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Effects of Nitrogen Concentration on Growth, Yield and Phytochemical Content of *Persicaria minor* Cultivated Using Hydroponics System

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Persicaria minor or locally known as kesum from the family Polygonaceae is a common herbal plant found in Malaysia. Hydroponic cultivation of kesum using deep water culture (DWC) system can be an alternative option for kesum growers in increasing crop yields. The deep water culture system is a method in which liquid fertiliser solution is given directly to the plant roots without any medium and water flow. The study's main objective was to determine the effects of nitrogen concentration on growth, yield and phytochemical content of kesum cultivated using hydroponic system. Plants were grown under one of four nitrogen concentration solution regimes and the experiment was conducted under a side netted rain shelter. The four treatments comprised of 200 mg/L, 100 mg/L, 50 mg/L and 0 mg/L of nitrogen concentrations. The experiment was arranged in Randomized Complete Block Design (RCBD) with 4 replications. Plants were selected randomly

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and harvested eight weeks after planting. Plants grown supplemented with 100 mg/L of nitrogen concentration gave the best growth performance and biomass yield. They produced the highest plant height (78 cm) and fresh biomass yield (174 g per plant). Meanwhile plants supplemented with 50 mg/L of nitrogen concentration produced the highest Quercetin-3-glucuronide (Q3G) (98.261 μg/mL) and Quercitrin (61.367 μg/mL) compound compared to the other treatments. However, plants treated with 0 mg/L of nitrogen concentrations exhibited major nutrients deficiencies symptoms and die after 3 weeks of planting. Hence, it can be concluded that the kesum plants cultivated using hydroponics' deep water culture system supplemented with 100 mg/L of nitrogen concentration gave the highest plant growth and biomass yields. However, to obtain higher Quercetin-3-glucuronide and Quercitrin compound, 50 mg/L of nitrogen concentration is recommended.

Keywords: Hydroponics; nitrogen; quercetin-3-glucuronide; quercitrin; Persicaria minor.

1. INTRODUCTION

Persicaria minor or known as Kesum in Malaysia is herbal plant that originates from Southeast Asia (Malaysia, Indonesia, Vietnam, Thailand) and thrives in moist and watery areas. Kesum leaves are also the main ingredient in cooking because of their aromatic properties that can evoke the delicious taste of food. Kesum is also used as medicine to overcome health problems. Kesum is rich in micronutrients, total phenolics content (TPC) and natural antioxidants. This active ingredient gives curative medicinal benefits in terms of antioxidants, anti-inflammatory, anti-aging, improves memory and promotes the body's immune system [1,2]. Kesum is a shrub plant that has a height of approximately 45 - 60 cm. Kesum leaves are small, long leaves (5 - 7 cm) and pointed, green in colour and has a reddish cylindrical shape stem. Kesum has short node sections and is easily rooted. It has flowers that are purple in colour. Basically, there are two types of kesum which are creeping and vertical. For commercial cultivation, vertical types of kesum plants are suitable for facilitating farm management and leaves harvesting. Kesum is usually grown using conventional soil planting and there is interest in cultivating the plant using hydroponic culture [3].

Deep water culture (DWC) or raft technique is the easiest hydroponic technique to carry out and cost-effective to manage for vegetable production [4,5]. In these techniques, liquid fertilizer is given directly to the plant roots without any medium and water flow [6]. This technique is very practical for planting leafy vegetables such as mustard, kalian, kale, pak choy and leafy herbs such as basil and mint. There is potential to increase the growth and yield of kesum using a hydroponic system based on significant increase in yields of leafy and fruity vegetables grown on a hydroponic system. Fertilizer is one of the main components in hydroponic culture.

Plant nutrition management in hydroponic cultivation is more effective and accurate compared to conventional soil planting [7]. Several studies have shown that nitrogen nutrition plays an essential role in fruit growth, development and production processes [8]. Nitrogen concentration in fertilizer might also influence plant secondary synthesized and metabolism [9,10,11]. Previous studies shown that nitrogen fertilizers have a significant effect on the level of total phenols and flavonoids [12]. Application of nitrogen able to increase lettuce cultivar biomass in hydroponic culture [13]. However, there are certain plants active compounds did not influence by nitrogen level [14]. Thus, this study was conducted to determine the effects of nutrient solution on growth, yield and beneficial pythochemical compound of *Persicaria minor* cultivated using hydroponics system. The main objective was to determine the optimum nutrient solution for kesum cultivation using hydroponic system.

2. MATERIALS AND METHODS

2.1 Study Area

A side-netted rain shelter of 30 m long x 10 m wide x 4.5 m high located in MARDI Station Serdang, Selangor, Malaysia was used in the study. All structures were made of galvanised steel frame with transparent polyethylene film (180 μm thick) roofing and insect repellent net (0.1 x 0.1 mm^2) side cladding. Entrance into the shelter must be through double doors to reduce the chance of insect entry. Deep water culture (DWC) or raft technique was the hydroponic technique used to carry out the experiment. Hydroponic containers made of fiberglass were used in the study. The hydroponic tank size is 90 cm wide × 300 cm long × 25 cm high. Kesum plant cuttings were placed on polystyrene with a thickness of 2.5 cm and floated on the surface of the nutrient solution like a raft. The polystyrenes were tightly packed to cover the entire hydroponic container. Plant holes with a diameter of 1.6 cm with a distance of 17 cm each were made on polystyrene. Each hydroponic container was equipped with a 60-watt submersible water pump connected to a PVC pipe to provide uniform fertiliser mixing in the hydroponic container. Other equipment and materials needed are two units of 100 L fertiliser stock barrels, an EC meter to measure the fertiliser concentration and a sponge as a germination medium for the cutting.

2.2 Planting Materials

Kesum plants were propagated through stem cuttings. Shoot cuttings with a length of 30 cm with 7-9 nodes were used as planting material. The leaves and side shoots on the cuttings were removed leaving only the leaves on the main shoots. Stem cuttings were clamped with a wet sponge as a germination medium was included into planting hole on the polystyrene. The 15 cm half of the cutting was immersed in the fertiliser solution, while the other 15 cm was on the top of the polystyrene of the hydroponic container. The planting activity was done in the evening to reduce the stress on the plant material. Kesum cuttings started to root after three days.

2.3 Nutrient Supplementation

The fertiliser was formulated by MARDI based on the needs of the vegetables and leafy plant [15]. All the fertiliser components were water soluble. The fertiliser stocks were prepared according to [16]. The macro and micro nutrients were prepared separately as A and B stock solutions respectively, at 100x dilution. Solution Iron and calcium nitrate were present in solution A, but all other components were present in solution B. To make sure that they all dissolved thoroughly in the water, each component was introduced one at a time. Calcium nitrate was added to a container of tap water (pH 5.5–6.5) to make stock A solution. After stirring the mixture until the calcium nitrate dissolved, the solution was placed into a 100-litre jar. A other container containing tap water was filled with iron powder, which was then added and swirled until it entirely dissolved. The stock B solution was made using the same method. Stock A and stock B were added into the hydroponic container at 1:1 ratio until the needed electricity conductivity (EC) was achieved. After the fertilizer solution were added to the hydroponic container, the submersible pump is turned on to ensure that the fertilizer solution mixed inside the hydroponic container. The submersible pump was turned on for five minutes to ensure that the fertilizer solution were completely mixed in the hydroponic container. The concentration of the fertilizer solution were checked periodically at least once every two weeks or when the volume of the fertilizer solution in the container begins to decrease due to sublimation and were used by the plants. The concentration of fertilizer solution for kesum plants were maintained at EC 2000 - 2400 μS/cm throughout the cultivation period.

2.4 Treatments and Experimental Design

The treatments were arranged in a randomised complete block design (RCBD) with four levels of treatment with four replicates and 80 plants per treatment. There were four nitrogen concentration used as treatments in this study. These treatments were as follows: the four treatments comprised of T1: 0 mg/L, T2: 50 mg/L, T3: 100 mg/L and T4:200 mg/L of the nitrogen concentrations. Routine horticultural practices for pest and disease were as followed: insecticide (*Malathion*) and fungicide (*Benlate*) were applied once every 2 weeks.

2.5 Parameter Measurement

The growth of the kesum plants was measured eight weeks after planting by measuring plant height, stem diameter, plant canopy, fresh weight of leaves and shoot, dry weight of leaves and shoot and SPAD value. The plants were randomly selected and harvested after eight weeks of planting to determine the yield and growth of kesum. The weight was measured immediately after harvest to prevent desiccation and water loss from the leaves.

2.6 Extraction

Samples were washed with running tap water to remove surface pollutants and cut into small pieces. They were then dried under hot air oven at temperature of 50°C for 72 h. After drying, the samples were ground into a fine powder. The samples were extracted with 70% methanol under sonication for 1 h. Following this, the samples were centrifuged at 10,000 rpm for 15 minutes to separate the supernatant from the sediment. Extraction was repeated three times under identical conditions. The filtrates were combined and brought to complete dryness using a rotary evaporator to obtain the crude extracts.

2.7 Phytochemical Analysis

The methanolic crude extracts were filtered through a 0.22 µm pore size nylon membrane filter prior to analysis. Identification of quercetin-3-glucoronide and quercitrin was performed on high performance liquid chromatography (HPLC). The compound was chromatographically separated using a C18 (150 mm x 4.6 mm x 3.5 µm) column. The mobile phase consisted of water and acetonitrile. The flow rate was set at 1.3 mL/min and the injection volume was 5 µL. The UV–vis absorption chromatogram was detected at 375 nm using DAD detector. The amount of quercetin-3-glucoronide and quercitrin in the extracts was calculated using the regression equation of their peak area to peak area of known concentration of the standards from the calibration curves.

2.8 Statistical Analysis

Data obtained were subjected to statistical analysis using analysis of variance (ANOVA) procedures to test the significant effect of all the variables investigated using SAS version 9.1. Means were separated using Duncan Multiple Range Test (DMRT) as the test of significance at $p \leq 0.05$.

3. RESULTS AND DISCUSSION

3.1 Effects on Plant Growth

Table 1 showed the results of plant growth parameters for plant height, stem diameter, plant canopy, SPAD value, and fresh and dry biomass per plant. Data indicated that there were highly significant ($p < 0.05$) differences among the nitrogen concentration treatments tested. T3 or 100 mg/L of nitrogen concentrations recorded the highest value in plant height (75 cm) compared to other concentrations tested. However, there was no significant difference in terms of plant height between treatments. The highest stem diameter was observed in T2 or 50 mg/L of nitrogen concentration followed by T4 and T3. However, there was no significant difference between T3 and T4 in terms of stem diameter. There were also no significant differences in plant canopy between each treatment. The highest SPAD values were obtained from plants supplemented with 200 mg/L of nitrogen concentration followed by 100 mg/L and 50 mg/L nitrogen concentration respectively. However, there was no significant difference between T3 and T4 in terms of SPAD value. Studies showed that higher application of nitrogen concentrations tends to increase the SPAD value. The higher nitrogen concentration increased the SPAD value and kesum leaves tend to be darker green in colour. The nitrogen content of the solutions showed a significant positive relationship with SPAD value. Previous studies had shown that there was a significant correlation between SPAD values and the contents of chlorophyll and nutrient concentration in plant leaves [17]. Meanwhile, Sakamoto and Suzuki [18], stated that a deficiency of nutrient resulted in lower chlorophyll in leaves, resulting in earlier plant death. Plant treated with 0% of nitrogen concentration exhibited major deficiency symptoms and plants died after 3 weeks of planting. These studies showed that kesum plants needed at least 50 mg/L of nitrogen concentration to grow.

There were significant nitrogen concentration effects on the fresh and dry weight of leaves and shoots between treatments. The highest leaves and shoots per plant fresh weight was observed in T3 (174 g) followed by T2 (159 g) and T4 (152 g). The study revealed that 100 mg/L of nitrogen concentration resulted in the optimum vegetative biomass. There was a 9.4% to 14.5% increment in vegetative biomass obtained in 100 mg/L of nitrogen concentration compared to other treatments. The dry weight of leaves and shoots per plant increased proportionally with fresh weight of leaves and shoots per plant. The highest dry weight of leaves and shoots per plant was also found to have the highest fresh weight of leaves and shoots per plant. Results revealed that plant height attributes contributed to the vegetative biomass, since higher plant height resulted in higher vegetative biomass. Application of nitrogen at rate of 80 – 100 kg/ha had increased kesum yield compared to 180 – 200 kg/ha in conventional soil planting [3]. Previous reports showed that hydroponic grown plants have different growth characteristics compared with soil grown plants [19]. The responsiveness level of hydroponic grown plant to the nitrogen concentration might be different from soil grew plants [20]. Sufficient nutrients availability are the main factors that influence plant growth and biomass production in hydroponic culture [21]. Previous studies on the increasing nitrogen concentration on a hydroponic culture of lettuce showed an increased in plant biomass [22].

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^{ab}	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^{ab}	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 $p < 0.05$

3.2 Effects on Phytochemical Content

The positive effects of nutrient concentration in plants have been widely studied, on both morphological and physiological characteristics. Nutrient concentration affected the growth, yield, fruit quality, and chemical composition of plants [23]. Numerous studies have shown that *P. minor* phytochemical compounds had benefits to the pharmaceutical and health industry [24]. The leaves of kesum contain a high concentration of aromatic metabolites as compared to the other herbs [25]. Several studies have demonstrated that there are high flavonoids compounds that can be found in *P. minor* including quercetin and Quercetin-3-glucoronine [26]. These two compounds had shown high antioxidant activity, elastase inhibitory activity that can act as anti-aging, delaying the process of collagen breakdown and subsequently the wrinkling process [25,27].

"Various studies have shown the presence of phenolic compounds and secondary metabolites, in particular flavonoids in P. minus leaves as found also in this study" [28,29,30]. There was significant nutrient concentration effects on quercitrin and quercetin-3-glucoronine content between treatments (Fig. 1). Plants treated with T2 or 50 mg/L of nitrogen concentration showed the highest quercitrin and quercetin-3-glucoronine content among treatments. Plants treated with T3 or 100 mg/L of nitrogen concentration produced the lowest quercitrin compound, while plant supplemented with T4 or 200 mg/L of nitrogen exhibited the lowest quercetin-3-glucoronine content. Plants supplemented with 50 mg/L of nitrogen concentration resulted in lower vegetative biomass compared to 100 mg/L of nitrogen concentration. Plants treated with 100 mg/L of nitrogen concentration gave rise to the highest vegetative biomass with lower compound of interest content.

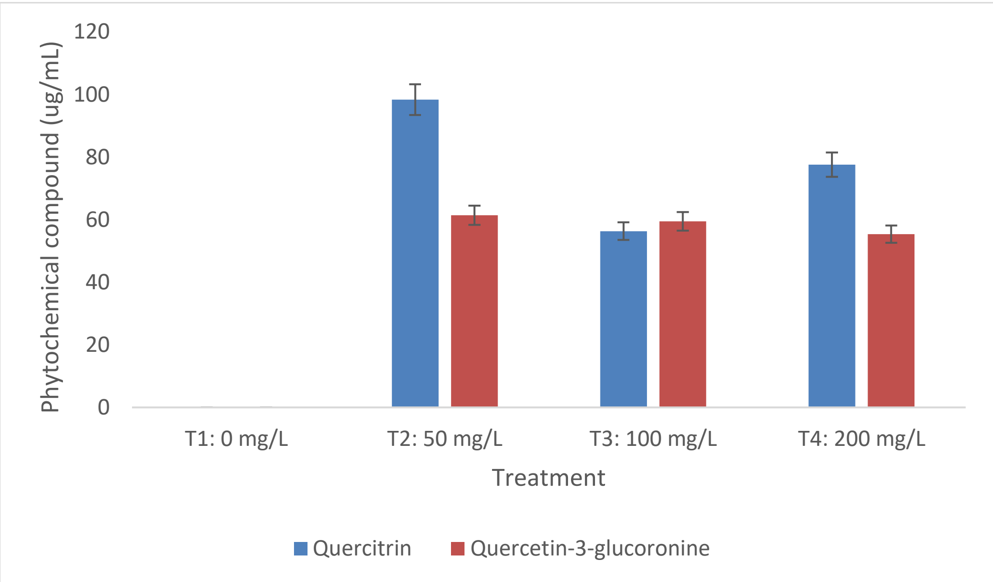

Fig. 1. Effect of nitrogen concentration on phytochemical compound quercitrin and quercetin-3-glucoronine content in *Persicaria minor*

Results suggested that nitrogen concentration have a significant effect on the level of quercitrin and quercetin-3-glucoronine. Nitrogen levels promote flavonol glycosides biosynthesis by gene regulation and the accumulation of substrate carbohydrates as seen in *Camellia sinensis*, while nitrogen deficiency and excess nitrogen have inhibitory effects [31]. The contents of quercetin and quercetin-3-glucoronine showed a similar pattern in this study, and it is necessary to further investigate the mechanisms at play. Previous research discovered that whether Allium cepa was treated with high or low nitrogen fertilizer levels, the plant's quercetin concentration was not significantly different [32]. Previous studies revealed that increased nutrient concentration can enhance strawberry, cucumber, lettuce, tomato, melon productivity and quality parameters in soilless conditions [33,13,34,35,36].

4. CONCLUSION

Findings from the study suggested that nitrogen concentration level exert notable effects on the growth and levels of active compounds in *Persicaria Minor*. Studies revealed that *Persicaria Minor* plants respond very well to 50 – 200 mg/L of nitrogen concentration. The plant was able to grow and produced vegetative biomass when supplemented with nutrient concentrations ranging between 50 – 200 mg/L of nitrogen concentration. Plants grown supplemented with 100 mg/L of nitrogen concentration gave the best growth performance and biomass yield. Meanwhile plants supplemented with 50 mg/L of nitrogen concentration produced the highest Quercetin-3-glucuronide and Quercitrin compound. Thus, it can be concluded that for plant growth and biomass yield, nutrient concentration at 100 mg/L of nitrogen concentration is the efficiency rate of application for *Persicaria Minor* grown using hydroponic system. However, 50 mg/L of nitrogen concentration can be considered to obtain high Quercetin-3-glucuronide and Quercitrin compound content.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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