***Original Research Article***

**Optimizing Hydroponic Nutrient Solutions for Enhanced Growth, Root Biomass, and Essential Oil Yield in the Endemic Medicinal Herb (*Plectranthus vettiveroides)***

ABSTRACT

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| *Plectranthus vettiveroides* is a valuable medicinal and aromatic plant native to the Western Ghats, known for its essential oil extracted from roots. Its natural populations are vanishing due to habitat distraction. The present study explores hydroponic cultivation as a sustainable alternative and it was conducted at the Saraswathy Thangavelu Extension Centre, Jawaharlal Nehru Tropical Botanic Garden and Research Institute, Puthenthope, Thiruvananthapuram, Kerala, India, between January 2023 and May 2023.Apical shoot cuttings of *P. vettiveroides* with ~15cm length having four nodes with apical buds and a minimum of two fully expanded juvenile leaves were harvested from 75-day-old field-grown plants and they were hydroponically grown for 70 days in six different nutrient solutions—Hoagland, Cooper, Hewitt, Steiner, Knoop, and a control with distilled water—under ambient greenhouse conditions. Among the tested media, Hoagland solution supported the most vigorous shoot growth, including increased leaf number and branching. Cooper medium showed a notable effect on pigment synthesis, yielding the highest levels of chlorophyll and carotenoids. Early and enhanced root formation was observed in Hoagland and Hewitt media, although prolonged exposure to static conditions led to excessive root elongation and tip browning. Overall, morphological assessments indicated that plants grown in nutrient-rich media exhibited superior growth and biomass accumulation compared to the control. Additionally, a strong positive correlation was observed between root biomass, photosynthetic pigment concentration, and essential oil yield, emphasizing the role of nutrient availability in regulating key physiological traits. Hydroponic cultivation with optimized nutrient media significantly enhances the growth and essential oil production. This method offers a sustainable strategy for large-scale production of root biomass and essential oils, contributing to the conservation of wild populations. |

*Keywords: [Plectranthus vettiveroides, Hydroponics, Nutrient media, Root biomass, Essential oil]*

1. **INTRODUCTION**

Medicinal plants are the cornerstone of traditional medicine and the bedrock of novel therapeutics. Plants continuously produce novel secondary metabolites, which have unique structures and favourable safety therapeutic profiles (Koehn & Carter, 2005). Even though more than six million phytochemicals have been found, only a small portion—less than 20%—of the estimated 400,000 plant species have been studied for their chemicals, showing there is a lot of opportunity to find new bioactive compounds. Nutrient availability, alongside environmental factors such as altitude, temperature, humidity, soil composition, and light intensity, plays a crucial role in regulating secondary metabolite production, particularly terpenoids, in medicinal plants. Specific microclimatic and nutrient conditions can enhance the concentration and diversity of phytochemicals in many species. The role of nitrogen (N) and phosphorus (P) on terpenoid synthesis in medicinal plants have been reviewed (Ormeno & Fernandez., 2012). Nitrogen and phosphorus have key role in the synthesis of amino acids and acetyl-CoA, which are the precursors in terpenoid synthesis (Ram *et al.,* 2006). Optimum level of N influence secondary metabolite production. For instance, low N levels reduce saponin synthesis in *Panax notoginseng* (Wei *et al.,* 2020) and excessive N suppresses saponin accumulation in *P. ginseng* and *Gynostemma pentaphyllum* (Liang *et al.,*2025; Long *et al.,* 2008; Meng *et al.,* 1999). Similarly, in *Cannabis sativa* (Cockson *et al.,* 2020) P levels modulate terpene geraniol production. In *Artemisia annua* potassium supplementation increased artemisinin content, a key sesquiterpenoid lactone (Ferreira and Janick, 1996). Drought-induced nutrient stress in *Aloe vera* enhances the biosynthesis of aloins and emodin (Liu *et al.,* 2013; Kumar *et al.,* 2017). In many medicinal and aromatic plants micronutrients enhance vegetative growth and essential oil yield (Kumar *et al.,* 2022).

*Plectranthus vettiveroides* (K.C. Jacob) N.P. Singh & B.D. Sharma, (Syn. *Coleus vettiveroides*), is an herbaceous medicinal plant belonging to the family Lamiaceae. As endemic to South India, it thrives in a unique habitat characterized by sandy soil with high moisture content and exposure to direct sunlight. The plant’s roots produce a highly aromatic, orange-red essential oil known for its broad-spectrum antimicrobial properties. This essential oil is widely recognized and utilized as a natural hand sanitizer and is an active ingredient in over 75 Ayurvedic and herbal formulations marketed both locally and internationally (Nisheeda *et al.,* 2016). Despite its medicinal value, *P. vettiveroides* is now considered extinct in the wild, primarily due to habitat destruction. However, a few farmers in Kollidam, located in the Cuddalore district of Tamil Nadu, continue to cultivate the plant to meet religious and traditional demands (Safeer *et al.,* 2013, Murugan *et al.,* 2015 & Nisheeda *et al.,* 2016). The increasing demand for its root-derived essential oil has led to issues of adulteration and substitution in the herbal drug industry, owing to a scarcity of authentic raw material.

Since 2007, *P. vettiveroides* has been conserved at the Saraswathy Thangavelu Extension Centre of Jawaharlal Nehru Tropical Botanic Garden & Research Institute, Puthenthope, Thiruvananthapuram, where the agro climatic conditions closely resemble its native habitat in Kollidam. A cultivation protocol was standardized to support its growth under these conditions (Safeer *et al.,* 2013). However, harvesting its thin, fibrous roots from the soil remains a labour-intensive process, with an estimated 30–40% of the roots lost during harvesting.

In response to these challenges, the authors explored *in vitro* root and hairy root culture techniques. The primary results indicate that, these approaches were not found to be effective, and the acceptability of *in vitro*-produced roots in herbal formulations remains limited. As an alternative, this investigation aims to establish a suitable hydroponic cultivation system for *P*. *vettiveroides*. In the first phase of this study, a nutrient medium was optimized to enhance both root biomass and essential oil yield under hydroponic conditions.

1. **MATERIALS AND METHODS**

**2.1 Plant material**

The experiment was conducted in the polyhouse of Saraswathy Thangavelu Extension Centre of JNTBGRI, Puthenthoppe, Thiruvananthapuram, Kerala (Lat 8.580697o, long 76.83503o). Top shoot cuttings of ~15cm length having four nodes with apical buds and minimum two fully expanded juvenile leaves were harvested from 75 days old field grown plants cultivated following the method suggested by Safeer *et al*. (2013) used for the hydroponic experiments. The average fresh weight of the top shoot cuttings used for the experiment was 3.5 g.

For hydroponic culture five nutrient media with varying nutrient formulations such as Cooper (Sayed *et al.,* 2019), Hewitt (Kusmiyati *et al.,* 2023), Hoagland & Arnon solution-2 (van Delden *et al.,* 2020), Knop four salt solution (Almeselmani & Moaed., 2022) and Steiner’s media (Tarin *et al.,* 2024) were selected. The nutrient contents of these media were depicted in table 1. To study the influence of different nutrient compositions, distilled water devoid of any trace element were used as a control (C).

**Table 1: Composition of nutrient media used for hydroponics cultivation of plants**

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Elements contributed | Cooper | | Hewitt | | Hoagland & Arnon solution-2 | | Knop | | Steiner | |
| Chemicals | Amount  (Gm/L) | Source | Amount  (Gm/L) | Source | Amount  (Gm/L) | Source | Amount  (Gm/L) | Source | Amount  (Gm/L) |
| Ca  N | Ca (NO3)2 | 1.003 | Ca (NO3)2 | 0.826 | Ca (NO3)2 | 0.944 | Ca (NO3)2 | 0.8 | Ca (NO3)2 | 0.68 |
| K  P | KH2 PO4 | 0.263 | KH2 PO4 | 0.218 | NH4H2PO4 | 0.115 | KH2 PO4 | 0.2 | KH2 PO4 | 0.136 |
| K  P | - | - | K2HPO4 | 0.07 | - | - | - | - | - | - |
| K  N | KNO3 | 0.583 | KNO3 | 0.303 | KNO3 | 0.606 | KNO3 | 0.2 | KNO3 | 0.62 |
| N | - | - | NH4NO3 | 0.16 | - | - | - | - | - | - |
| Mg  S | MgSO4 | 0.513 | MgSO4 | 0.37 | MgSO4 | 0.493 | MgSO4 | 0.2 | MgSO4 | 0.46 |
| Mn  Cl | - | - | MnCl2 | 0.0018 | MnCl2 | 0.00181 | - | - | - | - |
| Mn  S | MnSO4 | 0.0061 | - | - | - | - | - | - | MnSO4 | 0.002 |
| B | H3 BO3 | 0.0017 | H3BO3 | 0.00286 | H3BO3 | 0.00286 | - | - | H3 BO3 | 0.00269 |
| Cu  S | CuSO4 | 0.00039 | CuSO4 | 0.000176 | CuSO4 | 0.0008 | - | - | CuSO4 | 0.0007 |
| Na  Mo | - | - | - | - | Na2Mo O4 | 0.00012 | - | - | Na2MoO4 | 0.000126 |
| N  Mo | (NH4)6Mo7O24 | 0.00037 | (NH4) 6Mo7O24 | 0.000258 | - | - | - | - | - | - |
| Zn  S | ZnSO4 | 0.00044 | ZnSO4 | 0.000219 | ZnSO4 | 0.00022 | - | - | ZnSO4 | 0.00011 |
| Fe | Fe. EDTA | 0.079 | Fe. EDTA | 0.003 | Fe. EDTA | 0.002 | - | - | Fe. EDTA | 0.003 |
| Fe  P | - | - | - | - | - | - | FePO4 | Trace | - | - |

**2.2 Experimental Setup**

Initial studies were conducted using a non-circulating nutrient solution system. Plastic containers with a capacity of three liters were employed for cultivating the experimental plants. These containers measured 72 cm in diameter at the top, 50 cm at the base, and had a height of 19.5 cm. Each container was covered with a concave clay lid, designed with five ventilation holes (1 cm in diameter) to promote adequate aeration. One of these openings served as an entry point for introducing the shoot cuttings, ensuring that approximately 4–5 cm of the basal stem was submerged in the nutrient solution.

To maintain the upright position of the shoot cuttings and to avoid mechanical damage, non-absorbent cotton was gently used to secure them at the point of insertion. At the beginning of the experiment, each container was filled with 2.5 liters of nutrient solution, and the pH was adjusted to 5.8. Regular monitoring of pH was carried out on a weekly basis using a hand-held pH meter (Eutech- PCStester 35), and adjustments were made as required to keep the pH steady at 5.8 using 1N HCl.

Following the initial 30-day period, additional nutrient solution was added to each container as needed to maintain the original volume of 2.5 liters. This top-up process, rather than complete replacement, was repeated weekly throughout the culture period to ensure consistent nutrient availability and volume stability.

**2.3 Monitoring of Plant Growth and Estimation of Photosynthetic Pigments**

Throughout the experimental duration, the growth of the plants was carefully observed. To safeguard them from insect and pest infestations, routine manual inspections were carried out. Any insect eggs or remnants found on the plant surfaces were removed by hand to minimize interference with plant development.

At the end of the 70-day cultivation period, key morphological traits were recorded. These included plant height, number of leaves, leaf dimensions (length and width), petiole length, number and length of branches, and root length. These parameters were assessed to evaluate overall plant growth and development under the experimental conditions.

The concentration of photosynthetic pigments, specifically chlorophyll and carotenoids, was assessed in plants grown hydroponically for 70 days. Pigment extraction was performed using 80% acetone, and absorbance readings were obtained with a spectrophotometer (Tecan Spark 10M). The quantities of pigments were calculated using standard equations of Boyer,1990 based on absorbance values at specific wavelengths:

Carotenoids, which include pigments such as xanthophylls and carotenes, play essential roles in light absorption and protection against oxidative damage in plant tissues.

**2.4 Estimation of Root Biomass and Essential Oil Content**

After 70 days of cultivation, plants’ roots were carefully harvested to assess morphological characteristics and quantify total biomass. Root length was measured, and fresh roots were gently blotted using absorbent paper to remove excess surface moisture. The cleaned roots were then weighed, and the fresh biomass obtained from each treatment group was recorded for further analysis.

Subsequently, 100 grams of fresh root material from each treatment were processed for essential oil extraction. Hydro-distillation was carried out using a Clevenger-type apparatus operated at 80 °C for duration of six hours, as described by Remya et al. (2022). The distillate was collected in sterile specimen bottles. To ensure complete recovery, any residual oil adhering to the apparatus was rinsed with dimethyl ether. The oil-containing bottles were then placed in a controlled water bath maintained between 60 and 70 °C until the solvent completely evaporated, yielding pure essential oil.

Following extraction, physical attributes of the oil—including colour, consistency, density, and aroma—were noted. The final yield was expressed as volume per gram of fresh root material (v/w). Oil yield and qualitative characteristics were compared across different treatments and benchmarked against control plants grown in soil at the STEC-JNTBGRI, located in the coastal region of Thiruvananthapuram.

**2.5 Statistical Analysis**

The experiment was designed following a completely randomized design (CRD), with five replicates maintained for each treatment. To ensure reproducibility and reliability, the experiment was repeated thrice under identical conditions. Data obtained from all replicates were subjected to analysis of variance (ANOVA) to evaluate the significance of treatment effects. Post-hoc comparisons among treatment means were carried out using the Critical Difference (CD) test. All statistical analyses were performed using R software (version 4.4.1).

1. **RESULTS AND DISCUSSION**

Hydroponic cultivation offers a promising alternative for growing plant species that demand specific agroclimatic conditions, especially medicinal plants known for synthesizing pharmacologically important secondary metabolites. Unlike traditional soil-based farming, hydroponics provides a highly controlled environment where critical parameters such as nutrient availability, pH, temperature, and light intensity can be fine-tuned to match or even surpass the plant’s native habitat. This level of precision is particularly beneficial for medicinal plants, as the biosynthesis of many secondary metabolites is closely influenced by environmental stimuli (Venkatasai *et al.,* 2025).

In addition to environmental control, hydroponics conserves water, reduces the need for arable land, and lowers the risk of soil-borne diseases and contamination (Surendran *et al.,* 2017). Moreover, it allows uninterrupted cultivation throughout the year, unaffected by seasonal changes. Empirical studies have demonstrated the potential of this system in enhancing biomass and secondary metabolite yield in certain medicinal plants (Cavar Zeljkovic *et al.,* 2022). For example, *Echinacea purpurea* cultivated under tailored hydroponic conditions showed elevated levels of cichoric acid (Ahmadi *et al.,* 2021), while *Stevia rebaudiana* grown hydroponically accumulated higher concentrations of steviol glycosides (Ahmadirab *et al.,* 2024). These findings underscore the effectiveness of hydroponic systems in not only supporting plant growth but also in optimizing the production of bioactive compounds. Venkatasai *et al.,* (2025) reviewed about the external influencing factors that enhance biomass and secondary metabolite production in various plant species. The root derived secondary metabolite production through hydroponics system is also reported. For instance, high yield of ginsenosides from *Panax ginseng* (Park *et al.,* 2024), and antioxidants withanolide A and withaferin A from *Withania somnifera* have been reported (Singh *et al.,* 2023).

In the present study, apical shoot cuttings of *Plectranthus vettiveroides* were cultured in different hydroponic nutrient media to evaluate root and shoot biomass production along with the yield of root-derived essential oils. Each cutting, consisting of four nodes, and the basal portion was immersed in the nutrient solutions. Initial root development was observed at the basal stem surface, while the basal cut surface release exudates for up to two weeks, then healed and turned brownish colour, no roots were initiated from the cut surface. The sign of rhizogenesis was first noted as white tissue patches at the basal portion near the wound site, which later extend upwards to next node from the base cut ends.

The comparative growth rate of shoots and roots after 70 days of cultivation is depicted in table 2. The growth of roots from initiation to maximum elongation period observations are as follows. In Hoagland's medium, root emergence occurred within five days, with roots reaching an average length of 2.3 cm, by the 42nd day, the maximum average root length of 30.9 cm was achieved, after which root tip blackening and disintegration were observed. Similar growth patterns were noted across other media: Cooper medium: 4.04 cm (day 7), 35.8 cm (day 42), and reduced to 27.6 cm by day 70. Hewitt medium: 2.54 cm (day 7), peaking at 36.5 cm (day 49), and reduced to 25.9 cm by day 70. Knop medium: 2.36 cm (day 7), 40.0 cm (day 47), later declining to 23.5 cm. Steiner medium: 2.0 cm (day 7), 30.5 cm (day 42), reduced to 17.9 cm by day 70. Control (distilled water): 1.42 cm (day 7), with a maximum of 39.8 cm by day 70. Notably, no blackening was observed in the control, but biomass accumulation was significantly lower than in nutrient media. This indicates that days required for blackening of root tips and arresting root elongation depends on the media composition.

Among all formulations, Hoagland’s solution facilitated the earliest and most efficient root initiation. Compared to soil-based propagation method proposed in the same location by Safeer *et al.,* (2013), where root emergence typically began after two weeks, hydroponic conditions enabled visible rhizogenesis within one week. This early rooting response can be attributed to the continuous availability of dissolved oxygen and nutrients and the absence of soil-related physical resistance (Saini *et al.,* 2024; Sonneveld & Voogt., 2009; Resh., 2022).

During the initial 28–42 days of growth, roots exhibited healthy elongation due to the favourable supply of water and nutrients. However, stagnant hydroponic conditions led to diminished oxygen diffusion, eventually impairing aerobic respiration required for cellular energy production in root meristematic tissues (Buwalda *et al.,* 1994; Drew.,1997; Morard *et al.,* 2000). After this phase, prolonged stagnation induced hypoxic stress, facilitating the generation of ethylene, reactive oxygen species (ROS), and reduced ions like Fe²⁺. These stressors triggered oxidative damage, resulting in root tip necrosis and blackening—a common sign of cell death (Armstrong & Drew, 2002; Li *et al.,* 2011; Sachdev *et al.,* 2021). Such damage hindered further root elongation and compromised plant health. These results emphasize the significance of maintaining water circulation and adequate aeration in hydroponic systems to support optimal root growth and prevent physiological disorders caused by oxygen deprivation. The 70-day cultivation period provided critical insights into media-specific growth dynamics and the importance of environmental parameters in sustaining the biomass and secondary metabolite yield *of P. vettiveroides*. The morphometric and oil yield of the experimental results after 70 days of cultivation are given below

**3.1 Morphometric Analysis of Plants Grown in Hydroponics System**

The morphometric characteristic features such as plant height (cm), number of leaves, length and width of leaves (cm), length of petiole (cm), number of branches, branch length (cm) and root length (cm) were documented after 70 days of culture in selected nutrient formulations at a pH range of 5.5 to 5.8 in open sunlight and atmospheric temperature of 30-36º C. (Figure.1)

 

F

B

CV

E

D

A

**Figure.1: Morphology of plants at different nutrient medias A- Hewitt, B-Cooper, C-Hogland & Arnon, D- Steiner, E- Knop, F- Control**

The documented data were analysed by ANOVA and the results were depicted in table 2.

**Table 2: The morphometric traits of *Plectarnthus vettiveroides* cultivated in different media after 70 days**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Media | Height (cm) | No. of leaves | Leaf length (cm) | Leaf width (cm) | Petiole length (cm) | No. of branches | Branch length (cm) | Root length (cm) |
| Control | 11.50±0.79c | 9.20±1.10e | 4.90±0.26d | 4.48±0.29e | 2.46±0.15d | 0.00±0.00c | 0.00±0.00d | 19.80±1.89a |
| Cooper | 53.30±2.22a | 82.40±3.29b | 9.44±0.57b | 8.50±0.35c | 10.46±0.46a | 12.00±1.41ab | 30.60±2.97a | 27.60±1.67b |
| Hewitt | 52.70±4.44a | 66.00±3.16c | 10.24±0.17a | 9.66±0.13ab | 11.04±1.13a | 12.80±1.10a | 29.90±3.85a | 25.90±2.4bc |
| Hoagland & Arnon | 55.00±2.45a | 95.60±4.56a | 10.28±0.46a | 9.52±0.36b | 10.40±0.23a | 12.80±1.10a | 30.60±1.39a | 25.20±1.10bc |
| Knop | 40.50±2.78b | 48.00±1.41d | 8.18±0.25c | 7.52±0.36d | 6.20±0.27c | 11.20±1.10b | 11.50±1.27c | 23.50±2.69c |
| Steiner’s | 51.40±3.27a | 50.80±3.03d | 10.54±0.15a | 9.92±0.11a | 8.00±0.70b | 11.60±1.67ab | 24.70±2.78b | 17.9±1.88d |
| F-value | 169.79 | 511.53 | 192.48 | 256.41 | 154.09 | 88.32 | 139.14 | 65.27 |
| *P-value* | *<0.001* | *<0.001* | *<0.001* | *<0.001* | *<0.001* | *<0.001* | *<0.001* | *<0.001* |
| CD value @ 5% | 3.76 | 3.92 | 0.45 | 0.38 | 0.78 | 1.54 | 3.15 | 2.62 |

The present study demonstrates the significant impact of nutrient media on the vegetative performance of *Plectranthus vettiveroides* under hydroponic cultivation. Among the six media formulations tested, the Hoagland and Arnon medium consistently supported superior plant growth in terms of shoot elongation, leaf number, branching, and overall vigour. Hewitt, Cooper, and Steiner’s media also performed well, but to a slightly lesser extent. In contrast, plants maintained in Knop medium and the control (distilled water) showed markedly reduced growth, emphasizing the essential role of balanced nutrient input in hydroponic systems.

Plant height (55.00±2.45 cm) was greatest in Hoagland and Arnon medium, which is known for its comprehensive and balanced supply of macro- and micronutrients. This supports previous findings in other medicinal and aromatic plants where Hoagland medium enhanced shoot elongation and biomass accumulation due to sufficient nitrogen and potassium availability—critical elements for cellular expansion and division (Li & Cheng, 2014; Rattan *et al.,* 2021). The statistically similar performance of Hoagland, Cooper, Hewitt, and Steiner’s media suggests that a well-rounded nutrient profile is necessary for sustained shoot development.

Leaf number, an important index of photosynthetic surface and plant productivity, was also maximized (95.60±4.56) in Hoagland medium, indicating its efficacy in stimulating lateral meristematic activity. The significant differences observed among treatments further highlight that leaf production is particularly sensitive to nutrient availability. This result is in agreement with earlier hydroponic studies on *Ocimum basilicum* and *Mentha arvensis*, where nitrogen-enriched media supported prolific leaf development (Kiferle *et al.,* 2013 & Olfati *et al.,* 2012). Potassium is also significantly influencing leaf production (Inthichack *et al.,* 2012; Levine *et al.,* 2021).

Interestingly, while leaf length (10.54±0.15 cm) was similar among plants grown in Steiner’s, Hoagland, and Hewitt media, leaf width (9.92±0.11 cm) was most enhanced in Steiner’s medium. This suggests that while several media may support leaf elongation, specific formulations like Steiner’s may be more conducive to lateral leaf expansion, possibly due to their distinct nutrient ratios. A broader leaf area is associated with improved light interception and photosynthetic efficiency, both of which are crucial for biomass and metabolite production (Taiz *et al.,* 2015).

Petiole length followed a similar trend to plant height and leaf traits, with longer petioles observed in Hoagland, Hewitt, and Cooper media. The enhancement of petiole growth under nutrient-rich conditions may be attributed to improved vascular development and turgor-driven cell elongation. Control plants consistently showed reduced petiole development, further affirming the role of nutrient sufficiency in promoting morphometric traits.

Branching, a key determinant of vegetative yield and canopy architecture were significantly affected by nutrient availability. Plants in nutrient-supplemented media, particularly Hoagland and Hewitt, exhibited enhanced branching and branch elongation. In contrast, control plants failed to initiate branching, likely due to suppression of axillary meristem activity under nutrient-limiting conditions. These findings are consistent with previous reports where nutrient-deficient environments negatively affected shoot system architecture and limit shoot branching in plant species (Francis *et al.,*2023).

Root length displayed an inverse trend, with the longest roots observed in the Cooper medium and surprisingly also in the control treatment. This pattern suggests a compensatory response by plants under sub-optimal nutrient availability, wherein root elongation is promoted to explore larger volumes for nutrient acquisition. Such plasticity in root architecture under nutrient stress has been previously documented in hydroponic studies of several aromatic plants (Lopez-Bucio *et al.,* 2003; Balliu *et al.,* 2021).

The results confirm that *Plectranthus vettiveroides* responds positively to hydroponic media enriched with a full spectrum of essential nutrients. Hoagland and Hewitt media emerged as the most suitable formulations for promoting vegetative growth, branching, and shoot biomass. Steiner’s medium, while less effective in shoot-related parameters, proved beneficial for increasing leaf area, which may have implications for enhanced photosynthetic potential and essential oil yield. The poor growth observed in the control emphasizes that nutrient supplementation is not only beneficial but essential for successful hydroponic cultivation of this species. These findings provide valuable insights into optimizing nutrient management strategies for *Plectranthus vettiveroides* cultivation under soilless systems. Tailoring media composition to the specific growth objectives—be it biomass accumulation, leaf expansion, or root development—could significantly enhance the efficiency and productivity of commercial hydroponic operations.

**3.2 Biochemical Parameter (Leaf pigments) Analysis**

Photosynthetic pigments, including chlorophylls and carotenoids, are reliable indicators of plant health, nutrient status, and overall physiological performance. The assessment of photosynthetic pigments of *Plectranthus vettiveroides* grown under different hydroponic nutrient media revealed significant variations in photosynthetic pigment concentrations (Table 2), including chlorophyll a (Chl a), chlorophyll b (Chl b), total chlorophyll (T Chl), and total carotenoids (T Cr). These variations were statistically significant (p < 0.001), highlighting the influence of nutrient composition on pigment biosynthesis and overall plant physiology. Among the treatments, plants grown in Cooper medium recorded the highest values for all pigment parameters: Chl a (0.38 ± 0.03 mg g⁻¹ FW), Chl b (0.50 ± 0.07 mg g⁻¹ FW), total chlorophyll (0.89 ± 0.10 mg g⁻¹ FW), and carotenoids (0.25 ± 0.01 mg g⁻¹ FW). Steiner’s medium followed closely, supporting high levels of both chlorophylls and carotenoids. The Hewitt medium exhibited moderate pigment accumulation, while Hoagland, Knop, and especially the control (without nutrients) resulted in significantly lower pigment contents.

Chlorophyll biosynthesis is highly responsive to the availability of essential nutrients, particularly nitrogen and magnesium. Nitrogen is a core component of chlorophyll molecules and is vital for the synthesis of chloroplast proteins, while magnesium is centrally positioned in the chlorophyll structure, influencing light absorption efficiency (Marschner, 2012). The superior performance of Cooper and Steiner’s media may be attributed to their balanced and bioavailable supply of these nutrients, promoting robust chloroplast development and pigment accumulation.

The enhanced carotenoid content in these media also indicates improved antioxidant capacity, which contributes to photo-protection and stabilization of the photosynthetic apparatus under varying environmental conditions. Carotenoids play a crucial role in dissipating excess light energy and scavenging reactive oxygen species (Taiz *et al.,* 2015, Sun *et al.,* 2022). Conversely, the control plants, grown without added nutrients, showed severely diminished pigment levels (Chl a: 0.03 ± 0.00 mg g⁻¹ FW; Chl b: 0.02 ± 0.00 mg g⁻¹ FW; T Chl: 0.05 ± 0.00 mg g⁻¹ FW; T Cr: 0.01 ± 0.00 mg g⁻¹ FW). This reflects a poor physiological state likely resulting from chloroplast degradation, impaired pigment synthesis, and oxidative stress due to nutrient deficiency. Such reductions in pigment content under nutrient-poor conditions have been widely reported in hydroponic studies of medicinal and aromatic plants (Naz *et al.,* 2020). The data also revealed that while Hoagland and Arnon medium is traditionally considered effective in supporting plant growth, it performed sub-optimally for pigment biosynthesis in this study. This outcome may be due to species-specific nutrient demands or suboptimal ratios of certain microelements essential for pigment stability in *Plectranthus vettiveroides*. Such findings underscore the importance of customizing nutrient formulations to suit the physiological needs of individual plant species (López-Bucio *et al.,* 2003). The results indicate that Cooper and Steiner’s media are highly effective in enhancing the photosynthetic pigment profile of *Plectranthus vettiveroides*, which is closely linked to improved photosynthetic efficiency, plant vigor, and potential secondary metabolite production. The clear decline in pigment content in the control group confirms that nutrient supplementation is essential for maintaining functional photosynthesis in hydroponic cultivation systems.

**Table 3: Effect of different nutrient media on chlorophyll and carotenoid content in *Plectranthus vettiveroides***

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Medium** | **Chl\_a** | **Chl\_b** | **T Chl** | **T Cr** |
| Control | 0.03±0.00c | 0.02±0.00b | 0.05±0.00c | 0.01±0.00e |
| Cooper | 0.38±0.03a | 0.50±0.07a | 0.89±0.10a | 0.25±0.01a |
| Hewitt | 0.19±0.01b | 0.06±0.01b | 0.25±0.02b | 0.09±0.01c |
| Hoagland & Arnon | 0.07±0.01c | 0.05±0.00b | 0.12±0.01c | 0.05±0.01d |
| Knop | 0.07±0.01c | 0.04±0.01b | 0.11±0.01c | 0.04±0.01d |
| Steiner’s | 0.34±0.07a | 0.47±0.02a | 0.81±0.06a | 0.17±0.02b |
| F-value | 65.12 | 176.12 | 193.67 | 174.82 |
| *P-value* | *<0.001* | *<0.001* | *<0.001* | *<0.001* |
| CD value @ 5% | 0.06 | 0.05 | 0.08 | 0.02 |

**3.3 Biomass and Essential Oil Yield**

The influence of various nutrient media on biomass accumulation and essential oil production in *Plectranthus vettiveroides* revealed significant differences across treatments (p < 0.05). Shoot biomass was highest in Hewitt (141.40 ± 19.68 g), Hoagland & Arnon (135.20 ± 19.49 g), and Cooper (135.20 ± 27.99 g) media, with no significant difference among them. These results indicate the efficacy of complete nutrient formulations in promoting vegetative growth (Marschner., 2012). Root biomass was highest in Hoagland & Arnon (21.28 ± 7.63 g), followed by Cooper (15.50 ± 3.81 g), reflecting improved nutrient uptake and root vigour under balanced nutrition (Taiz *et al.,* 2015). In contrast, control and Knop media showed significantly lower biomass accumulation, indicating poor nutrient support. The root-to-total biomass ratio was highest in the control (13.94 ± 3.60%), suggesting a stress-induced shift towards root investment for nutrient foraging (Hermans *et al.,* 2006). However, similar ratios in Hoagland & Arnon and Cooper media imply balanced allocation to both roots and shoots. Essential oil yield was maximized in Hoagland & Arnon (0.120 ± 0.00 ml) and Hewitt (0.080 ± 0.00 ml), likely due to the availability of key nutrients like nitrogen, potassium, and sulphur, which are essential for terpene biosynthesis (Gershenzon & Croteau, 1991). Despite high biomass, Cooper and Steiner’s media supported lower oil yields (0.020 ± 0.00 ml), indicating that oil biosynthesis is influenced by nutrient composition, not biomass alone. The control yielded minimal oil (0.004 ± 0.00 ml), reinforcing the importance of nutrient supplementation.

Hoagland & Arnon and Hewitt media are optimal for enhancing both vegetative growth and oil production in *P. vettiveroides*. While Cooper supports biomass accumulation, it is less efficient for oil synthesis. These findings emphasize the role of tailored nutrient regimes in optimizing hydroponic cultivation for medicinal and aromatic plants.

**Table 4: Effect of different nutrient media on biomass and essential oil yield in *Plectranthus vettiveroides***

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Medium** | **Shoot biomass (gm)** | **Root biomass (gm)** | **Root and total plant biomass ratio** | **Oil Yield**  **(ml)** |
| **Control** | 10.74±1.44d | 01.74±0.60e | 13.94±3.60a | 0.004±0.00e |
| **Cooper** | 135.20±27.99a | 15.50±3.81b | 10.28±3.29ab | 0.020±0.00d |
| **Hewitt** | 141.40±19.68a | 13.30±2.64bc | 08.59±0.82b | 0.080±0.00b |
| **Hoagland & Arnon** | 135.20±19.49a | 21.28±7.63a | 13.59±2.29a | **0.120±0.00a** |
| **Knop** | 81.00±12.21c | 07.00±2.81de | 07.95±2.94b | 0.010±0.00c |
| **Steiner’s** | 106.60±11.01b | 08.70±3.25cd | 07.55±2.00b | 0.020±0.00d |

**3.4 Correlation Analysis**

Correlation analysis revealed strong, positive relationships among key vegetative traits in *Plectranthus vettiveroides*. Plant height significantly correlated with number of leaves (r = 0.89\*\*), leaf length (r = 0.96\*\*), leaf width (r = 0.94\*\*), and branch number (r = 0.95\*\*), indicating synchronized shoot development. Shoot biomass also strongly correlated with plant height (r = 0.89\*\*), petiole length (r = 0.94\*\*), and leaf count (r = 0.88\*\*), suggesting these morphological traits are key contributors to above ground biomass accumulation (Poorter *et al.,* 2012).

Root length showed significant negative correlations with most shoot parameters (e.g., r = –0.79\*\* with plant height), reflecting a resource allocation shift under nutrient-limiting conditions—a known adaptive response (Hermans *et al.,* 2006). The shoot-to-root ratio also negatively correlated with shoot traits and chlorophyll levels, reinforcing the importance of balanced biomass distribution for optimal growth (Poorter & Nagel, 2000).

Photosynthetic pigment levels, particularly chlorophyll a, showed moderate correlations with vegetative traits and shoot biomass (r = 0.52\*), suggesting a supportive, though not dominant, role of pigment concentration in growth (Lichtenthaler *et al.,* 2007). Oil yield was positively associated with number of leaves (r = 0.68\*\*), root biomass (r = 0.73\*\*), and petiole length (r = 0.62\*), indicating that both structural and physiological vigour contribute to secondary metabolite production (Gershenzon & Croteau, 1991).

Table 5: Correlation analysis of plant characters at Hoagland & Arnon media.

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Table 5: Correlation analysis of plant characters at Hoagland & Arnon media**. | | | | | | | | | | | | | | | |
|  | Plant height  (cm) | No. of leaves | Leaf length (cm) | Leaf width (cm) | Petiole length (cm | No. of branches | Branch length (cm) | Root length (cm) | Chl\_a | Chl\_b | Total  Chl | Shoot biomass (g) | Root biomass (g) | Shoot and root ratio | **Oil yield** |
| Plant height (cm) | 1.00 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| No. of leaves | 0.89\*\* | 1.00 |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Leaf length (cm) | 0.96\*\* | 0.81\*\* | 1.00 |  |  |  |  |  |  |  |  |  |  |  |  |
| Leaf width (cm) | 0.94\*\* | 0.76\*\* | 0.99\*\* | 1.00 |  |  |  |  |  |  |  |  |  |  |  |
| Petiole length (cm | 0.90\*\* | 0.90\*\* | 0.85\*\* | 0.83\*\* | 1.00 |  |  |  |  |  |  |  |  |  |  |
| No. of branches | 0.95\*\* | 0.83\*\* | 0.94\*\* | 0.92\*\* | 0.82\*\* | 1.00 |  |  |  |  |  |  |  |  |  |
| Branch length (cm) | 0.90\*\* | 0.92\*\* | 0.87\*\* | 0.85\*\* | 0.94\*\* | 0.85\*\* | 1.00 |  |  |  |  |  |  |  |  |
| Root length (cm) | -0.79\*\* | -0.52\*\* | -0.83\*\* | -0.84\*\* | -0.49\*\* | -0.84\*\* | -0.60\*\* | 1.00 |  |  |  |  |  |  |  |
| Chl\_a | 0.53\* | 0.37NS | 0.53\* | 0.50\* | 0.49NS | 0.43\* | 0.59\* | -0.47\*\* | 1.00 |  |  |  |  |  |  |
| Chl\_b | 0.38NS | 0.28NS | 0.39NS | 0.36NS | 0.28NS | 0.32NS | 0.46NS | -0.44\* | 0.93\*\* | 1.00 |  |  |  |  |  |
| T\_Chl | 0.45NS | 0.32NS | 0.46NS | 0.42NS | 0.37NS | 0.37NS | 0.52\* | -0.46\*\* | 0.97\*\* | 0.99\*\* |  |  |  |  |  |
| Shoot biomass | 0.89\*\* | 0.88\*\* | 0.88\*\* | 0.84\*\* | 0.94\*\* | 0.84\*\* | 0.90\*\* | -0.56\*\* | 0.52\* | 0.36NS | 0.57\* | 1.00 |  |  |  |
| Root biomass | 0.66\*\* | 0.81\*\* | 0.65\*\* | 0.62\*\* | 0.74\*\* | 0.62\*\* | 0.74\*\* | -0.33\*\* | 0.12NS | 0.05NS | 0.21NS | 0.82\*\* | 1.00 |  |  |
| Shoot and root ratio | -0.30\* | 0.01NS | -0.31\* | -0.32\* | -0.14NS | -0.27\* | -0.14\* | 0.46\*\* | -0.55\*\* | -0.44\*\* | -0.45\*\* | -0.12\* | 0.39NS | 1.00 |  |
| Oil yield | **0.57\*** | **0.68\*\*** | **0.56\*** | **0.57\*** | **0.62\*** | **0.57\*** | **0.54NS** | **-0.28NS** | **-0.31NS** | **-0.43\*** | **-0.27NS** | **0.55\*** | **0.73\*\*** | **0.33NS** | **1.00** |

\*\* *Significant @ both 5% and 1% \* Significant @ 5% NS – Not significant*

1. **CONCLUSION**

This study highlights the effectiveness of hydroponic systems in cultivating *Plectranthus vettiveroides* under controlled conditions. The use of specific nutrient solutions, particularly Hoagland and Hewitt media, was found to significantly improve shoot and root development, while Cooper medium supported better pigment production. A notable observation was the strong association between root biomass and essential oil content, suggesting that optimizing root growth can enhance the yield of valuable plant compounds. Overall, hydroponics presents a reliable and sustainable approach for producing quality planting material and conserving this important medicinal plant, reducing the need for wild harvesting.

**DISCLAIMER (ARTIFICIAL INTELIGENCE)**

Author hereby declares that no generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

AcknowledgEments

The authors are grateful to Dr. C.K. Biju, Principal Scientist and the Director, KSCSTE – JNTBGRI, Palode, Thiruvananthpuram, Kerala for their consistent encouragements and administrative supports. We also thankful to the University Grants Commission for funding.

Competing interests

Authors have declared that no competing interests exist.

Authors’ Contributions

Both authors have contributed equally

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