# Effects of Nitrogen Concentration on Growth, Yield and Phytochemical and Aromatic Compounds of*Persicaria Minor* Cultivated using Hydroponics System


## ABSTRACT

*Persicaria minor*, locally known as kesum and belonging to the family Polygonaceae, is a widely used aromatic herb in Malaysia. Cultivating kesum hydroponically using a deep-water culture (DWC) system presents an alternative method for growers to enhance crop yields. In this system, a liquid fertilizer solution is delivered directly to the plant roots without any growth medium or water flow. Kesum contains phytochemical compounds such as quercetin-3-glucuronide and quercitrin, which have medicinal properties. The primary objective of this study was to assess the effects of nitrogen concentration on the growth, yield, phytochemical (quercetin-3-glucuronide and quercitrin) and aromatic (aldehyde and caryophyllene) content of kesum cultivated in a hydroponic system. Plants were grown under four nitrogen concentration regimes: 200 mg/L, 100 mg/L, 50 mg/L, and 0 mg/L. The experiment was conducted under a side-netted rain shelter and arranged in a Randomized Complete Block Design (RCBD) with four replications. The plants were harvested eight weeks after planting. Those supplemented with 100 mg/L of nitrogen exhibited the best growth performance and biomass yield, along with the highest levels of aromatic compounds (aldehyde C-10: 5.80% and aldehyde C-12: 12.23%). Plants treated with 50 mg/L of nitrogen produced the highest amounts of quercetin-3-glucuronide (98.261 µg/mL) and quercitrin (61.367 µg/mL) compared to other treatments. Additionally, plants receiving 200 mg/L of nitrogen had the highest caryophyllene content (12.53%). In conclusion, the deep-water culture system in hydroponics can effectively cultivate kesum, allowing for adjustments in nitrogen concentration to achieve optimal yields, phytochemical levels, or aromatic compound production based on specific objectives.



**Keywords:** Hydroponics, nitrogen, *Persicaria minor*, Quercetin-3-glucuronide, Quercitrin, aldehyde C-10 and aldehyde C-12




## INTRODUCTION

*Persicaria minus*, commonly known as kesum, is a herbal plant native to Southeast Asia that thrives in moist, watery environments. Its aromatic leaves are often used in cooking [1]. Kesum is rich in micronutrients, total phenolic content (TPC), and natural antioxidants, offering numerous medicinal benefits, including antioxidant, anti-inflammatory, and anti-aging properties. It also supports memory enhancement and boosts the immune system [2, 3]. This shrub grows to a height of 45-60 cm and features small, narrow leaves (5-7 cm) with pointed tips. The leaves are green, while the plant has reddish, cylindrical stems with short nodes that root easily. Kesum also bears purple flowers.

Kesum is classified as an aromatic herbal plant with notable nutraceutical, pharmaceutical, and therapeutic properties [4, 5]. Two key aromatic compounds, aldehyde and caryophyllene, are being actively researched in the essential oil of Persicaria species [6]. Aromatic aldehydes contribute to the fragrance and flavor of plants and include compounds such as aliphatic aldehydes, analogous alcohols, sulfanyl derivatives, sesquiterpenoids (like caryophyllene and its derivatives), and monoterpenoids [6]. Research on the essential oil of Persicariaspp has demonstrated its antibacterial properties [6, 7], hydrogen peroxide radical scavenging activities, antifungal properties [1], and inhibitory effects against pathogenic bacteria [8]. This study will focus on aldehydes and their derivatives, particularly in relation to their antimicrobial, antioxidative, anti-inflammatory, and immunomodulatory effects.

Kesum is traditionally cultivated using conventional methods in mineral or peat soils with efficient irrigation systems, as its growth heavily depends on soil moisture [9]. However, this method requires large areas and significant labor, particularly during planting and harvesting. Moreover, excessive fertilizer use in conventional farming can lead to environmental pollution, particularly through leaching into soil and groundwater. An alternative approach is deep water culture (DWC), also known as the raft technique, which is one of the simplest and most cost-effective hydroponic methods. In DWC, liquid fertilizer is delivered directly to the plant roots without any growth medium or water flow [10]. This technique is practical for growing leafy vegetables like mustard, kale, pak choy, and herbs such as pegaga, selom, kaduk, basil, mint, and even kesum. The use of hydroponic systems has shown potential to increase the growth and yield of kesum, as demonstrated by the significant yield improvements seen in other leafy and fruiting vegetables grown hydroponically.

Several studies have demonstrated the crucial role of nitrogen nutrition in fruit growth, development, and production processes [11, 12, 13]. Nitrogen concentration in fertilizers may also affect plant secondary metabolite synthesis and metabolism [14]. Previous research has indicated that nitrogen fertilizers significantly influence the production of active compounds in plants [13]. For instance, nitrogen application has been shown to enhance the essential oil yield in patchouli compared to plants grown without nitrogen [15]. Therefore, this study aims to assess the effects of nutrient solutions on the growth, yield, and aromatic compounds of *Persicaria minor* cultivated using a hydroponic system.

## MATERIALS AND METHODS

### *Study Area*

The research was conducted in a rain shelter at the MARDI Station in Serdang, Selangor, Malaysia. This structure measured 30 meters in length, 10 meters in width, and 4.5 meters in height. The shelter's framework consisted of galvanized steel, with a transparent polyethylene film (180 mm thickness) serving as the roof. The sides were enclosed with insect-repellent netting featuring 0.1 x 0.1 mm2 mesh openings. To minimize insect intrusion, access to the shelter was controlled through a double-door entry system.

### *Experiment unit*

The experiment utilized the deep-water culture (DWC) hydroponic technique, also known as the raft method. Fiberglass containers measuring 90 cm wide × 300 cm long × 25 cm high served as the hydroponic tanks. Kesum plant cuttings were placed in 1.6 cm diameter holes spaced 17 cm apart on 2.5 cm thick polystyrene sheets. These sheets floated on the nutrient solution surface, covering the entire container. Each tank was equipped with a 60-watt submersible water pump connected to a PVC pipe, ensuring uniform fertilizer distribution. The setup also included two 100 L fertilizer stock barrels, an EC meter for monitoring fertilizer concentration, and sponges as a germination medium for the cuttings.

### *Planting materials*

Kesum plants were propagated using stem cuttings measuring 30 cm in length, each containing 7-9 nodes. The cuttings were prepared by removing leaves and side shoots, leaving only the leaves on the main shoots. For planting, each cutting was clamped with a wet sponge to serve as a germination medium and inserted into a planting hole on the polystyrene. Half of the cutting (15 cm) was submerged in the fertilizer solution, while the other half remained above the polystyrene surface. To minimize stress on the plant material, the planting was carried out in the evening. The Kesum cuttings began to develop roots after a period of three days.

### *Treatments and Experimental Design*

The experiment employed a Randomised Complete Block Design (RCBD) with four treatments, each replicated four times and containing 80 plants per treatment. The treatments consisted of varying nitrogen concentrations: T1 at 0 mg/L, T2 at 50 mg/L, T3 at 100 mg/L, and T4 at 200 mg/L. To maintain plant health, routine horticultural practices were followed, including regular pest and disease management. This involved biweekly applications of Malathion as an insecticide and Benlate as a fungicide.

### *Nutrient supplementation*

The fertilizer used in this study was formulated by MARDI specifically for vegetable plants, based on research by Yaseer Suhaimi et al. (2009, 2011). All components were water-soluble and prepared as two separate stock solutions at 100 times the final concentration. Stock A contained calcium nitrate and iron, while Stock B included all other nutrients. Each component was dissolved individually in tap water (pH 5.5 - 6.5) to ensure complete dissolution before being combined in 100-liter vessels. For the hydroponic containers, Stocks A and B were mixed at a 1:1 ratio to achieve the desired electrical conductivity (EC). A submersible pump was used for five minutes to ensure thorough mixing. The fertilizer concentration was monitored at least biweekly, or when solution levels decreased due to evaporation and plant uptake. For kesum plants, the EC was maintained between 2000 - 2400 mS/cm throughout the cultivation period.

### *Parameter measurement*

The growth of kesum plants was assessed eight weeks after planting by recording plant height, stem diameter, canopy size, fresh and dry weights of leaves and shoots, and SPAD value. Randomly selected plants were harvested at the eight-week mark to evaluate their growth and yield. The weights were taken immediately after harvest to prevent desiccation and water loss from the leaves.

### *Preparation of extracts*

The aerial parts of P. minor were collected, rinsed under running tap water to remove surface contaminants, and cut into small pieces. The samples were then dried in a hot air oven at 50°C for 72 hours. Once dried, they were ground into a fine powder and extracted with 70% methanol (1:10) using sonication for 1 hour. The mixture was centrifuged at 10,000 rpm for 15 minutes, and the extraction process was repeated three times under the same conditions. The combined filtrates were concentrated to dryness using a rotary evaporator to obtain the crude extracts.

### *Phytochemicals compound analysis*

The methanolic crude extracts were filtered through a 0.22 µm nylon membrane filter before analysis. Quercetin-3-glucuronide and quercitrin were identified using high-performance liquid chromatography (HPLC), equipped with an auto-sampler, diode array detector (DAD), and column oven. Chromatographic separation was achieved using a Waters XBRIDGE-C18 column (150 mm x 4.6 mm x 3.5 µm) at 40°C. The mobile phase consisted of water with 0.1% formic acid (phase A) and acetonitrile with 0.1% formic acid (phase B), with a gradient elution. The flow rate was set to 1.3 mL/min, and the injection volume was 5 µL. UV–vis detection was performed at 375 nm using the DAD detector. The quantities of quercetin-3-glucuronide and quercitrin in the extracts were calculated by comparing the peak areas to those of standard compounds using calibration curves.

**Comment [I1]:** could you add the concentrations used for the calibration curve?

### *Aromatic content analysis*

After harvesting, the kesum plants were initially sun-dried and then air-dried for 48 hours to reduce moisture content by approximately 40%. The dried samples were ground into a powder and extracted with hexane for 24 hours. The extract was filtered and analyzed using a UV-spectrophotometer at a wavelength of 248 nm to assess aldehyde and caryophyllene content.

### *Statistical Analysis*

The data obtained were analyzed statistically using analysis of variance (ANOVA) to determine the significant effects of the variables under investigation, with SAS version 9.1 software. Mean differences were evaluated using the Duncan Multiple Range Test (DMRT) at a significance level of $p \leq 0.05$.

## RESULTS AND DISCUSSION

### *Effects on plant growth and biomass*

Table 1 summarizes the plant growth parameters, including plant height, stem diameter, plant canopy, SPAD value, and both fresh and dry biomass per plant. The results show that nitrogen concentration treatments had a significant effect ($p < 0.05$) on these parameters. T3 (100 mg/L nitrogen) led to the tallest plants at 75 cm, although there was no statistically significant difference in plant height among the treatments. The largest stem diameter was seen in T2 (50 mg/L nitrogen), followed by T4 and T3. Similarly, there were no significant differences in plant canopy between the treatments.

**Comment [I2]:** to add information on the methodology used to make this SPAD media

The highest SPAD values were observed in plants treated with 200 mg/L of nitrogen, followed by those receiving 100 mg/L and 50 mg/L, respectively. Research has demonstrated that increasing nitrogen concentrations tends to elevate SPAD values, leading to darker green leaves in kesum plants. Previous studies have found a significant correlation between SPAD values and the chlorophyll and nitrogen content in plant leaves [18]. Sakamoto and Suzuki (2022) also reported that nitrogen deficiency leads to reduced chlorophyll levels in leaves, resulting in premature plant death. Plants exposed to 0 mg/L of nitrogen exhibited severe deficiency symptoms and died within three weeks of planting. These findings indicate that kesum plants require a minimum of 50 mg/L of nitrogen for proper growth. Suitable nitrogen concentrations not only promote growth but also serve as effective fertilizers [20].

Significant effects of nitrogen concentration were observed on the fresh and dry weight of leaves and shoots across the treatments. The highest fresh weight of leaves and shoots per plant was recorded in T3 (174 g), followed by T2 (159 g) and T4 (152 g). The study showed that a nitrogen concentration of 100 mg/L produced the optimal vegetative biomass. Additionally, the dry weight of leaves and shoots per plant increased in proportion to the fresh weight. The findings also indicated that plant height contributed to the overall vegetative biomass, as taller plants yielded more biomass.

Nitrogen is a known nutrient that can influence plant growth and development by affecting photosynthesis and the uptake of minerals [21, 22]. Application of nitrogen at the rate of 80 – 100 kg/ha had increased kesum yield compared to 180 – 200 kg/ha in conventional soil planting [9]. Nitrogen fertilizer significantly increased the plant biomass, height, canopy and SPAD value as shown in the study [14]. Previous reports showed that hydroponically grown plants have different growth characteristics compared with soil grown plants [23]. The responsiveness level of hydroponically grown plants to nitrogen concentration might be different from soil grown plants [24]. Nitrogen promotes the number of meristematic cells and their growth leading to the formation of biomass. Furthermore, nitrogen application is known to increase the levels of cytokinin, which affects cell wall extensibility [25. 26].

Table 1. Effects of nitrogen concentration on plant growth after two months of cultivation

| Treatment | Plant height (cm) | Stem diameter (mm) | Plant canopy (cm) | SPAD value | Fresh weight of leaves and shoots per plant (g) | Dry weight of leaves and shoots per plant (g) |
|---|---|---|---|---|---|---|
| T1: 0 mg/L | nil | nil | nil | nil | nil | nil |
| T2: 50 mg/L | $75^a$ | $7.2^a$ | $51^a$ | $44^b$ | $159^b$ | $35.9^b$ |

| | | | | | | |
|---|---|---|---|---|---|---|
| T3: 100 mg/L | 78[a] | 6.3[b] | 52[a] | 48[a] | 174[a] | 40.5[a] |
| T4: 200 mg/L | 74[a] | 6.5[b] | 56[a] | 52[a] | 152[c] | 31.3[b] |

Mean values in the same column followed by the same letter are not significantly different at $p < 0.05$

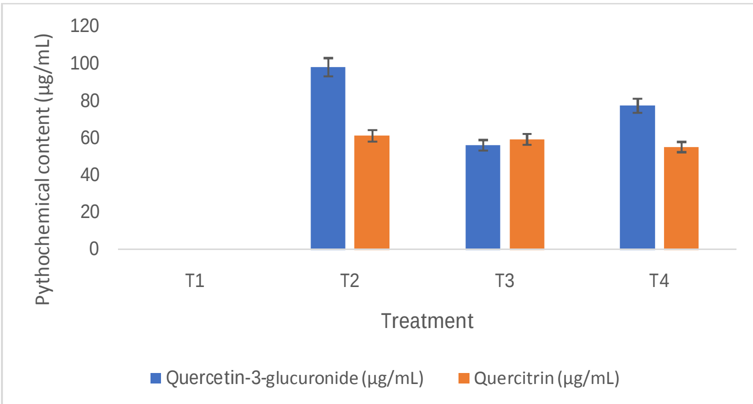
***Effects on phytochemical content***

Significant nitrogen concentration effects were on quercetin-3-glucuronide and quercitrin content between treatments (Figure 1). Plants treated with T2 or 50 mg/L of nitrogen concentration showed the highest quercetin-3-glucuronide (98.261 µg/mL) and quercitrin (58.467 µg/mL) content. Meanwhile, plants supplemented with 100 mg/L of nitrogen concentration gave the lowest value of quercetin-3-glucuronide (56.291 µg/mL) and plants cultivated with 200 mg/L of nitrogen produced the lowest quercitrin content (55.315 µg/mL). Data revealed that plants supplemented with 100 mg/L of nitrogen concentration raised the highest vegetative biomass but low in both quercetin-3-glucuronide and quercitrin content. Contrast to the plants supplemented with 50 mg/L of nitrogen concentration which gave the lowest biomass but high in both both quercetin-3-glucuronide and quercitrin content.

There is a trend between biomass and phytochemical content, where lowered biomass increases the plant's bioactive compound. Optimal nitrogen concentration application rate can increase the yield and active ingredients of *A. mongolica* [14]. Previous studies revealed that increased nutrient concentration can enhance strawberry, cucumber, lettuce, tomato, melon productivity and quality parameters in soilless conditions [27, 28,29, 30, 31]. The positive effects of nutrient concentration in plants have been widely studied, on the morphological and physiological characteristics. Nitrogen concentration affected the growth, yield, fruit quality, and chemical composition of plants [32]. Extract derived from the leaves of P. minor has been reported to contain quercetin-3-glucuronide and quercitrin which give the plant its antioxidant properties [33].



Figure 1:Effects of nitrogen concentration on quercetin-3-glucuronide and quercitrin content in plants after two months of cultivation




***Effects on aromatic content***

Significant effects of nutrient concentration were found on the levels of aldehyde-C10, aldehyde C-12, and caryophyllene across treatments (Figure 2). Plants treated with 100 mg/L of nitrogen (T3) had the highest aldehyde-C10 (5.80%) and aldehyde C-12 (12.23%) content, but the lowest caryophyllene content (9.13%) among all treatments. Conversely, plants treated with 200 mg/L of nitrogen (T4) showed the lowest aldehyde-C10 (0.75%) and aldehyde C-12 (2.11%) content, but the highest caryophyllene content (12.53%). The results indicate that plants given 100 mg/L of nitrogen produced the most vegetative biomass, along with high aldehyde C-10 and C-12 content. However, those treated with 200 mg/L nitrogen produced lower biomass but higher caryophyllene levels.

There is a trend between aldehyde and caryophyllene where a high content of aldehyde in the plant sample lowered caryophyllene content and vice versa. Similar studies on *Kickxiaaegyptiaca* essential oil revealed the same pattern linking aldehyde and caryophyllene trends, where high aldehyde with lower caryophyllene content [34]. Generally, nitrogen applications increase oil content and yield in aromatic plants by stimulating biomass, leaf area development and photosynthetic rate level [35]. The results of the present study are also supported by the findings of Daneshian et al. (2009) and Azizi et al. (2009) on plants other than *P. minor*.

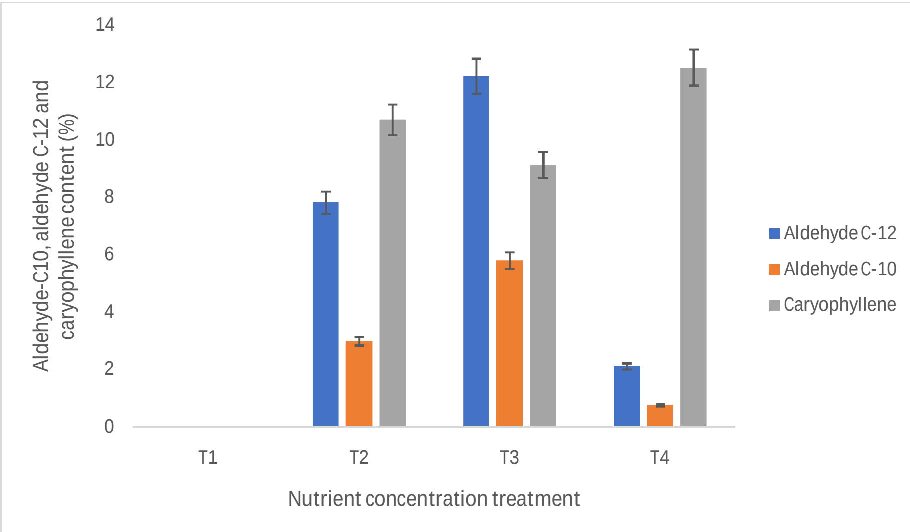



Figure 2:Effects of nutrient solution strength on aldehyde C-10, aldehyde C-12 and caryophyllene contents in plants after two months of cultivation

## CONCLUSION

Studies revealed that *Persicaria Minor* plants respond very well to 50 – 200 mg/L of nitrogen concentration. When supplemented with nitrogen concentrations ranging between 50 – 200 mg/L, the plant was able to grow and produce vegetative biomass. Plants supplemented with 100 mg/L of nitrogen concentration gave the best plant growth performance and the highest aldehyde-C10 and aldehyde C-12 content. Meanwhile, the best nitrogen concentration to obtain the highest quercetin-3-glucuronide and quercitrin contents was 50 mg/L of nitrogen concentration. Plants treated with 200 mg/L nitrogen produced lower biomass but higher caryophyllene levels. Thus, it can be concluded that for plant growth, biomass yield, aldehyde-C10 and aldehyde C-12 content, the nutrient concentration at 100 mg/L is the efficiency rate of application for *Persicaria Minor* grown using a hydroponic system. However, 50 mg/L of nitrogen concentration can be considered to obtain high quercetin-3-glucuronide and quercitrin content. Conversely, 200 mg/L of nitrogen concentration could be an option for high caryophyllene content.

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