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Ashvini M Bagal
Department of Horticulture, RCSM College of Agriculture, Kolhapur, Maharashtra, India

RD Pawar
Jr. Scientist, AICRP on Potato, ZARS, Ganeshkhind, Pune, Maharashtra, India

PR Londhe
Department of Horticulture, RCSM College of Agriculture, Kolhapur, Maharashtra, India

RA Karande
Assistant Professor, Plant Pathology Section, RCSM College of Agriculture, Kolhapur, Maharashtra, India

AB Jadhav
Associate Professor, Division of Soil Science, RCSM College of Agriculture, Kolhapur, Maharashtra, India

US Shinde
Associate Professor, Department of Horticulture, RCSM College of Agriculture, Kolhapur, Maharashtra, India

Sima A Sarvade
Assistant Professor, Department of Horticulture, RCSM College of Agriculture, Kolhapur, Maharashtra, India

Corresponding Author:
Ashvini M Bagal
Department of Horticulture, RCSM College of Agriculture, Kolhapur, Maharashtra, India

Impact of seeding density and cell spacing on biochemical parameters of spinach (*Spinacia oleracea* L.) under DWC technique of aquaponics

Ashvini M Bagal, RD Pawar, PR Londhe, RA Karande, AB Jadhav, US Shinde and Sima A Sarvade

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Abstract
A study was conducted to evaluate the effect of plant spacing and seeding density on various biochemical parameters of spinach variety All Green under Deep Water Culture technique of aquaponics during August to October, 2024. The experiment was laid out in a Factorial Completely Randomized Design (FCRD) with two replications. The experimental design included two factors *viz*., three plant spacings-S_1: 5 cm × 5 cm, S_2: 15 cm × 5 cm, and S_3: 15 cm × 15 cm and four seeding densities i.e. D_1: one seed per cup, D_2: two seeds per cup, D_3: three seeds per cup, and D_4: four seeds per cup. The biochemical parameters assessed in the study included nitrogen, phosphorus, potassium, ascorbic acid, and chlorophyll content. Among these, plant spacing and seeding density had no statistically significant effect on nitrogen, phosphorus, potassium and ascorbic acid content. However, chlorophyll content was significantly influenced by plant spacing.

Keywords: Spinach, *Spinacia oleracea*, Aquaponics, Deep water culture (DWC)

Introduction

Spinach (*Spinacia oleracea* L.), a cool-season leafy vegetable from the Amaranthaceae family is widely cultivated for its nutritional and medicinal value (Awan *et al*., 2016) [1]. It is rich in vitamins A and C, folic acid, iron, calcium, and antioxidants. It supports human health by reducing anaemia, constipation, and oxidative stress-related conditions (Yadav *et al*., 2020; Lomnitski *et al*., 2003) [17, 6]. Medicinally, it contains bioactive compounds like flavonoids, alkaloids, tannins, and glycosides, and is traditionally used to treat diabetes, asthma, inflammation, and neurological disorders (Roughani & Miri, 2019) [14].

It is a diploid (2n=12), dioecious species with tetramorphic sex expression-comprising extreme male, vegetative male, female, and rare monoecious forms (Yadav *et al*., 2020) [17]. Genetic studies link cultivated spinach to *S. turkestanica*, suggesting domestication likely occurred in Eastern or Southern Asia, including regions like Afghanistan and Pakistan (Ribera *et al*., 2021) [13].

Spinach is commonly used in traditional Indian cuisine by mixing its leaves into wheat flour to prepare dishes like roti, puri, and paratha. Due to its nutritional richness and adaptability, it shows great potential for agricultural diversification and addressing malnutrition (Yadav *et al*., 2020) [17]. The crop thrives with minimal inputs and can be cultivated even on marginal lands. Moreover, its high tolerance to cold makes it suitable for fresh-market production and protected cultivation systems like high tunnels (Black *et al*., 2008) [2].

Spinach productivity is affected by both plant spacing and seed density. Reducing spacing to increase planting density has been found to enhance both leaf yield and overall biomass production (Maboko & Du Plooy, 2009) [7]. Alternatively, wider spacing enhances vegetative characteristics including greater plant height and leaf expansion, attributed to better resource availability and microclimate conditions (Woldu *et al*., 2019) [16].

Soilless culture enables sustainable intensification by reducing dependence on arable land and freshwater, while ensuring high productivity. With controlled environmental parameters, it supports healthy plant growth and efficient nutrient uptake, leading to rapid development and higher yields. The system requires minimal inputs-using only one-fifth the land and onetwentieth the water compared to conventional farming making it more environmentally sustainable. Nevertheless, the closed-loop nature of the system demands vigilant monitoring and effective disease management (Fussy & Papenbrock, 2022) [4]. These factors are motivating the transition to innovative cultivation practices.

The soilless cultivation technique enhances vegetable crop production by enabling plant growth in mediums other than soil, with essential mineral nutrients administered directly to root systems through irrigation. Hydroponics, aeroponics, and aquaponics constitute the primary forms of this cultivation method.

Aquaponics, an integrated food production system combining aquaculture and hydroponics, facilitates efficient use of resources by reducing water usage to about 10 per cent of that in traditional soil-based horticulture. This method is well-suited for urban and resource-limited rural settings, sharing advantages with hydroponics and recirculating aquaculture systems. Aquatic animal waste is biologically processed by bacteria to generate nutrients absorbed by plants, which in turn clean the water before it is recycled back to the aquatic tanks (Ranawade *et al*., 2017) [12]. The synergistic integration of these components enhances overall system productivity. Considering the critical dependence of future agriculture on water and land availability, aquaponics presents a promising sustainable solution (Verma *et al*., 2020) [15].

Despite its potential, limited studies exist on optimizing spinach production in aquaponic systems under Indian conditions. Thus to address this, the present study was conducted to determine its feasibility within aquaponics, at the Landcraft Agro Aquaponics Unit located A/p. Hatkanangale, Tal. Hatkanangale, Dist. Kolhapur.

Materials and Methods

The present research was conducted to evaluate the effect of plant spacing and seeding density on various biochemical parameters of spinach (*Spinacia oleracea* L.) variety All Green under Deep Water Culture technique of aquaponics during August to October, 2024.

Experiment comprised two factors, *i.e.* three cell spacings {(S_1: 05 cm x 05 cm (72 cell sheet), S_2: 15 cm x 05 cm (36 cell sheet), S_3: 15 cm x 15 cm (18 cell sheet)} and four seeding densities {(D_1: 01 seed/cup, D_2: 02 seeds/cup, D_3: 03 seeds/cup, D_4: 04 seeds/cup)}. The seeds of selected variety All Green were sown during August, 2024 according to different treatments of seeding densities in netpots. The netpots were then placed on raft sheets in last quarter of August as per the spacing treatments making it to twelve different treatment combinations {T_1-S_1D_1 (Seeding density of 1 seed/cup with cups spaced at 05 cm x 05 cm), T_2- S_1D_2 (Seeding density of 2 seeds/cup with cups spaced at 05 cm x 05 cm), T_3-S_1D_3 (Seeding density of 3 seeds/cup with cups spaced at 05 cm x 05 cm), T_4- S_1D_4 (Seeding density of 4 seeds/cup with cups spaced at 05 cm x 05 cm), T_5- S_2D_1 (Seeding density of 1 seed/cup with cups spaced at 15 cm x 05 cm), T_6- S_2D_2 (Seeding density of 2 seeds/cup with cups spaced at 15 cm x 05 cm), T_7- S_2D_3 (Seeding density of 3 seeds/cup with cups spaced at 15 cm x 05 cm), T_8- S_2D_4 (Seeding density of 4 seeds/cup with cups spaced at 15 cm x 05 cm), T_9- S_3D_1 (Seeding density of 1 seed/cup with cups spaced at 15 cm x 15 cm), T_{10}- S_3D_2 (Seeding density of 2 seeds/cup with cups spaced at 15 cm x 15 cm), T_{11}- S_3D_3 (Seeding density of 3 seeds/cup with cups spaced at 15 cm x 15 cm), T_{12}- S_3D_4 (Seeding density of 4 seeds/cup with cups spaced at 15 cm x 15 cm)}.

Plants evaluated in Factorial Completely Randomized Design (FCRD) with two replications. The five plants were selected randomly for recording various observations on biochemical parameters including leaf nutrient analysis for the major nutrients of nitrogen, phosphorus and potassium with leaf Chlorophyll content (mg/100 g), and Ascorbic Acid Content (mg/100 g)}.

Results and Discussion

The analysis of variance revealed non-significant differences among the factors and treatments for the traits studied. Due to the continuous circulation of the nutrient-enriched solution across the hydroponic system, uniform nutrients availability is ensured for all plants. As a result, biochemical parameters measured across treatments did not differ significantly.

Leaf N Content (%)

Cell spacing recorded non-significant influence on total Nitrogen content in spinach leaves (Table 1). Maximum N content (3.23%) was recorded at a cell spacing of 05 x 05 cm (S_1) and a minimum nitrogen content (3.00%) observed at cell spacing of 15 x 15 (S_3). The influence of seeding density also not statistically significant. Among the treatments, D_3 (three seeds/cup) resulted in the higher nitrogen concentration (3.32%), followed by D_1 (3.28%), D_2 (3.12%), and D_4 (2.84%). These outcomes align with previous studies in lettuce by Gonnella *et al*. (2003) [5]. A non-significant interaction effect between spacing and seeding density was recorded for nitrogen.

Leaf P Content (%)

Cell spacing had no significant impact on total phosphorus content in spinach leaves, with a uniform value of 0.05% recorded across all spacing treatments. Similar findings were reported by Maboko and Du Plooy (2013) [8] in tomatoes and Maludin *et al*. (2020) [9] in Pakchoy under raft-based and closed hydroponic systems, respectively. No significant influence of seeding density was recorded on total phosphorus levels in spinach leaves. Although the D_2 treatment (two seeds per cup) recorded a slightly elevated value (0.06%), the remaining densities produced similar readings (0.05%). These results corroborate those of Gonnella *et al*. (2003) [5] in lettuce grown at varying densities (Table 2). There was no notable interactive impact of plant spacing and seeding density on the phosphorus content of spinach.

Leaf K Content (%)

Variations in inter-plant spacing did not result in significant differences in total potassium content in spinach leaves, which ranged from 2.01% to 2.09%. The higher potassium concentration was recorded under the densest spacing (S_1: 05 × 05 cm), while the lowest was found at the widest spacing (S_3: 15 × 15 cm). These findings are supported by Maboko & Du Plooy (2013) [8]. No statistically significant differences were observed in potassium content as a result of varying seeding densities. A peak K content of 2.15% was noted in the D_4 treatment (four seeds per cup), followed by D_2 with 2.06%. While D_3 treatment (three seeds per cup) recorded the minimum potassium content (1.99%) (Table 3). These results are in agreement with the findings of Gonnella *et al*. (2003) [5] in lettuce cultivated under hydroponic conditions. While Potassium were unaffected by the interaction of spacing and seeding density.

1.4 Leaf Chlorophyll Content (mg/100 g)

Plant spacing had a significant impact on chlorophyll accumulation in spinach leaves. The highest chlorophyll content (3.75 mg) was recorded at the widest spacing of 15 × 15 cm (S_3), followed by 15 × 5 cm spacing (S_2), which yielded 3.39 mg chlorophyll content. In contrast, the significantly lowest chlorophyll content (2.76 mg) was observed under the closest spacing of 5 × 5 cm (S_1). These results indicate that wider spacing facilitates better chlorophyll development in spinach leaves (Table 4). A similar trend was observed by Noboa *et al*. (2022) [10] in kale. No statistically significant variation was observed across different seeding densities. The maximum chlorophyll concentration (3.36 mg/100 g) was observed at a seeding density of four seeds per cup (D_4), while minimum chlorophyll levels (3.26 mg/100 g) were found in both one seed (D_1) and three seeds per cup (D_3) treatments. These findings are in agreement with results earlier reported by Dalave (2022) [3] in dill. The interaction between spacing and seeding density did not significantly influence chlorophyll content in spinach leaves (Table 4).

1.5 Ascorbic Acid Content (mg/100 g)

Ascorbic acid content ranged across different cup spacings, with no statistically significant differences. The maximum value was recorded at 15 × 15 cm spacing (S_3), while the minimum was observed at 5 × 5 cm (S_1) (Table 5). Wider spacing enhanced ascorbic acid content in spinach, likely due to better light exposure and reduced stress, supporting findings by Quy *et al*. (2016) [11] in hydroponic lettuce. Seeding density had no significant impact on the ascorbic acid levels in spinach leaves. The maximum content (27.51 mg per 100 g fresh weight) was recorded in the treatment with three seeds per cup (D_3), followed by four seeds (D_4) and two seeds per cup (D_2), which yielded 26.91 mg and 26.30 mg, respectively. The minimum value (25.53 mg/100 g) was observed in the single-seed treatment (D_1). No significant interactive effect of spacing and seeding density on ascorbic acid content was observed in spinach.

Table 1: Effect of cell spacing and seeding density on leaf N (%) content in spinach

	S_1 (05 x 05 cm)	**S_2 (15 x 05 cm)**	**S_3 (15 x 15 cm)**	**Mean (D)**
D_1 (01 seed per cup)	3.46	3.44	2.93	3.28
D_2 (02 seed per cup)	3.30	2.72	3.34	3.12
D_3 (03 seed per cup)	3.37	3.37	3.22	3.32
D_4 (04 seed per cup)	2.79	3.21	2.52	2.84
Mean (S)	3.23	3.19	3.00	3.14
	SEm(±)	CD at 5%	CV (%)	
Spacing (S)	0.11	NS	9.57	
Density (D)	0.12	NS		
Interaction (S x D)	0.21	NS		

Table 2: Effect of cell spacing and seeding density on leaf P (%) content in spinach

	S_1 (05 x 05 cm)	**S_2 (15 x 05 cm)**	**S_3 (15 x 15 cm)**	**Mean (D)**
D_1 (01 seed per cup)	0.05	0.05	0.07	0.05
D_2 (02 seed per cup)	0.06	0.06	0.06	0.06
D_3 (03 seed per cup)	0.05	0.05	0.05	0.05
D_4 (04 seed per cup)	0.05	0.06	0.04	0.05
Mean (S)	0.05	0.05	0.05	0.05
	SEm(±)	CD at 5%	CV (%)	
Spacing (S)	0.002	NS	13.00	
Density (D)	0.003	NS		
Interaction (S x D)	0.005	NS		

Table 3: Effect of cell spacing and seeding density on leaf K (%) content in spinach

	S_1 (05 x 05 cm)	**S_2 (15 x 05 cm)**	**S_3 (15 x 15 cm)**	**Mean (D)**
D_1 (01 seed per cup)	2.02	1.94	2.04	2.00
D_2 (02 seed per cup)	1.99	2.20	1.98	2.06
D_3 (03 seed per cup)	1.98	1.97	2.04	1.99
D_4 (04 seed per cup)	2.40	2.05	2.00	2.15
Mean (S)	2.09	2.04	2.01	2.05
	SEm(±)	CD at 5%	CV (%)	
Spacing (S)	0.06	NS	8.78	
Density (D)	0.07	NS		
Interaction (S x D)	0.13	NS		

Table 4: Effect of cell spacing and seeding density on leaf chlorophyll content (mg/100 g) in spinach

	S_1 (05 x 05 cm)	S_2 (15 x 05 cm)	S_3 (15 x 15 cm)	Mean (D)
D_1 (01 seed per cup)	2.90	3.37	3.52	3.26
D_2 (02 seed per cup)	2.87	3.38	3.70	3.32
D_3 (03 seed per cup)	2.65	3.16	3.98	3.26
D_4 (04 seed per cup)	2.62	3.66	3.81	3.36
Mean (S)	2.76	3.39	3.75	3.30
	SEm(±)	CD at 5%	CV (%)	
Spacing (S)	0.07	0.20	5.68	
Density (D)	0.08	NS		
Interaction (S x D)	0.13	NS		

Table 5: Effect of cell spacing and seeding density on ascorbic acid (mg/100 g) content in spinach

	S_1 (05 x 05 cm)	S_2 (15 x 05 cm)	S_3 (15 x 15 cm)	Mean (D)
D_1 (01 seed per cup)	25.11	25.80	25.69	25.53
D_2 (02 seed per cup)	26.26	26.49	26.15	26.30
D_3 (03 seed per cup)	27.98	26.50	28.05	27.51
D_4 (04 seed per cup)	25.57	27.07	28.10	26.91
Mean (S)	26.23	26.46	26.99	26.56
	SEm(±)	CD at 5%	CV (%)	
Spacing (S)	0.45	NS	4.82	
Density (D)	0.52	NS		
Interaction (S x D)	0.91	NS		

Conclusion
The uniformity in environmental conditions and unrestricted nutrient availability within the system ensured equal nutrient access for all plants, thereby resulting in statistically non-significant differences among treatments for the biochemical parameters studied.

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