adv Multidiciplin Res 11 (04): 9714-9724.

Mutua CM, Ogweno JO, Gesimba RM. 2021. Effect of NPK fertilizer rates on growth and yield of field and greenhouse grown Pepino melon (*Solanum muricatum* Aiton). J Horticult Plant Res 13 (11): 10-23. DOI: 10.18052/www.scipress.com/JHPR.13.10.

Nur S, Thohari. 2007. Tanggap dosis nitrogen dan pemberian berbagai macam bentuk bolus terhadap pertumbuhan dan hasil tanaman bawang merah (*Allium ascalonicum* L). Jurnal Ilmiah Ilmu-Ilmu Pertanian 4 (1): 30-33. [Indonesian]

Nuraini A, Hamdani JS, Suminar E, Ardiansyah D. 2017. Aplikasi chitosan untuk meningkatkan hasil benih kentang G0 (*Solanum tuberosum* L.) kultivar granola pada berbagai jenis media tanam. Kultivasi 16 (3): 466-473. DOI: 10.24198/kultivasi.v16i3.14374. [Indonesian]

Nuryani E, Haryono G, Historiawati. 2019. Pengaruh dosis dan saat pemberian pupuk P terhadap hasil tanaman buncis (*Phaseolus vulgaris* L.) tipe tegak. Jurnal Ilmu Pertanian Tropika dan Subtropika 4 (1): 14-17. [Indonesian]

Paramita S, Nuryanto MK. 2018. Anti-inflammatory activity of bawang Dayak (*Eleutherine bulbosa* (Mill. Urb.)) ethanol bulb extracts. J Vocational Health Stud 2: 51-55. DOI: 10.20473/jvhs.V2.I2.2018.51-55.

Parvin K, Rahman MA, Islam MR, Jahan MS, Uddin MN, et al. 2019. Exogenous calcium alleviates salinity-induced oxidative stress in mustard (*Brassica juncea* L.). Plants 8: 151. DOI: 10.3390/plants8060151.

Patoni. 2000. Pengaruh Cekaman Kekeringan terhadap Pertumbuhan, Hasil, dan Kandungan Vitamin C Buah Tanaman Tomat (*Lycopersicum esculentum* Mill.). [Hon. Thesis]. Fakultas Biologi, Universitas Gadjah Mada, Yogyakarta. [Indonesian]

Perez-de-Luque A. 2017. Interaction of nanomaterials with plants: What do we need for real applications in agriculture? Front Environ Sci 5: 12. DOI: 10.3389/fenvs.2017.00012.

Rahman M, Mukta JA, Sabir AA et al. 2018. Chitosan biopolymer promotes yield and stimulates accumulation of antioxidants in strawberry fruit. PLoS One 13 (9): e0203769. DOI: 10.1371/journal.pone.0203769.

Rosmawaty T, Jumin HB, Mardaleni M, Sinaga C. 2019. Produksi dan kandungan flavonoid umbi tanaman bawang Dayak (*Eleutherine palmifolia*) dengan pemberian NPK 16: 16: 16 pada berbagai umur panen. Dinamika Pertanian 35 (3): 111-118. DOI: 10.25299/dp.2019.vol35(3).4574. [Indonesian]

Saharan V, Pal A. 2016. Properties and Types of Chitosan-Based Nanomaterials. SpringerBriefs in Plant Science. Springer, New Delhi. DOI: 10.1007/978-81-322-3601-6_3.

Sari VI, Saleh I, Ekawati R. 2020. Respons pertumbuhan, produksi, dan kandungan flavonoid bawang dayak (*Eleutherine palmifolia*) terhadap pengendalian gulma dan jarak tanam. Agrotechnol Res J 4 (2): 92-98. DOI: 10.20961/agrotechresj.v4i2.41725. [Indonesian]

Sathiyabama M, Indhumathi M. 2022. Chitosan thiamine nanoparticles intervene innate immunomodulation during Chickpea-*Fusarium* interaction. Intl J Biol Macromol 198: 11-17. DOI: 10.1016/j.ijbiomac.2021.12.105.

Sharif R, Mujtaba M, Rahman MU, Shalmani A, Ahmad H, Anwar T, Tianchan D, Xiping Wang X. 2018. The multifunctional role of chitosan in horticultural crops: A review. Molecules 23 (4): 872. DOI: 10.3390/molecules23040872.

Singh AK, Singla P. 2020. Root phenolics profile modulates microbial ecology of rhizosphere. Plant Phenol Sustain Agric 1: 555-578. DOI: 10.1007/978-981-15-4890-1_24.

Stankovic MS. 2011. Total flavonoid content in plant extracts using aluminum chloride colorimetric assay. J Med Plant Res 5 (25): 5555-5559. DOI: 10.5504/BBEQ.2011.0020.

Suci DAW. 2020. Pengaruh Kitosan Terhadap Kandungan Flavonoid pada Kalus Kesambi (*Schleichera oleosa* (Lour.) Merr) Secara in vitro. [Dissertation] Universitas Islam Negeri Maulana Malik Ibrahim, Malang. [Indonesian]

Sumarni N, Sopha GA, Gaswanto R. 2012. Respons tanaman bawang merah asal biji true shallot seeds terhadap kerapatan tanaman pada musim hujan. Indones Agency Agric Res Dev 22 (1): 23-28. DOI: 10.21082/jhort.v22n1.2012.p23-28.

Sun Q, Yang F, Liu M, Han Y, Dong M. 2022. Nitrogen metabolism and its relationship with plant growth under nitrogen supply. Front Plant Sci 13: 847650. DOI: 10.3389/fpls.2022.847650

Tamal MA, Aryanto D. 2020. Efektivitas air rebusan bawang dayak (*Eleutherine palmifolia* (L.) Merr) dalam menghambat pertumbuhan bakteri *Escherichia coli* pada daging sapi. Teknologi Pangan: Media Informasi Dan Komunikasi Ilmiah Teknologi Pertanian 11 (1): 16-26. DOI: 10.35891/tp.v11i1.1880. [Indonesian]

Xing HY, Cai YQ, Wang XF, Wang LL, Li P, Wang GY, Chen JH. 2015. The cytoprotective effect of hyperoside against oxidative stress is mediated by the Nrf2-ARE signaling pathway through GSK-3β inactivation. PLoS One 10 (12): e0145183. DOI: 10.1371/journal.pone.0145183.

Yamika W, Gunawan R, Riyadi P, Hasyim S. 2021. Response of growth and yield of shallot to potassium fertilization on entisol soil. IOP Conf Ser Earth Environ Sci 648: 012008. DOI: 10.1088/1755-1315/648/1/012008.

Yuswi NCR. 2017. Ekstraksi antioksidan bawang Dayak (*Eleutherine palmifolia*) dengan metode ultrasonic bath (kajian jenis pelarut dan lama ekstraksi). Jurnal Pangan dan Agroindustri 5 (1): 71-79. [Indonesian]