

Evaluation of Substrate Composition on Germination, Growth and Biomass of Spinach (*Spinacia Oleracea*) and Beetroot (*Beta Vulgaris*)

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Abstract

Innovative cultivation methods are required because of the growing strain that increased urbanisation and climate variability are placing on land, water resources, and agricultural sustainability. For urban food production, hydroponics—a soilless growth technique—offers a resource-efficient substitute. Under regulated hydroponic settings, the current study assessed the effects of various substrate compositions on the germination, growth, and yield performance of beetroot (*Beta vulgaris* L.) and spinach (*Spinacia oleracea* L.). Cocopeat, perlite, vermiculite, sand, LECA, and oasis cubes were among the seven substrate treatments (T1–T7) that were evaluated. The highest germination rates (71.4% in spinach and 85.7% in beetroot) as well as better vegetative growth, root development, and overall plant quality were seen in T1, which was composed of cocopeat (50%), perlite (25%), and vermiculite (25%). While imbalanced substrates produced lower performance, substrates with balanced water retention and aeration (T1–T3) greatly outperformed other treatments. The study comes to the conclusion that optimal substrate composition—specifically, the air-water balance—plays a critical role in boosting hydroponic productivity and efficiency, underscoring its potential as a scalable and sustainable solution for urban agriculture in resource-constrained areas like central India.

Keywords: Spinach, beetroot, Substrate Composition, soilless cultivation

1. Introduction

Rapid urbanization, climate variability, declining soil fertility, and limited freshwater resources pose major challenges to sustainable agriculture, particularly in developing countries like India. These factors intensify land and water competition, increase droughts and erratic weather, and reduce crop productivity (Sharma, 2025; Farooq et al., 2021). Conventional farming is further constrained by inefficient water use, soil degradation, and vulnerability to pests (Yang et al., 2025; Alemnnew & Alemayehu, 2020), while increasing water scarcity threatens food security (He & Rosa, 2023). These challenges highlight the need for innovative, resource-efficient agricultural technologies. Hydroponics is a soilless cultivation method where plants grow in nutrient-rich solutions, allowing precise control of nutrients, pH, and oxygen. This improves nutrient efficiency, plant growth, and crop quality while reducing resource losses (Savvas & Gruda, 2018; Walters & Currey, 2019). It can reduce water use by up to 80–90% and increase yields due

to optimized conditions (Graamans et al., 2018; Nikolaou et al., 2020), making it ideal for urban and peri-urban agriculture with limited land and water resources.

A comparative hydroponic experiment was conducted on beetroot (*Beta vulgaris* L.) and spinach (*Spinacia oleracea* L.) to assess the effect of seven growing substrates on early growth and yield. Substrate selection is crucial in soilless systems as it influences germination, root development, water retention, aeration, and nutrient availability, thereby affecting plant performance (Neocleous & Savvas, 2017; Barrett et al., 2016). This study aimed to identify the most suitable substrate by evaluating germination, vegetative growth traits, and biomass accumulation, key indicators of productivity in hydroponic leafy vegetables (Rouphael & Colla, 2020). Leafy vegetables and short-duration crops are well suited for hydroponics due to rapid growth, high value, and efficient nutrient uptake. Spinach (*Spinacia oleracea* L.) is rich in iron, vitamins, and antioxidants, while beetroot (*Beta vulgaris* L.) provides fibre, betalains, and polyphenols with health benefits (Bunea et al., 2020; Clifford et al., 2015). Both respond well to controlled conditions, improving growth, yield, and quality, making them ideal for evaluating substrate performance in hydroponic systems (Kyriacou et al., 2017; Rouphael & Colla, 2020).

In central India, including cities like Bhopal, research on hydroponics is limited despite rising interest in sustainable urban agriculture. The region's subtropical climate supports leafy vegetable cultivation, while increasing demand for pesticide-free produce enhances hydroponic potential (Sanyal et al., 2021). However, successful adoption requires region-specific data on crop performance, substrate suitability, and growth under local conditions to optimize systems and support sustainable urban food production (Eigenbrod & Gruda, 2015). This study evaluates the growth and yield of hydroponically grown spinach (*Spinacia oleracea* L.) and beetroot (*Beta vulgaris* L.) using different substrates under controlled conditions. By analyzing germination, vegetative growth, morphological traits, and biomass, it aims to identify optimal substrates for soilless cultivation. The findings will support hydroponics as a resource-efficient and sustainable approach for urban and peri-urban agriculture in central India (Gruda, Bisbis, & Tanny, 2022; Kozai, Niu, & Takagaki, 2020).

2. Methodology

2.1. Seed Selection: The seeds of beetroot (*Beta vulgaris* L.) and spinach (*Spinacia oleracea* L.) were chosen because of their high nutritional content, short growth cycles, and adaptability for hydroponic growing. Because of their significant sensitivity to controlled nutrient supply and environmental conditions, both crops are frequently employed in controlled environment agriculture, making them suitable model species for assessing substrate performance (Cho et al., 2020; Petropoulos et al., 2016).

2.2. Seed Sterilization: After rinsing the seeds with tap water and then distilled water, they were surface-sterilized for five minutes using 70% ethanol. To get rid of any remaining sterilising agent, the seeds were then thoroughly rinsed many times with sterile distilled water. In order to reduce bacterial and fungal interference during germination investigations, chemical surface sterilisation is a common procedure (Singh et al., 2018).

2.3. Seed Soaking: Seeds were immersed in distilled water for 1.5–2 hours to improve germination and hydration. Under hydroponic conditions, seed soaking has been demonstrated to boost water imbibition, stimulate metabolic processes, and enhance uniform germination (Paparella et al., 2015; Farooq et al., 2019).

Figure 1 – Seed Selection and Sterilization

2.4. Substrate Treatment: Sand and heavy contaminants were removed from the cocopeat by thoroughly washing it with tap water and then distilled water, allowing it to settle before being decanted (Abad et al., 2016; Barrett et al., 2016). After being washed using the same method as cocopeat, LECA balls were soaked in distilled water for an hour (Gruda et al., 2017). To get rid of fine ash particles, perlite was sieved (Savvas & Gruda, 2018). Before being used, oasis cubes were soaked in distilled water (Barrett et al., 2016). To make sure there were no pieces of rock or other coarse contaminants, vermiculite was manually examined (Gruda et al., 2017).

2.5. Substrate Preparation: To assess their impact on seed germination and early plant growth, seven distinct substrate compositions (T1–T7) were created. Due to their widespread application in hydroponic and soilless cultivation systems and their disparate physical attributes, such as porosity, water-holding capacity, drainage, and root-zone aeration, coco peat, perlite, vermiculite, sand, LECA (Lightweight Expanded Clay Aggregate), and oasis cubes were chosen (Raviv, Lieth, & Bar-Tal, 2019; Shahzad et al., 2020; He et al., 2021).

2.6. Sowing: Pre-treated seeds were sown in substrate-filled trays at a uniform depth of 1–2 cm with consistent spacing to ensure homogeneous emergence and minimize inter-seed competition. Appropriate sowing depth is a critical factor influencing seedling emergence, root establishment, and early vigor in soilless cultivation systems, as it regulates moisture availability and mechanical resistance during germination (Finch-Savage & Bassel, 2016).

2.7. Planting Conditions: The trays were put in a chamber with a controlled environment after they were sown. The temperature range for spinach (*Spinacia oleracea* L.) was kept between 18 and 22 °C, which is thought to be ideal for quick germination and consistent seedling establishment. In order to facilitate effective emergence and early growth, beetroot (*Beta vulgaris* L.) trays were kept at slightly higher temperatures of 20–25 °C. For all crops, relative humidity was controlled between 60 and 70 percent to ensure sufficient moisture availability and lessen evaporative stress during the germination stage. In hydroponic and soilless culture methods, it has been demonstrated that crop-specific temperature and humidity optimisation under controlled conditions greatly enhances germination uniformity and early seedling vigour (Hernández & Kubota, 2016; Walters & Currey, 2019).

2.8. Environmental Control: Seeds were maintained under dark, moist, and well-ventilated conditions for 7 days to ensure uniform germination (Nonogaki, 2017). After germination, a 16 h light/8 h dark photoperiod was provided for 14 days to enhance photosynthesis and vegetative growth (Bantis et al., 2018). For pot trials, plants were transferred to a covered polyhouse and acclimatized under natural conditions to reduce transplant shock and support stable growth (Shamshiri et al., 2020). Temperature (20–25 °C) and relative humidity (60–70%) were maintained throughout, as these conditions favor optimal growth, photosynthesis, and nutrient uptake in hydroponic leafy vegetables (Pennisi et al., 2020).

2.9. Nutrient Solution Preparation: In order to supply vital macronutrients like nitrogen (N), phosphorus (P), and potassium (K), as well as secondary nutrients and micronutrients like calcium (Ca), magnesium (Mg), iron (Fe), and other trace elements needed for early vegetative growth, a balanced hydroponic nutrient solution was created. The total volume of the nutrient solution was adjusted to 10 L using distilled water after stock solutions A, B, and C were made independently and mixed in a ratio of 6:3:6 g, respectively. In order to prevent fertiliser precipitation and to guarantee ionic compatibility and consistent nutrient availability, it is usual practice in hydroponics to prepare nutrient solutions using distinct stock components. Leafy vegetables grown hydroponically can benefit from such balanced nutrient formulations (Hochmuth & Hochmuth, 2018; Li et al., 2022).

2.10. Nutrient Application: During the first 7 days, distilled water was applied via fine spraying to maintain moisture and prevent desiccation (Paparella et al., 2015). After germination, seedlings received 10 mL distilled water per plant daily for 14 days to support early establishment and minimize osmotic stress (Carvalho & Amancio, 2019). Subsequently, a diluted nutrient solution (5 mL/plant/day) was applied for 25 days to promote root development and nutrient acclimation (Silva et al., 2020), after which plants were transferred to larger planters. During the pot trial phase, nutrient solution was supplied at 250 mL/plant/day for 30 days to support vegetative growth. The nutrient solution was maintained at pH 5.8–6.2, with TDS 490–800 mg L⁻¹ and EC 0.8–1.0 mS cm⁻¹ to ensure optimal nutrient availability and avoid osmotic stress, conditions suitable for spinach and beetroot in hydroponic systems (Massa et al., 2017; Trejo-Télez & Gómez-Merino, 2018).

Figure 2 – Plants growth from initial seedling phase to mature seedling and pre-harvesting period



3. Observations records

A systematic experimental schedule was followed to monitor germination, early growth, vegetative development, and key physicochemical parameters of the hydroponic system. The details are presented below:

Table 1- Experimental Design and Growth Conditions for Cultivation of Spinach and Beetroot

Stage	Duration & Dates	Conditions / Environment	Inputs / Treatment	Observation Frequency
Germination Observations	Day 0–14 (04/12/25 – 17/12/25)	Dark, moist, well-ventilated (first 7 days)	Distilled water spray	Daily until completion of germination
Early Seedling Growth	Day 15–25 (18/12/25 – 12/01/26)	Controlled conditions	Distilled water: 10 mL/day (up to Day 14); Nutrient solution: 5 mL/day (Day 15–25)	Observations at 7-day intervals

Vegetative Growth (Pot Trial Stage)	Day 26–55 (13/01/26 – 11/02/26)	Covered polyhouse (natural photoperiod)	Nutrient solution: 250 mL/day/plant	Regular monitoring
Physiochemical Monitoring Parameters	Throughout experiment	Controlled environment	pH: 5.8–6.2; EC: 800–1000 $\mu\text{S cm}^{-1}$; TDS: 0.490–0.800 ppm; Temperature: 20–25°C; Relative Humidity: 60–70%	Daily monitoring

3.1. Post Harvest data collected from pot trials plant – Spinach (*Spinacia oleracea*)

Table 2- Method: Visual and manual assessment during harvesting

Plant S. No.	No. of Buds	No. of leaves (in count)	Stem Length (in cm.)	Leave Length (in cm.)	Leave Breadth (in cm.)	Root Length (in cm.)
1	4	7	7.4, 8.5, 5, 6.4, 9, 5.5, 9.3	7, 7, 5, 8.4, 9, 6.2, 8.5	5, 5.3, 3.2, 4.5, 5.1, 2.7, 2.6	21.5
2	2	10	15, 12.2, 10.5, 10.8, 12.2, 13.5, 12, 9, 8.5, 9.5	6.5, 9.9, 11.2, 12, 8.8, 7.5, 9.2, 6.5, 7.3, 5	5.6, 4, 4, 5, 3.7, 3, 3.9, 3, 4, 3	7.5
3	2	7	10, 10.5, 12, 7.2, 7.5, 12.5, 8	8, 8, 8.5, 5.5, 5.5, 7.5, 4	4, 4.5, 3.5, 2, 2.5, 3, 2.5	8
4	4	12	8.5, 9.5, 6, 7.5, 10, 6.5, 10.3, 14.4, 9.4, 13.4, 11, 11.9	8, 8, 6, 9.5, 9, 5.2, 7.4, 8, 6.7, 9.4, 7.4, 11.5	5, 5.3, 3.2, 4.5, 5.1, 2.7, 23, 3, 2.1, 4.5, 4, 4.1	9
5	2	9	11.5, 13.5, 11, 13.5, 11, 11.9, 10.9, 13, 11.5	11.5, 10.5, 7, 10, 11, 11, 11.5, 12.6, 10	4.7, 5, 4, 3.9, 13.7, 5, 3.5, 4.5, 3.2	9.5

3.2 Post Harvest data collected from pot trials plant – Beetroot (*Beta vulgaris*)

Table 3- Method: Visual and manual assessment during harvesting

Plant S. No.	No. of Buds	No. of leaves (in count)	Stem Length (in cm.)	Leave Length (in cm.)	Leave Breadth (in cm.)	Root Length (in cm.)
1	0	4	8.5, 9, 6.5, 10	6.4, 6.8, 5.5, 5	3.5, 5, 5, 3.5	7.5
2	2	7	14.4, 14.5, 13.4, 11.2, 10.3, 12.9, 13.4	5.7, 6.2, 13.9, 12.7, 13.7, 12.8, 12	2.5, 4.5, 7, 4.5, 4.5, 5.8, 6	10.6
3	2	5	7.5, 11.5, 7, 9, 6	5.5, 11, 6, 9.5, 5.9	2, 3.5, 2, 2.9	12
4	5	4	9, 4.1, 9.5, 13.5	6.8, 4.7, 7.2, 7.3	4, 2.8, 3.5, 3.3	8.2

5	0	3	6.2, 10, 6.4	6.2, 6.8, 5.9	2.5, 4.2, 2.6	6.9
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3.3. Substrate Performance Assessment: Across all stages (T1–T7 substrates)

Table 4 – Method: Visual and manual assessment during transplanting and harvesting

Code Name	T1	T2	T3	T4	T5	T6	T7
Water retention behavior	Medium	Medium	Low	Medium	High	Low	Very Low
Aeration and drainage characteristics	Medium	High	High	Medium	Low	Medium	Very High
Root penetration and anchorage	Very High	High	High	Medium	Very Low	Low	Low
Substrate compaction or degradation	Low	Low	Low	Low	-	Medium	High
Ease of plant removal at transplanting	Excellent	Excellent	Excellent	Good	Medium	Good	Good

4. Results

4.1. Germination performance: T1 showed highest germination: 71.4% spinach (5/7 seeds) and 85.7% beetroot (6/7 seeds) within 4-5 days. T5 (oasis cube) showed very poor germination in both crops. T6 and T7 (LECA mixes) performed good but inferior to T1-T3.

4.2. Growth parameter: T1 plants exhibited maximum plant height, leaf number, root length, and highest quality scores in both spinach and beetroot. Coco peat + perlite + vermiculite combinations (T1-T3) significantly outperformed other treatments.

4.3. Substrate physical properties: T1 and T2 showed a balanced combination of moderate water retention and adequate aeration, while T3 exhibited high aeration but lower water retention. T5 had high water retention but low aeration, whereas T7 showed very high aeration with very low water retention. Coco peat dominant mixes (T1, T4, T6, T7) showed excellent moisture retention while maintaining aeration.

4.4. Root development and handling: Root penetration and anchorage were highest in T1, followed by T2 and T3, while T5, T6, and T7 showed reduced root development. Substrate compaction was minimal in T1–T4 but increased in T6 and was highest in T7. Ease of plant removal during transplanting was rated excellent in T1–T3, while other treatments ranged from good to moderate.

4.5. Overall performance: Among all treatments, T1 demonstrated the best overall performance across germination, growth parameters, and substrate characteristics. A organic farming substrate composed of cocopeat (50%), perlite (25%), and vermiculite (25%) was utilized to provide adequate aeration, moisture retention, and root support.

5. Discussion

The current study's findings show that, in organic farming settings, the composition of the substrate has a major impact on spinach and beetroot germination and early establishment. The maximum germination rate seen in T1 indicates that a suitable environment for seed imbibition and metabolic activation is created by substrates with balanced moisture retention and aeration. On the other hand, relatively poor performance in LECA-based treatments (T6 and T7) may be caused by limited moisture availability in the

early stages, whereas poor germination in T5 may be linked to excessive water retention and decreased oxygen availability.

Substrate composition also had a significant impact on vegetative growth parameters, such as plant height, leaf count, and root length. Combinations of cocopeat, perlite, and vermiculite create ideal physical conditions for plant development, as demonstrated by T1's constant superior growth, which was followed by T2 and T3. The need of preserving a balanced air–water ratio in the root zone is highlighted by the fact that, despite T3's strong aeration, its comparatively reduced water retention may have somewhat inhibited growth in comparison to T1.

The observed differences in plant performance might also be explained by the physical characteristics of the substrate. High water retention was shown by cocopeat-dominant mixtures, however the best outcomes were only obtained when paired with ingredients that enhance aeration. The necessity for equilibrium between moisture availability and oxygen diffusion for effective root functioning was highlighted by the suboptimal conditions produced by treatments with imbalanced features, such as T5 (high moisture, low aeration) and T7 (very high aeration, very low moisture).

These results were corroborated by root development and handling traits, with T1 exhibiting better root penetration, anchoring, and transplantability. While more compaction in T6 and T7 probably limited root growth, minimal compaction in T1–T4 allowed for improved root expansion. Overall, by guaranteeing sufficient aeration, moisture retention, and structural support, the substrate composition of cocopeat (50%), perlite (25%), and vermiculite (25%) (T1) offered the best conditions for organic farming development.

6. Conclusion

The current study shows that in organically produced spinach and beetroot, substrate composition is critical to germination, growth performance, and total plant development. In terms of germination rate, vegetative growth, root development, and ease of handling, the substrate combination of cocopeat (50%), perlite (25%), and vermiculite (25%) (T1) consistently shown superior performance across the assessed treatments. It was discovered that substrates with balanced aeration and water retention were better at promoting early seedling establishment and long-term plant growth, but unbalanced substrates performed worse because of either too much moisture or not enough water. The results emphasise how crucial it is to maximise the physical characteristics of growing media in order to create effective root-zone conditions in soilless farming systems. Overall, the study shows that organic farming production and resource-use efficiency can be greatly increased with an optimised substrate mix. These findings lend credence to organic farmings' potential as a workable and expandable approach to sustainable urban agriculture, especially in areas like central India that have limited water and land resources.

7. Future Scope

The long-term performance of optimised substrates under various environmental circumstances and across several crop cycles can be the subject of future research. Deeper understanding of system optimisation would come from in-depth research on nutrient dynamics, microbial interactions within the root zone, and the financial viability of various substrate combinations. The scalability and commercial viability of organic farming systems for urban and peri-urban agriculture could further be improved by extending the study to cover a larger variety of crops and including automation or precision nutrient management techniques.

8. References

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