**Effect of Nitrogen Concentration on Plant Growth and Yield of Black Ginger Cultivated in Soilless Culture System**

**Abstract**

This study investigated the influence of nitrogen concentration on the growth, yield, and phytochemical profile of black ginger (*Kaempferia parviflora*) cultivated in a soilless culture system. Black ginger, widely recognized for its medicinal properties and high domestic demand, faces production challenges that limit its supply. To address this, the experiment employed a completely randomized block design using 8‐month-old rhizomes subjected to five nitrogen treatments (50, 100, 150, 250, and 300 ppm) while maintaining constant phosphorus and potassium levels. Morphological parameters such as plant height, vegetative fresh weight, number of tillers, SPAD value, fresh rhizome yield, and the rhizome-to-shoot ratio were measured to assess growth performance. Although plant height was statistically similar across treatments, both vegetative fresh weight and fresh rhizome yield increased with higher nitrogen levels, peaking at 250 ppm before declining at 300 ppm. The optimal rhizome-to-shoot ratio was also achieved at this intermediate concentration, indicating effective biomass partitioning toward the valuable storage organs. Phytochemical analyses were conducted to quantify total phenolic content (TPC), total flavonoid content (TFC) and the concentration of 4,5,7‐trimethoxyflavone, a key methoxyflavone linked to various therapeutic activities. Interestingly, while the highest TPC and TFC were observed at 50 ppm nitrogen, the level of 4,5,7‐trimethoxyflavone was maximized at 250 ppm. Antioxidant activities measured via DPPH radical scavenging and FRAP assays supported these findings, suggesting that both low and moderate-high nitrogen supplies benefit the plant’s secondary metabolism. Overall, the study demonstrates that optimizing nitrogen fertilization in a soilless culture system can significantly enhance both the yield and medicinal quality of black ginger.

**Introduction**

Black ginger, scientifically referred to as *Kaempferia parviflora*, is a member of the Zingiberaceae family, which thrives in tropical and sub-tropical regions. Native to Southeast Asia, this perennial plant is characterized by its thick, dark purple rhizomes. It has been traditionally cultivated both as a spice and for its medicinal properties. The plant features oblong leaves measuring approximately 6 to 8 cm in length and produces distinctive purple and white flowers [1]. Black ginger is highly valued in alternative medicine for its therapeutic benefits, which include treating fungal infections, gastrointestinal issues, low vitality, allergies, body pains, oral diseases, male impotence, and promoting overall health [2,3].

A soilless culture system is an innovative agricultural method that mimics the role of soil by providing physical support to plants and creating an optimal root environment with precise water and nutrient delivery. This system has demonstrated significant yield improvements, with crops such as chillies, rock melons, and tomatoes showing 3 to 5 times higher productivity compared to traditional soil-based cultivation [4,5]. For plants like black ginger, efficient fertilizer use is essential for sustainable agricultural practices. The soilless culture system enhances mineral use efficiency by delivering nutrient solutions directly to the plant’s root zone, making it a promising technique for cultivating black ginger and similar crops.

Fertilizers are widely used in agriculture to increase crop production. Nitrogen (N), Phosphorus (P) and Potassium (K) are nutrients which maintain the growth, yield and quality of the plants. Among chemical fertilizers, nitrogen (N), phosphorus (P), and potassium (K) are the major elements required for supporting plant growth and development in black ginger [6]. In the soilless culture system, fertilizers are supplied as ions form in the nutrient solution [7]. Several formulations of essential macro and micronutrients have been developed to enhance nutrient uptake and plant growth in the soilless culture system [8]. N is required for chlorophyll formation and it influences stomatal conductance and photosynthetic efficiency[9]. Sufficient supplies of fertilizers for crops can improve the performance and yield of plants but also determine plant quality [10]. In a soilless culture, different concentrations of macronutrients were tested and results indicated that plants respond differently [11].

Phytochemical studies revealed that black ginger’s rhizomes contain phenolic and flavonoid compounds including flavones, flavanones and chalcones [12, 13]. The rhizomes of *K. parviflora* contain a variety of methoxyflavones, which contribute to its medicinal potential [14]. Among these methoxyflavones, 4,5,7-trimethoxyflavone stands out as a representative compound with a wide range of pharmacological properties [3]. These make 4,5,7-trimethoxyflavone a promising candidate for developing therapeutic agents targeting various health conditions, including inflammation, oxidative stress, cancer, microbial infections, cardiovascular diseases, and neurodegenerative disorders [14]. Domestic demand for black ginger is high and has increased significantly as people become more interested in its medicinal properties. However, the demand for black ginger rhizomes in Malaysia can hardly be fulfilled due to the low production yield [15].

Cultivating ginger using a soilless culture system could be an alternative method to increase rhizome yields and address the supply shortage problem. The potential to enhance the growth and yield of black ginger rhizomes using a soilless system is supported by significant yield increases observed in chillies, rock melons, tomatoes and other leafy and fruity vegetables grown on various media [4,5]. Therefore, the present investigation was conducted to study the effects of N on the growth, yield and rhizomes quality of black ginger grown using a soilless culture system. The main objective was to determine the optimum N concentration for black ginger cultivation using a soilless culture system.

**Materials and method**

**2.1 Planting materials**

Black ginger is propagated vegetatively using 8-month-old rhizomes, with shoots emerging 2–3 weeks after planting. The rhizomes were cut into smaller sections, each approximately 4 cm in length and 30 g in weight. Each seed rhizome contained 2–3 bud points. Before planting, the seed rhizomes were treated with propamocarb. The black ginger plants can be harvested as fully developed rhizomes after 8 months.

**2.2 Study area**

The study was conducted in a side-netted rain shelter measuring 30 meters in length, 10 meters in width, and 4.5 meters in height, located at the MARDI Serdang, Selangor, Malaysia. The structure was constructed using galvanized steel frames, with a transparent polyethylene film roof (180 µm thick) and insect-repellent net side cladding (mesh size 0.1 x 0.1 mm²). Access to the shelter was restricted to double doors to minimize the entry of insects.

**2.3 Treatments and experimental design**

The experiment employed a complete randomized block design with three replications. Each treatment plot contained 50 plants. The study involved five different nitrogen (N) concentration levels, while phosphorus (P) and potassium (K) levels were maintained constant at 150 ppm and 200 ppm, respectively. The fertilizer formulations, developed by the Malaysian Agricultural Research and Development Institute (MARDI), were tailored to the nutrient requirements of the plant rhizomes, with nitrogen concentrations adjusted according to the treatments. Five treatments (T1–T5) were applied, each with varying N levels (50, 100, 150, 250, and 300 ppm) mixed into the nutrient solution. Ammonium nitrate (NH₄NO₃) was used as the nitrogen source, with its content calculated and incorporated into the nutrient solution. A 100 L stock solution of the nutrient solution was prepared, using water-soluble fertilizers. Prior to application, the pH of the nutrient solution was adjusted to a range of 5.5–6.5.

**2.4 Irrigation set-up**

The irrigation system, built in the side-netted rain shelter, consisted of a 1,500-litre tank, 1.5 Hp water pump, water filter, pressure meter and four lateral lines (28 m each) looped to each other. Each lateral line was equipped with 100 drippers placed into 100 polyethylene bags, side by side. The distance between each line was 1.5 m and between each dripper point in the lateral line was 0.3 m. A valve was attached to an inlet to control the amount of the irrigated solution pumped in. A small valve was also attached to each lateral line to maintain the flow through the drip line. The nutrient solutions were supplied through 0.3 m micro tubes and arrow drippers.

**2.5 Nutrient concentrations and irrigation frequencies**

The irrigation solutions were prepared in a 1,500-litre tank. Fertilizer stock were added into the tank until the needed electricity conductivity (EC) was achieved. The EC of the fertigation solution was between 1800 µS and 2400 µS. The irrigation scheduling was automatically implemented by a digital timer, two times per day in the first 3 months (0800 h and 1600 h), three times per day in the 4th – 7th months (0800 h, 1000 h and 1600 h), and once per day in the last month (0800 h). The irrigation duration was 3 minutes and an identical amount of fertiliser solution was applied to all polyethene bags. The daily irrigation volumes per plant were 500 ml in the first 3 months, 750 ml in the 4th – 7th months, and 250 ml in the last month. Routine horticultural practices for pest, disease and weed control were followed. Insecticide (Malathion) and fungicide (Benlate) were applied once every 2 weeks.

**2.6 Parameters measurements**

The growth of the black ginger plants was measured monthly by measuring the height and weight of leaves/shoots and rhizomes. The black ginger plants were randomly selected, and the rhizomes were harvested after eight months of sowing to determine their growth, yield and bioactive compound. The weight was measured immediately after harvest to prevent desiccation and water loss from the rhizomes.

**2.7 Preparation of extracts**

Rhizome samples were obtained and washed with running tap water to remove surface pollutants and cut into thin slices. They were then dried under hot air oven at 60 °C for 48 h. After drying (moisture content of 8-10% dry basis), the samples were ground into a fine powder and kept in an air-tight container before extraction. The samples were extracted with 70% methanol (1:10) under sonication for 1 h. The samples were centrifuged at 5,000 rpm for 10 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. The crude extracts were stored at 4°C till the following determinations.

**2.8 Identification and quantification of 4,5,7-trimethoxyflavone**

Before analysis, the methanolic crude extracts were filtered through a 0.22 μm pore size nylon membrane filter. Identification of 4,5,7-trimethoxyflavone was performed on high-performance liquid chromatography (HPLC). The compound was chromatographically separated using a XBRIDGE (150 mm x 4.6 mm x 3 μm) column and maintained at 40 °C. A linear binary gradient of water (0.1% formic acid) and acetonitrile (0.1% formic acid) was used as mobile phases A and B, respectively. The flow rate was set at 1 mL/min and the injection volume was 1 μL. The UV–vis absorption chromatogram was detected at 265 nm using a DAD detector. The amount of 4,5,7-trimethoxyflavone in the extracts was calculated using the regression equation of its peak area to peak area of known concentration of the standard from the calibration curve.

**2.9 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 the Duncan Multiple Range Test (DMRT) as the test of significance at p ≤ 0.05.

**Results and discussions**

**3.1 Effect of nitrogen on plant growth and yield**

The results indicate that nitrogen levels had a pronounced effect on the vegetative growth and rhizome yield of black ginger. Although plant height remained statistically similar across treatments (56–58 cm), vegetative fresh weight increased markedly with nitrogen supply, rising from 475 g at 50 ppm to a peak of 682 g at 250 ppm before slightly declining at 300 ppm. Similarly, average fresh rhizome yield per plant increased from 354 g at 50 ppm to 605 g at 250 ppm, with the rhizome‐to‐shoot ratio improving from 0.75 to 0.89. Notably, the number of tillers peaked at 150 ppm (23 tillers) and the SPAD values—a proxy for chlorophyll content and photosynthetic efficiency—were highest at 150 ppm (58), suggesting that moderate nitrogen levels optimize both leaf quality and resource allocation. The increase in vegetative fresh weight and fresh rhizome yield with nitrogen levels up to 250 ppm suggests that adequate nitrogen enhances photosynthetic activity and the partitioning of assimilates toward the economically valuable rhizomes. The slight reduction in yield at 300 ppm implies that excessive nitrogen might disrupt nutrient balance or lead to inefficient biomass allocation, a phenomenon observed in other crops [17] (Wang and Li, 2004). Moreover, the optimal rhizome-to-shoot ratio at 250 ppm underscores that there is a threshold beyond which additional nitrogen does not translate into proportional yield gains.

Investigation of nitrogen fertilization impact on *Ziziphora clinopodioides* found that nitrogen at moderate rate significantly improved plant height, fresh and biomass [18] (Hazrati et al., 2024). These observations are consistent with previous studies on ginger and other medicinal rhizome crops where optimal nitrogen fertilization enhanced yield and quality without affecting plant growth and yield [19, 20, 21] (Suhaimi et al., 2021; Mohd et al., 2023; Suhaimi et al., 2023). The proper use of nitrogen fertilizer is a proven method to enhance plant growth, boost crop yield, and improve quality [22] (Sen et al., 2020). Such findings underscore the importance of fine-tuning nitrogen application in black ginger cultivation to maximize rhizome development and overall productivity.

Table 1: Effect of nitrogen concentration on black ginger plant growth and yield after eight months of cultivation periods

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Treatment | Plant height (cm) | Vegetative fresh weight (g) | Number of tillers | SPAD value | Diameter of tiller (cm) | Average Fresh rhizome yield per plant (g) | Rhizome-to-shoot ratio |
| T1 | 56a | 475e | 22a | 44c | 0.12a | 354e | 0.75c |
| T2 | 56a | 520d | 19b | 54b | 0.12a | 410d | 0.79b |
| T3 | 58a | 558c | 23a | 58a | 0.14a | 458c | 0.82b |
| T4 | 58a | 682a | 20b | 50b | 0.13a | 605a | 0.89a |
| T5 | 58a | 657b | 21b | 52b | 0.13a | 510b | 0.77c |

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

**3.2 Effect of nitrogen on rhizome quality**

The results show that nitrogen fertilization significantly influenced the phytochemical composition of black ginger extract. At the lowest nitrogen level (50 ppm), the total phenolic content (TPC) was highest (23.582 mg CGAE/g) along with high total flavonoid content (TFC, 1.524 mg RE/g). In contrast, treatments at 100 and 150 ppm resulted in a marked reduction in both TPC (8.705 and 6.156 mg CGAE/g, respectively) and TFC (0.608 and 0.462 mg RE/g, respectively). Interestingly, the treatment at 250 ppm showed a substantial increase in the specific flavonoid compound, 4,5,7‐trimethoxyflavone (8.588 µg/mg), compared to the other treatments, suggesting that moderate to higher nitrogen levels may specifically induce the biosynthesis of this compound. Antioxidant activity, as assessed by DPPH radical scavenging (IC50) and ferric-reducing antioxidant power (FRAP), also varied with nitrogen levels. Lower IC50 values and higher FRAP values indicate enhanced antioxidant capacity. In this study, the 50 ppm treatment (IC50 of 40.495 mg/mL and FRAP of 2.557 µmol Fe/g) and the 250 ppm treatment (IC50 of 49.128 mg/mL and FRAP of 2.699 µmol Fe/g) exhibited superior antioxidant performance compared to the 100, 150, and 300 ppm treatments. These observations imply that lower and moderate-high nitrogen supplies help maintain higher levels of phenolic and flavonoid compounds, which are known contributors to antioxidant activity, while excessive or intermediate nitrogen levels may suppress these bioactive metabolites. However, research by Liava et al. (2021) found that nitrogen fertilization increased plant growth and yield but did not significantly affect oil and silymarin accumulation in *Silybum marianum.* Constract to study conducted by Seif Sahandi et al. (2019) demonstrated that nitrogen fertilization significantly influenced soluble protein content, antioxidant enzyme activity, and essential oil yield of peppermint.

In summary, the data indicate that both low (50 ppm) and moderate-high (250 ppm) nitrogen fertilization enhance the phytochemical profile and antioxidant activity of black ginger extract. The 50 ppm treatment maximized overall phenolic and flavonoid contents, whereas the 250 ppm treatment specifically boosted the accumulation of 4,5,7‐trimethoxyflavone—a compound linked to potent bioactivity. Similar studies on brown rice showed that moderate-high nitrogen concentrations at 210-260 kg/100 m2 able to increase the content of Total Flavonoid Content (TFC), β-sitosterol, stigmasterol, DPPH and OH free radical scavenging [25] (Ma et al., 2023). These findings support the idea that optimal nitrogen management is crucial for modulating secondary metabolism in medicinal plants like black ginger, aligning with recent studies that emphasize the role of nutrient supply in enhancing the production of bioactive compounds [26, 27, 28] (Chung et al., 2010; Stumpf et al., 2015; Marisa et al., 2018).

Table 2: Phytochemical content and antioxidant activity of black ginger extract

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Treatment | Total Phenolic Content (TPC)  (mg CGAE/g) | Total Flavonoid  Content  (mg RE/g) | 4,5,7-trimethoxyflavone  (µg/mg) | DPPH radical scavenging activity,  IC50 (mg/mL)\* | Ferric-reducing antioxidant power (FRAP) (μmol Fe/g) |
| T1 | 23.582 a | 1.524 a | 2.774 b | 40.495 a | 2.557 a |
| T2 | 8.705 d | 0.608 b | 2.562 b | 53.691 c | 0.868 b |
| T3 | 6.156 d | 0.462 b | 2.227 b | 71.188 e | 0.601 c |
| T4 | 17.575 b | 1.543 a | 8.588 a | 49.128 b | 2.699 a |
| T5 | 10.612 c | 0.542 b | 2.406 b | 57.186 d | 0.683 bc |

\*A lower IC50 value indicates higher antioxidant activity.

Min labeled with the same letter is not significant at P<0.05 according to Tukey’s test.

**Conclusion**

Based on the results, optimal nitrogen fertilization plays a crucial role in enhancing the phytochemical profile and antioxidant capacity of black ginger extract. Both low (50 ppm) and moderate-high (250 ppm) nitrogen levels yielded superior bioactive properties: the 50 ppm treatment maximized overall phenolic and flavonoid contents, while the 250 ppm treatment specifically increased the concentration of 4,5,7‐trimethoxyflavone, a compound associated with potent antioxidant activity. These findings indicate that fine-tuning nitrogen input can modulate secondary metabolism in black ginger, thereby potentially improving its medicinal quality and efficacy. Future research should explore the physiological mechanisms underlying these responses and consider potential interactions with other macro- and micronutrients to further optimize fertilization strategies.

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