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**Growth and Nutritional Value of Broccoli in “substrate-based soilless culture”
under an Improved Nutrient Solution**

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ABSTRACT:

This study aimed to optimize the Brassica solution in various growing media (coco-peat+perlite, silty-sand, and sand). The study was conducted at the Sari Agricultural science and Natural resource university of Sari, Iran. The first factor was growing media (coco-peat+perlite, silty-sand, and sand) and second factor was the nutrient solution consisting Hoagland (N1), Brassica-SANRU solution (N2), Brassica-SANRU solution at the vegetative stage plus additional mono-potassium phosphate at the reproductive stage (N3), Brassica-SANRU solution with extra mono-potassium phosphate at both the vegetative and reproductive stages (N4), and Brassica-SANRU solution in the vegetative stage combined with extra potassium sulfate in the reproductive stage (N5). Yield related traits and leaf nutrient of cultivated in these circumstances Broccoli (*Brassica oleracea* var. *italica*) was measured. Results showed that the highest curd fresh weight (246.37 g) was achieved in plants grown in coir-perlite medium combined with N4 solution, although there was not significance difference among N2 and N3 in this media and N4 in sand. In term of curd fresh weight, all combined treatments including N2, N3, N4 and N5 in coir-perlite medium showed a significant superior compared to the control Hoagland in sand and silty- sand medium. Plants grown in silty-sand using N1 showed significantly delayed in harvest time (88.66 days after transplant) compared to most of other treatments however this difference was significant with

plants grown in coir-perlite with N4 solution that harvested 82.66 days after transplant. Plants in coir-perlite and silty-sand medium with N4 solution had the highest leaf potassium content (5.052 %). Moreover, plants in silty-sand with N4 solution in silty-sand and coir perlite had higher levels of leaf calcium (2.25 and 1.56 %) respectively. Also, the highest leaf magnesium (0.57 %) was recorded with the application of N4 in silty-sand medium. According to obtained results, in sand and silty-sand media, increasing the phosphorus and potassium levels in the nutrient solution throughout the growing period enhances the broccoli yield. Whereas, coir-perlite medium with improved solution; N2 (Brassica-SANRU solution) throughout the growth period is sufficient for optimal broccoli growth without any need for extra potassium and phosphorus application.

Keywords: Brassica, Soilless culture, Growing medium, Plant nutrition, Mineral deficiency

INTRODUCTION

Broccoli (*Brassica oleracea* var. *italica*) is an important vegetable from cruciferous family that is cultivated worldwide. As one of the most nutritious crops in the cabbage family, it contains significant amounts of vitamin C, vitamin A, thiamine, riboflavin, niacin, and essential minerals such as calcium, phosphorus, potassium, and iron (KUMAR *et al.* 2011).

High yields and quality of broccoli depend on the adequate supply of water and minerals in the growing medium. In agriculture, due to various and significant constrains, soilless cultivation offers high potentials. Nowadays, soilless systems are widely used especially for production of vegetable crops, mainly due to factors such as the limited availability of suitable soil for intensive vegetable production (KHOSHGOFTARMANESH *et al.* 2016). Even in regions where soil is not a limiting factor, soilless systems offer several advantages over traditional soil cultivation for growing a variety of vegetables (SAHIN 2023). These benefits include increased yields, healthier and more uniform crop production, conservation of water and land, control of environmental pollution, and reduced labor requirements (ERDAL *et al.* 2023).

The growing medium plays a crucial role in the soilless or hydroponic systems. Different substrates, alone or in mix, may be used for hydroponic production of crops. Such medium should possess adequate water-holding capacity and cation exchange capacity without any adverse or harmful effects on plant growth (EBRAHIMI *et al.* 2012). On the other hand, the hydroponic medium should be easily drained to provide adequate aeration, and it needs to be available at a low cost (CANTLIFFE *et al.* 2007). The type and ratio of components in a mix medium can alter its properties such as the needed solution applications, water use efficiency and overall plant yield (SILBER & BAR-TAL 2008). In a study conducted by SARKAR *et al.* (2021), it was found that growing lettuce in a mixture of coco-peat, sawdust, and rice husk yielded the highest, while lettuce grown in sawdust produced the least fresh yield. In another study, the highest biomass of lettuce leaves was achieved using the coir-perlite medium among different substrates of wood fibers, sheep wool, mineral fibers and coir-perlite (FERBY *et al.* 2023). The maximum tomato fruit yield was also recorded in the 100% coco-peat treatment compared to other treatments including charcoal, biochar, rice husk, and corn straw in combination with coco-peat (SHARDA *et al.* 2023).

Phosphorus is an essential mineral and a significant component of photosynthetic metabolism in plant cells, particularly in the form of sugar phosphates. Phosphorus is also necessary for energy

transformation and efficiency in the form of ATP synthesis (TAIZ & ZEIGER 2002). In general, phosphorus deficiency suppresses plant growth and photosynthesis in various species (MENG *et al.* 2021). In Romaine lettuce, the yield as well as photosynthetic performance, including net photosynthesis, stomatal conductance, quantum yield, and electron flow were significantly reduced by low levels of phosphorus in nutrient solution (NEOCLEOUS & SAVVAS 2019).

Potassium serves as an activator in various metabolic processes, including carbon and nitrogen metabolism. It also influences protein synthesis, sugar transport and crop yield (UTHMAN & GARBA, 2023). Potassium plays a crucial role in the transport of sucrose from source to sink, stomatal conductance, cytoplasmic ion movement and cell elongation (MOURA *et al.* 2023; AHMED *et al.* 2023). Potassium stimulates and regulates ATPase activity in the plasma membrane. This activity leads to the creation of acidic conditions, which subsequently initiate cell wall loosening, cell expansion and plant growth (MOURA *et al.* 2023; OOSTERHUIS 2014). In a study conducted by ALMEIDA *et al.* (2020) using two treatments of complete nutrient solution and macronutrient removal on broccoli, the results showed that the removal of potassium resulted in an increase of 40% in soluble sugar content and a 60% increase in starch levels in broccoli leaves. Deficiencies in nitrogen, potassium, and calcium can cause severe damage to the chlorophylls and photosynthetic production of plants that finally results in stunted growth, low quality and disrupted nutritional balance of broccoli (ALMEIDA *et al.* 2020).

The Brassica-SANRU solution was first developed in 2014 at Sari University of Agricultural Sciences and Natural Resources (SANRU). This nutrient solution was specifically designed for the outdoor soilless cultivation of broccoli, although it can also be applied to other members of the Brassica family. It was subsequently evaluated in different pilot outdoor “substrate-based soilless cultures” (GHASEMI *et al.* 2014). The elements contained in the Brassica-SANRU solution are shown in Table 1. The objectives of this study were to further optimize this specific nutrient formula for broccoli in “substrate-based soilless culture” production, by selecting coco-peat+ perlite, silty-sand, and sand as cost-effective and natural material as well as to recognize in which growth stage the critical roles of potassium and phosphorus in broccoli plant is more important.

MATERIALS AND METHODS

EXPERIMENTAL DESIGN AND GROWTH CONDITIONS This experiment was conducted in a factorial format based on a randomized complete block design (CRBD) with two factors and three replications at the Sari Agricultural Sciences and Natural Resources University, Iran (36.5659° N, 53.0586° E; altitude –10 m). Factorial arrangement was used to investigate main and interactive effects of medium and nutrient solution and CRBD to control environmental heterogeneity.

The first factor involved the cultivation medium, which consisted of coco-peat 70% + perlite 30% (by volume), silty-sand, and sand. The second factor was the type of nutrient solution in five levels including Hoagland (N1), 1950 version, Brassica-SANRU solution (N2), Brassica-SANRU solution applied in the vegetative stage and with extra mono potassium phosphate (KH_2PO_4) in the reproductive stage (N3), Brassica-SANRU solution with extra KH_2PO_4 in both vegetative and reproductive stages (N4), Brassica-SANRU solution in the vegetative stage + extra potassium sulfate (K_2SO_4) in the reproductive stage (N5). The macro and micronutrients in different nutrient solutions and the electrical conductivity (EC) and pH of the nutrient solutions are given in Table 1.

Broccoli seedlings of the BARON F1 Hybrid variety, obtained from Elite Seed in the Netherlands, were transplanted from seedling trays to black plastic pots (5 L volume) at the two-true leaf stage. After transplantation, the seedlings were irrigated with tap water for one week, and then the application of nutrient solution was commenced. The nutrient solution was applied every three days throughout the broccoli growth period until harvest. In general, after three applications of the nutrient solution, the pots were washed thoroughly with extra water to prevent nutrient accumulation and salinity.

Before using nutrient solutions for the pots, the pH and electrical conductivity (EC) of the solutions were measured, and the pH was adjusted to a range of 6.5 to 7.0. Each pot received 400 mL of nutrient solution (in each application), until the curd formation stage. The volume of applied nutrient solution was increased to 600 mL/pot at the onset of curd development, and by the end of the growing season, due to the increased need of the plants, the solution volume was further raised to 800 mL for each pot. We studied broccoli growth and yield in substrate-based soilless culture with different nutrient solution from two-true leaf stage to harvest.

MORPHOLOGICAL TRAITS MEASUREMENT At the curd harvesting stage, yield-related traits such as curd diameter, curd fresh and dry weight, stem length, leaf fresh and dry weight, and leaf length were measured. The curd harvesting time was defined as the swelling of the first buds (FELLER 1995).

LEAF CHLOROPHYLL, NUTRIENT AND CURD TOTAL PHENOL ASSAY Total leaf chlorophyll content was determined using the method presented by CARTER & KNAPP (2001). The absorbance of the extracts was measured at 665.2 nm, 652.4 nm, and 470 nm using spectrophotometer. The chlorophyll content was subsequently calculated based on the method described by LICHTENTHALER & BUSCHMANN (2001) using Equation (1):

$$\text{Chlorophyll a} : 16.72 A_{665.2} - 9.16 A_{652.4}$$

$$\text{Chlorophyll b} : 34.09 A_{652.4} - 15.28 A_{665.2}$$

The nutrient content of the leaves was measured from samples taken from leaves after curd formation stage. After being washed with distilled water, the leaves were dried in an oven at 60°C. The dried samples were then ashed in a furnace at 550°C for 4 hours to eliminate organic matter. Following this, the ashes samples were digested with 25% hydrochloric acid and 10 mL of warm deionized water. The mixture was filtered using Whatman filter paper in preparation for measuring mineral elements. The potassium and calcium content in the leaf extract was measured using the flame photometry method (STEPHAN *et al.* 2013). The leaf nitrogen content was measured by spectrometry, using a Nitrogen domas analyzer NDA 702, Vep, Italy. To measure phosphorus, 10 mL of ammonium Vanado-molybdate reagent was added to 10 mL of tissue extract in a 100 mL flask, and the solution was brought to volume. After 30 minutes, the absorbance was measured at 410 nm (OLSEN 1954). The ASTM spectrophotometric method and test were used to measure magnesium in leaves using an Agilent model 240 FS atomic absorption spectrophotometer. Toxicity, optimum and deficiency range of nutrient elements adapted from UCHIDA (2000). Total phenol in curd represents the total antioxidant agent, was assessed using the colorimetric Folin-Ciocalteu method. 20 µL of the curd extract was added to a tube, and 1.6 mL of ultrapure water

and 100 µL of 2-fold diluted Folin-Ciocalteu reagent were added. Then were incubated for 5 min, at 37 °C, and 300 µL of saturated Na₂CO₃ (1Mol) was added, the mix was put for 30 minutes in a water bath set at 40 C. The absorbance at 765 nm was measured against a reagent blank using a spectrophotometer (Hanon instrument i3), and gallic acid was used as the reference standard to measure total phenol in samples (SLINKARD & SINGLETON 1977).

DATA ANALYSIS All data was checked for normality and after that statistical analysis was conducted. The statistical analysis software SAS (V.9.2, Cary, NC, USA) was used to determine significant differences by analysis of variance (ANOVA), and mean separation was performed using LSD multiple range test at a probability of $p < 0.05$. Graphs were drawn using Excel 2016 software.

RESULTS

MORPHOLOGY, BIOMASS, AND PHENOL CONTENT

The results of analysis of variance showed that the interaction between culture medium and nutrient solution on curd fresh weight, stem length, and leaf total dry weight was significant at ($P < 0.01$) probability level. Additionally, their interaction on leaf fresh weight, curd harvesting time (at a given size) and the curd total phenol content was significant at ($P < 0.05$) probability level (Table 2).

The highest curd fresh weight in broccoli was achieved using coir-perlite medium combined with the N4 solution (Brassica-SANRU solution with extra mono-potassium phosphate in both the vegetative and reproductive stages). However, there was no significant difference in curd fresh weight among the N4, N2 and N3 solutions when applied in coir-perlite medium and with plants grown in sand medium with the N4 solution. In comparison with control Hoagland solution in silty- sand and sand medium, all combined treatments including N1, N2, N3, N4 and N5 in coir-perlite medium were significantly superior (Figure 1).

Due to results of simple effects of medium and nutrient solution on curd dry matter, plants grown in the silty- sand substrate showed significantly more dry matter compared to the coir- perlite medium (Figure2). In terms of nutrient solution, plants grown in N5 solution exhibited the significant highest curd dry matter percentage, while N2, N1 and N3 solution revealed the lowest dry matter percentage respectively which did not show any significant difference from each other (Figure 3).

Due to significant interaction effects of medium and nutrient solution on stem length, the shortest broccoli stem (18.73 cm) observed in the plants N4 solution with the coir-perlite medium and the longest broccoli stem recorded in plants grown in coire-perlite medium with N1 (Figure 4).

The highest leaves fresh weight observed in plants grown in coir-perlite medium with N4 solution; however, it showed no significant difference with the N2 and N3 in the same medium, and with N3 and N4 solutions in the silty-sand medium. The leaves fresh weight produced in the sand medium was significantly lower than that in the other two substrates (Figure 5). Plants grown in different mediums with Hoagland solution (N1), showed no significant difference from each other

in plant leaf fresh weight (Figure 5). Coire-perlite with N2, N3 and N4 solutions; and silty- sand under N4 solution resulted in significantly improved plant leaf fresh weight respectively.

Based on significant interaction effects of medium and nutrient solution, the highest leaf dry weight observed in plants treated with N4 solution in silty-sand and coir-perlite medium, followed by N3 and N2 solutions in the coir-perlite medium. Conversely, the N5 solution consistently resulted in the lowest plant leaf dry weight across all the used media, as illustrated in Figure 6.

The shortest DAT as an indicator of earliness was observed in plants grown in coir-perlite medium fertigated with the N4 solution, with an average of 82.66 days that significantly was shorter than the plants grown in silty-sand with the Hoagland solution, with an average of 88.66 days (Figure 7).

The highest curd total phenol content was recorded in the plants treated with N3 solution in sand media, which did not differ significantly from the plants in the coir-perlite media with N3 solution (Figure 8). Plants grown in sand medium supplied with N1 solution had the lowest curd total phenol content.

Leaf total Chlorophyll and Nutrients elements

The results of the analysis of variance indicated that interaction between growing medium and nutrient solution on leaf total chlorophyll, as well as the leaf N, K, P, Ca and magnesium concentrations was significant ($P < 0.01$) (Table 3).

The highest leaf chlorophyll content was observed in Coir-perlite + N4 and Sand+ N5 respectively. On the contrary, the lowest leaf chlorophyll content was observed in plants grown in the silty-sand medium with N1 solution, which did not differ significantly from the N1 solution in coir-perlite and sand media (Figure 9).

The results showed that based on the interaction effects of medium and nutrient solution, the highest leaf nitrogen concentration was observed in plants grown in sand with N2 and in silty-sand with N4 solution (Figure 10) that showed only significant difference to leaf nitrogen concentration in plants grown in silty-sand with N2.

The highest leaf phosphorus was obtained in plants grown in coir-perlite with N4 nutrient solution, which was not significantly different with plants grown in silty-sand and sand with the same nutrient solution (Figure 11). The lowest leaf phosphorus was observed in plants grown in silty-sand and sand media with Brassica-SANRU solution.

Due to interaction effects of medium and nutrient solution, the highest leaf K was in silty-sand and coir-perlite media and with N4 solution (Figure 12). Conversely, the lowest leaf K recorded in plants grown in sand medium with both N2 and N3 solutions.

The highest leaf calcium concentration was recorded in the in silty-sand medium with N4 solution and the lowest leaf calcium concentrations observed in plant grown in silty sand medium with N5 solution. (Figure 13)

Plants grown in silty-sand with N4 solution had the highest leaf magnesium level, which did not showed significant different with plants grown in coir-perlite with N2, N3, and N4 and silty-sand with N1, and N3 solutions (Figure 14).

All plant had normal nitrogene content (3-5%) in leaves (Figure 15). The plants grown in Coir-perlite and silty-sand media with N4 solutions had the highest leaf potassium content (more than 4%) while plants grown in silty- sand with N1, N2, N5 and coire-perlite with N5 and sand with

N2, N3 and N5 showed deficiency. Other treatments leaf K was in normal range among all treatments.

In case of calcium plants in silty-sand+ N3, N4 and coir-perlite with N2, N3, N4 and N5 had optimum calcium in their leaves. Whereas, plants in silty-sand with N1, N2, N5, coir-perlite+N5 and sand+ N1, N2, N3, N4, N5 showed calcium deficiency. The leaf Mg in different treatments was in normal conditions (ranged from 0.2-0.7) except for plants grown in the sand with N1 and silty-sand with N2, and N5 treatments that exhibited magnesium deficiency (Figure 16).

CORRELATION ANALYSIS

The correlation analysis of traits (Table 4) shows a positive and significant correlation between curd dry weight and total leaf chlorophyll content ($R = 0.68$). Curd dry weight ($R=0.43$) and aerial parts dry weight ($R=0.75$) showed a positive and significant correlation with leaf potassium. On the other hand, leaf magnesium showed a significant positive correlation with both curd fresh weight ($R = 0.48$) and curd dry weight ($R = 0.50$).

DISCUSSION

In the present study, the interaction of different media with nutrient solutions significantly influenced broccoli plant growth. With two substrates of silty-sand and sand, the Brassica-SANRU solution enriched with KH_2PO_4 demonstrated improved plant growth performance, probably due to mineral loss through leaching. The modified Brassica-SANRU solution did not significantly increase the curd yield of broccoli in the coir-perlite medium, probably due to its ability and capacity to retain minerals. As coir have high cation exchange capacity, causes to adsorb cations such as potassium, calcium, and magnesium and gradually release that offers buffering capacity and supports nutrient retention (MLIK & TLUSTOS 2025) so, no need for extra phosphorus and potassium for plants grown in this medium. High retention capacity (SACHIN *et al.* 2020) and the presence of potassium, calcium, and magnesium nutrients and high exchange capacity of coir fibers for many cations and anions (ABAD *et al.* 2002; KRISHNA PILLAI *et al.* 2020; EBRAHIMI *et al.* 2012), eliminates the need for strong nutrient solutions.

From the growing medium point of view, plants in coir-perlite media showed more curd fresh weight, because coir have more water holding capacity and moisture content than other media so in this media less water losses occurred and provide more water to plant. KALAIVANI & JAWAHARLAL (2019) investigated physical characterization of cocopeat in combination with different organic amendments and found that coco peat alone showed higher water holding capacity and moisture content in compared with combination with bio compost and vermicompost. Also, on a study on cucumber by PANDEY *et al.* (2026) with different portion of coco peat and coco chips on cucumber yield indicated that highest yield was obtained in higher ratios of coco peat with cocochips and cocopeat alone that attributed to superior physical properties, including high water-holding capacity, excellent aeration, uniform salinity distribution, and high porosity (WALLACH 2019). The decrease in curd fresh weight and leaf fresh and dry weight in plants grown in sand media is due to leaching of nutrient because sand have low adsorption capacity, which provide intensive nutrient leaching (PRADO *et al.* 2023). So, its low water holding capacity, causes leaching of nutrient elements involved in photosynthesis and growth in this media.

1 The increase in broccoli growth and yield under SANRU solution could be due to a better presence
2 of both K and P, as the maximum yield was not observed in the nutrient solution enriched with
3 K_2SO_4 . In other words, high phosphorus and potassium levels in nutrient solutions are essential
4 throughout the growth period to enhance broccoli growth and yield, particularly in sand and silty-
5 sandy soils. The N5 nutrient solution with a higher concentration of K_2SO_4 led to a reduction in
6 leaf biomass production. The increased sulfur content in the nutrient solution likely encourages the
7 plant to produce secondary metabolites, as broccoli is a sulfur-loving species (PARK *et al.* 2020)
8 and many of its phytochemicals contain sulfur. In general, reallocation of plants' photosynthetic
9 products to the secondary metabolites implies a stop or significant reduction in vegetative growth
10 (GHASEMI *et al.* 2021).

11 Earliness in broccoli is an important trait that is influenced by several factors including production
12 of adequate photoassimilates in source and their transfer to the sink (the curds). In this study, the
13 enriched nutrient solution with KH_2PO_4 was able to improve plant growth induce early maturity in
14 all three used culture media.

15 Higher curd dry weight in N5 nutrient solution attributed to potassium present in this treatment.
16 As high potassium levels play a crucial role in the photosynthetic process and its efficiency in
17 producing more carbohydrates (MARSCHNER 2012). The beneficial effects of potassium on
18 carbohydrate biosynthesis are manifested in two primary ways: through the stomatal mechanism,
19 which involves the opening of stomata and the provision of adequate CO_2 , and through the non-
20 stomatal mechanism, which activates photosynthetic enzymes and biochemical reactions (ZORB *et al.*
21 2013). Potassium plays a crucial role in phloem loading and sugar transport (BIKHISH *et al.*
22 2024). Potassium can regulate the allocation of carbohydrates between the source and sink organs
23 of crops. Appropriate potassium levels can increase the supply of photosynthates at the source
24 organ also increases sink strength and increases plants yield (LI *et al.* 2022). In this context, ASIF
25 *et al.* (2017) studied potassium deficiency on wheat growth and found that potassium deficiency
26 was detrimental to leaf photosynthesis, also affects on the yield and quality of plant by increasing
27 specific leaf weight and accumulation of soluble sugar in leaves as source organ and thereby
28 affecting carbohydrates transport to the sink organ as observed in the form of decrease in grain
29 yield as sink.

30 A study conducted by GHASEMI *et al.* (2019) on radishes, a member of the Brassicaceae family,
31 demonstrated that foliar spraying with mixture of sucrose and potassium sulfate significantly
32 enhanced the weight and carbohydrate content of radish roots (edible part). Higher rates of K in
33 nutrient solution resulted in shorter stem length of broccoli plants probably due to this fact that
34 curds represented stronger sink for photoassimilates, so the increase in stem length was minimal
35 under high K rates, while the yield of the edible parts was significantly high. Nevertheless, due to
36 higher bioavailability of lower K rates in sand medium, nutrient solution rich in K, didn't result in
37 higher curd yields, probably due to other limiting factors such as its low retention capacity for
38 water and minerals.

39 In a similar way, extra phosphorus showed positive effects on plant biomass probably due to better
40 leaves photosynthesis condition. In the high phosphorus treatment, plants had higher leaf P,
41 photosynthetic efficiency and biomass than the control plants. It was shown in tomato that under
42 hydroponic conditions root growth and biomass in the treatment with a low phosphorus (15 mg/L)
43 decreased by 38% compared to the control (adequate minerals with P of 30 mg/L) (NACIRI *et al.*
44 2022).

1 Increase in broccoli yield in extra phosphorus solution attributed to the presence of phosphorus in
2 the structure of vital molecules like adenosine triphosphate (ATP) as regulating plant energy,
3 nucleic acid and phospholipids. So is needed for plants' metabolism of ATP and nucleic acid that
4 can promote plant biological processes like, growth, development and photosynthesis (LU *et al.*
5 2023). High yield of plants in extra phosphorus can be associated with to increase in photosynthesis
6 and photoassimilate production, that affect curd yield. In this relation a study conducted by
7 KAYOUMU *et al.* (2023) to investigate effects of phosphorus on growth and photosynthesis in low
8 P- tolerant cotton, results showed that phosphorus deficiency reduced plant growth, leaf area and
9 photosynthesis by decline in ATP and NADPH molecules content. In the present study, K was the
10 enriched element in both N4 and N5 solutions, and it can be inferred that potassium plays a crucial
11 role in enhancing the growth, biomass and yield of broccoli. The extra potassium in nutrient
12 solution; however, should not be limited only to the reproductive growth stage, and its addition
13 during routine plant growth could have beneficial effects on broccoli curd. Potassium has also roles
14 in increasing leaf photosynthesis efficiency as well as in the transfer of carbohydrates from source
15 to sink (ZORB 2014).

16 Brassica-SANRU solution, whether basic or modified, demonstrated superior leaf chlorophyll
17 content compared to Hoagland treatment. The leaf chlorophylls serve as the primary site for light
18 reception in photosynthesis, higher rates of leaf Chls are generally linked with enhanced the
19 photosynthetic efficiency. An increased chlorophyll content allows for greater absorption of light
20 energy, leading to the generation of more photo-assimilates and dry matter (WANG *et al.* 2022 a;
21 ORT *et al.* 2015). Minerals levels and their ratio are important influencers of leaf photosynthetic
22 efficiency that consequently can result in higher plant growth and quality (EBRAHIMI *et al.* 2012).
23 The regulation of stomatal movement by K helps maintain optimal CO₂ levels for photosynthesis,
24 preventing water loss, and produces sufficient sugar in plants (WANG *et al.* 2022b; YANG *et al.*
25 2020). As potassium has a positive role in stomatal conductance, it would be reasonable to justify
26 the increase in curd yield by increasing photosynthetic efficiency.

27 In this study, the broccoli leaves had a N concentration within the normal range, and the treatments
28 did not cause nitrogen deficiency or toxicity (Figure 15). However, plants treated with the
29 Hoagland nutrient solution had a lower leaf nitrogen percentage than other treatments, which could
30 be probably due to the low phosphorus concentration in this solution compared to the Brassica-
31 SANRU solution. The synergistic role of phosphorus in increasing nitrogen uptake has been shown
32 in various studies (GRACIANO *et al.* 2006 STEVENS *et al.* 2019; XIA *et al.* 2023).

33 Leaf phosphorus concentration was recorded in the range of 0.1-0.2 %, lower than the normal range
34 (0.3-0.7%) provided by UCHIDA *et al.* (2000). The plants grown in all combined treatments showed
35 lower phosphorus concentrations than the efficiency range (Figure 15). Low levels of leaf P and
36 even P deficiency may occur due to low temperatures during the growth period of broccoli in winter
37 season. In corn, investigating the effects of temperature on plant roots revealed that both root
38 growth rate and phosphorus uptake were higher at 25°C than at 18°C (MACKAY & BARBER 1984).
39 Additionally, research on cucumbers demonstrated that phosphorus uptake was significantly
40 reduced at 10°C compared to 20°C (YAN *et al.* 2012).

41 Less leaf potassium in plants grown in silty- sand and sand medium is due to this fact that potassium
42 is a mobile element that can leached easily from root zone so in sand based medium plants cannot
43 absorb sufficiently K in their leaves. The potassium deficiency is more prevalent in acidic, sandy,

waterlogged, saline or calcareous soils as well as under adverse climatic conditions such as water and heat stress (MENGEL & KIRKBY 2001; ZIVDAR *et al.* 2016). Leaf potassium deficiency resolved with application of SANRU solution with extra KH_2PO_4 in both vegetative and reproductive stages in silty- sand and sand medium due to high potassium content in this nutrient solution composition. In an experiment with different levels of potassium, calcium, nitrogen, and sulfur and via the standard Steiner nutrient solution, it was shown that low potassium levels in the nutrient solution significantly reduced the broccoli plants leaf K levels (RANDT & LOUIS 2011).

Magnesium has direct and indirect roles in leaf greening and photosynthesis activity (MARSCHNER 2012). Silty-sand medium treated with Hoagland nutrient solution and additional mono potassium phosphate exhibited the highest leaf magnesium concentration compared to other treatments. This could be due to synergistic effects of additional K and P on Mg uptake and efficiency, instead of a general antagonistic competition that may exist in many cases (EBRAHIMI *et al.* 2012; MARSCHNER 2012). The synergistic effects of magnesium and phosphorus on increasing the crop yield of wheat have been previously reported (WEIH *et al.* 2021). Nevertheless, plants grown in Coir-perlite medium, regardless of the nutrient solution type, maintained a normal leaf magnesium concentration.

In the present study, nutrient solutions with high phosphate concentrations (N3 and N4) enhanced leaf calcium uptake, except in sandy soil. Leaf calcium levels in plants grown in silty-sand with N3 and N4 solutions, as well as in coir-perlite with N1, N2, N3, and N4 solutions, ranged from 1.1 to 2.25 %, that is consistent with the normal levels reported for broccoli (UCHIDA 2000). In contrast, plants receiving additional potassium in nutrient solutions (N5), regardless of the growing medium, exhibited lower levels of leaf calcium, which could be due to the antagonistic effects of potassium and calcium on root uptake (JAKOBSEN 1993). Reduced calcium uptake by roots and decreased calcium accumulation in sugar beet leaves have been reported with the application of potassium fertilizer (RHODES *et al.* 2018). Additionally, SAKAIGAICHI *et al.* (2014) reported an increase in calcium and magnesium concentrations with reduced potassium fertilizer application, which was associated with the suppression of calcium uptake at elevated potassium levels.

The correlation between leaf potassium and curd dry matter can be attributed to potassium's role in photo-assimilate transfer to the target organ, as demonstrated in several studies. GHASEMI *et al.* (2024) found that foliar application of potassium and magnesium on pepper increased yield and fruit biomass. Potassium plays an important role in regulating photosynthesis, enzyme activation, cation transfer to sink organs, carbohydrate metabolism, and turgor pressure of plant cells (LEMOINE *et al.* 2013).

The role of potassium in enhancing photophosphorylation, as well as its involvement in the dark reactions of photosynthesis, has been shown to promote greater accumulation of carbohydrates and facilitate the transfer of carbohydrates from sources to destinations (BONS & SHARMA 2023). The positive correlation between potassium levels and the fresh weight of the aerial parts of broccoli can be attributed to potassium's role in maintaining cellular water content and supporting metabolic processes within plant tissue. This relationship positively influences and enhances the fresh weight of the aerial parts (SHAREF 2016).

CONCLUSION

In the present study, a previously approved (Brassica-SANRU) nutrient solution suitable for the growth and production of cole crops was included and treated for further improvement toward a more specific NS that could be suitable for broccoli cultivation. It was found that increasing the phosphorus and potassium is recommended for better performance of the Brassica-SANRU nutrient solution in terms of broccoli growth and yield attributes, and particularly when is cultivated in sand and silty-sand media. Our results revealed that the Brassica-SANRU solution works perfectly in coir-perlite medium, with no need to increase the levels of P and K in the form of mono-potassium phosphate. Our findings indicated that the Brassica-SANRU solution with a lower strength than the Hoagland solution worked perfectly with coir-perlite medium, while in the silty-sandy and sandy soils that represent locally available and cheap substrates, this solution needed additional minerals. Also, our results showed that broccoli grown under N4 with silty-sand had the highest leaf K, Ca, and Mg contents. However, adding extra phosphorus to the nutrient solution is not a sustainable treatment due to side effects and nutrient imbalances. In our experiment conditions, potassium is leached from the root environment more rapidly in silty- sand and sandy medium, resulting in reduced potassium absorption by the roots of broccoli plants.

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflict of interest.

DATA AVAILABILITY

The authors confirm that the data supporting the findings of this study are available within the article.

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Table 1. Chemical properties, macro and micro elements concentration in different nutrient solutions formula used in the study

Nutrient elements concentration	Nutrient solution			
	Hoagland	SANRU	SANRU+KH ₂ PO ₄	SANRU + K ₂ SO ₄
EC (ms/m)	1.8±0.08	1.8±0.06	1.9±0.05	1.9±0.04
pH	6.3-6.8	6.4-6.9	6.3-7.4	6.6-7.1
Nitrogen (mg/L)	210	157.5	157.5	157.5
Phosphorus (mg/L)	31	70.5	102.5	70.58
Potassium (mg/L)	78.1	221.5	241.01	240.55
Magnesium (mg/L)	48	49.5	49.5	49.5
Calcium (mg/L)	160	136.0	136.0	136.0
Sulfur	32.1	65.76	65.76	73.76
Iron (mg/L)	0.48	4.8	4.8	4.8
Zinc (mg/L)	0.04	0.27	0.27	0.27
Manganese (mg/L)	0.14	0.98	0.98	0.98

Molybdenum (mg/L)	0.03	0.109	0.109	0.109
Copper (mg/L)	0.01	0.08	0.08	0.08
Boron (mg/L)	0.1	0.70	0.70	0.70

Table 2. Analysis of variances of factors and their interactions on morphology, biomass, and phenol content of broccoli plants

Source of variance	df	Mean Square						
		Curd fresh weight	Curd dry matter	Stem length	Total leaf fresh weight	Total leaf dry weight	Harvest time (DAT)	Total phenols of curd
Medium (M)	2	5506.09**	11.07 **	4.75 **	6759.57**	416.84**	11.62 ^{ns}	0.0033 ^{ns}
Nutrient Solution (NS)	4	9625.10 **	133.07 **	5.24 **	3980.57**	515.74**	36.22 **	0.059 **
M×NS	8	505.18 **	22.41 ^{ns}	7.64 **	1599.95*	146.36**	5.62 *	0.0129 *
Error	30	726.56	14.75	1.46	714.04	25.16	9.20	0.0067
Coefficient of Variation (%)	-	14.49	15.14	5.62	18.56	21.23	3.59	14.54

ANOVA: ns, *, ** not significant, or significant at $P < 0.05$ or $P < 0.01$, respectively

Table 3. Analysis of variances of factors and their interactions on total chlorophyll and nutrient elements of broccoli

Source of variance	df	Mean Square					
		Total chlorophyll	Nitrogen	Phosphorus	Potassium	Calcium	Magnesium
Medium (M)	2	0.0039 **	0.564 ns	0.0014 *	6.245 **	0.870 **	7917282.6 **
Nutrient Solution (NS)	4	0.0111 **	0.451 ns	0.0067 **	10.022 **	0.0577 **	3116704.1 *
M×NS	8	0.00267 **	0.793 **	0.0016 **	1.892 **	0.365 **	3544207.7 **
Error	30	0.0056	6.73	0.0004	0.427	0.0842	1369298.8
Coefficient of Variation (%)	-	15.89	20.43	15.24	25.14	22.18	22.30

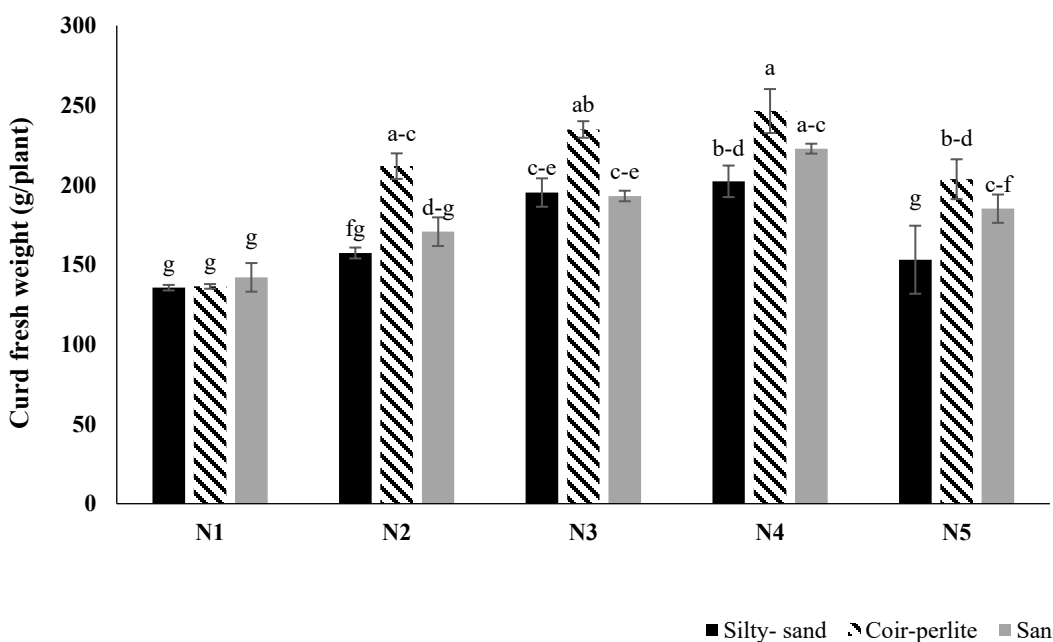
ANOVA: ns, *, ** not significant, or significant at $P < 0.05$ or $P < 0.01$, respectively

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8 **Table 4.** The correlation analysis of morphological, physiologic and nutritional value traits in
9 broccoli plants grown in different media and nutrient solutions

	Curd diameter (mm)	Curd dry matter (%)	Curd fresh weight (g)	Aerial fresh weight (g)	Aerial dry weight (g)	Leaf chlorophyll (mg.g FW- l)	Curd phenol (mg.g DW- l)	Leaf fresh weight (g)	Leaf nitrogen (%)	Leaf potassium (%)	Leaf magnesium (ppm)
Curd diameter (mm)	1										
Curd dry matter (%)	0.74**	1									
Curd fresh weight (g)	0.76**	0.86**	1								
Aerial fresh weight (g)	0.66**	0.75**	0.91 **	1							
Aerial dry weight (g)	0.58 *	0.71**	0.81 **	0.93	1						
Leaf chlorophyll (mg.g FW- l)	0.60 *	0.68 *	0.68 **	0.55 *	0.51*	1					
Curd phenol (mg.g DW- l)	0.40 ^{ns}	0.24 ^{ns}	0.37 ^{ns}	0.39 ^{ns}	0.38 *	0.20 ^{ns}	1				
Leaf fresh weight (g)	0.37 ^{ns}	0.50*	0.67*	0.85**	0.87**	0.38 ^{ns}	0.20 ^{ns}	1			
Leaf nitrogen (%)	-0.05 ^{ns}	0.02 ^{ns}	-0.07 ^{ns}	-0.05 ^{ns}	-0.04 ^{ns}	0.33 ^{ns}	-0.41 *	0.15 ^{ns}	1		
Leaf potassium (%)	0.16 ^{ns}	0.43 ^{ns}	0.59 *	0.69*	0.68 ^{8*}	0.27 ^{ns}	-0.07 ^{ns}	0.79 **	0.05 ^{ns}	1	
Leaf magnesium (ppm)	0.21 ^{ns}	0.48*	0.50*	0.61*	0.81**	0.21 ^{ns}	0.25 ^{ns}	0.71 *	0.11 ^{ns}	0.50 *	1

1 **, *, ns: Correlation is significant at the $P < 0.01$, $P < 0.05$ and not significant respectively.



11 **Fig 1.** Curd fresh weight of broccoli as affected by medium and nutrient solutions interaction. Values are
12 means $\pm$ SD (n=3). Columns with the same letter are not statistically different at the ($P < 0.05$, LSD test).
13 N1- N5 represent different nutrient solution and growth stage of application (see material and methods for
14 detail).

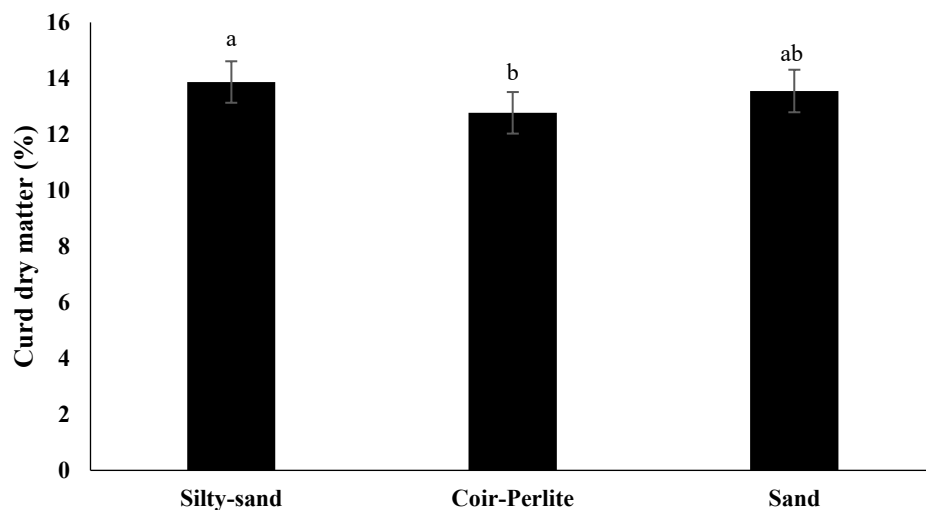


Fig 2. Curds dry matter of broccoli as affected by medium. Values are means $\pm$ SD (n=3). Columns with the same letter are not statistically different at the ($P < 0.05$, LSD test).

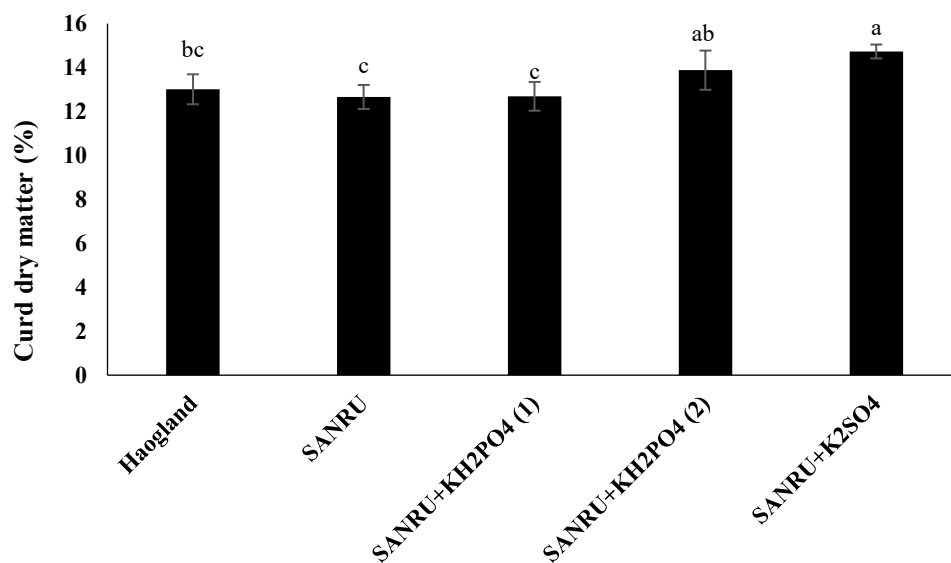


Fig 3. Curds dry matter of broccoli as affected by nutrient solutions. Values are means $\pm$ SD (n=3). Columns with the same letter are not statistically different at the ($P < 0.05$, LSD test). N1- N5 represent different nutrient solution and growth stage of application (see material and methods for detail).

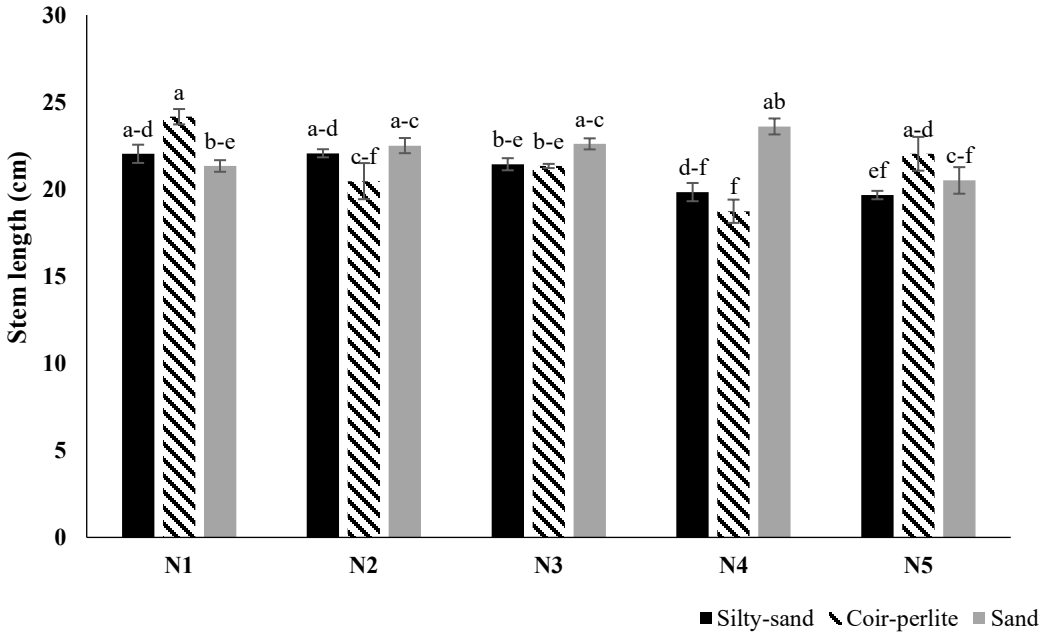


Fig 4. Stem length of broccoli as affected by medium and nutrient solutions interaction. Values are means $\pm$ SD (n=3). Columns with the same letter are not statistically different at the ($P < 0.05$, LSD test). N1- N5 represent different nutrient solution and growth stage of application (see material and methods for detail).

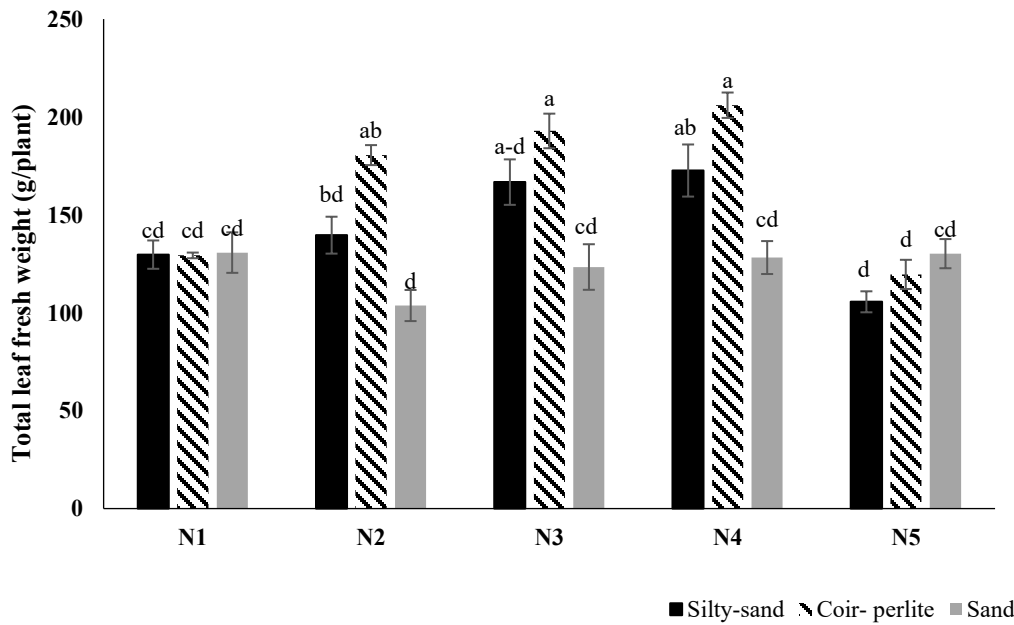


Fig 5. Total leaf fresh weight of broccoli as affected by medium and nutrient solutions interaction. Values are means $\pm$ SD (n=3). Columns with the same letter are not statistically different at the ($P < 0.05$, LSD test). N1- N5 represent different nutrient solution and growth stage of application (see material and methods for detail).

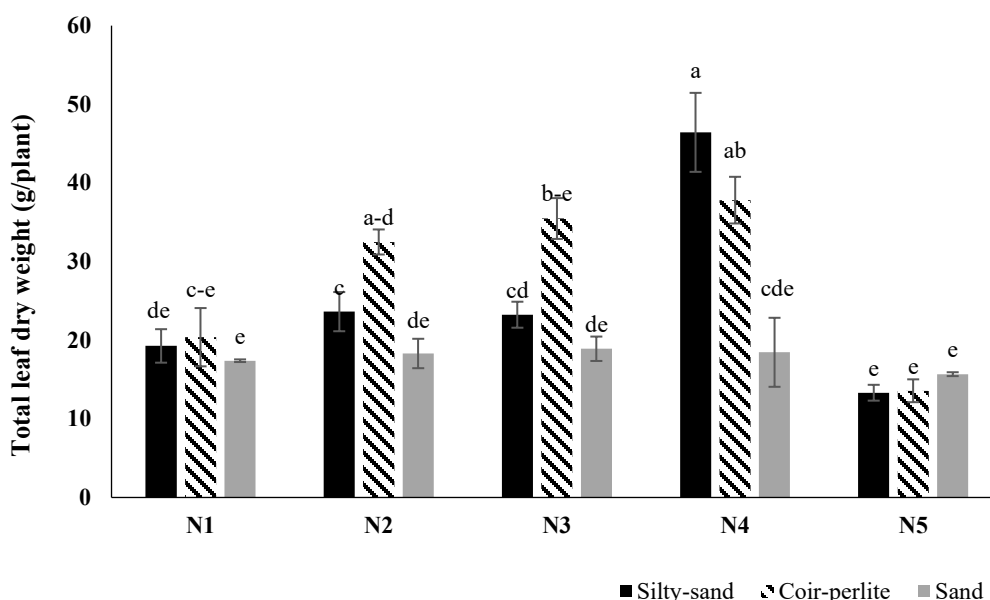


Fig 6. Total leaf dry weight of broccoli as affected by medium and nutrient solutions interaction. Values are means $\pm$ SD (n=3). Columns with the same letter are not statistically different at the ($P < 0.05$, LSD test). N1- N5 represent different nutrient solution and growth stage of application (see material and methods for detail).

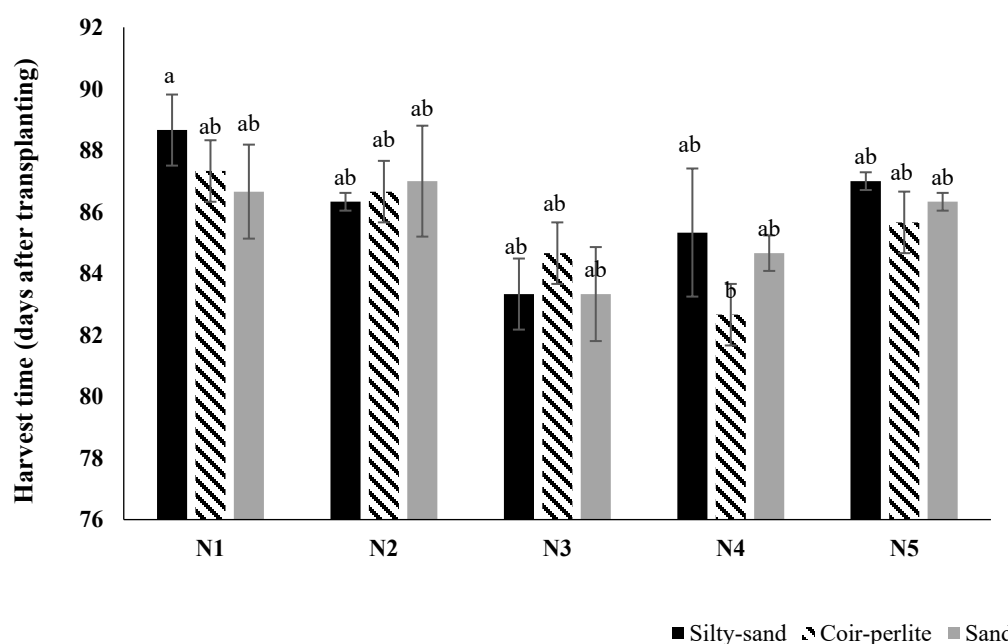


Fig 7. Harvest time of broccoli curds as affected by medium and nutrient solutions interaction. Values are means $\pm$ SD (n=3). Columns with the same letter are not statistically different at the ($P < 0.05$, LSD test). N1- N5 represent different nutrient solution and growth stage of application (see material and methods for detail).

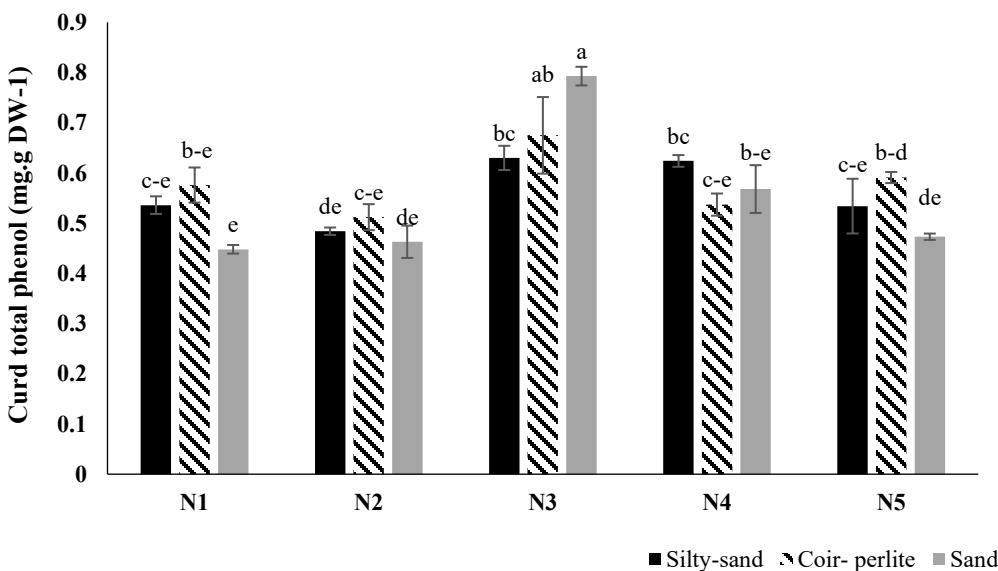


Fig 8. Curd total phenols content of broccoli as affected by medium and nutrient solutions interaction. Values are means $\pm$ SD (n=3). Columns with the same letter are not statistically different at the ($P < 0.05$, LSD test). N1- N5 represent different nutrient solution and growth stage of application (see material and methods for detail).

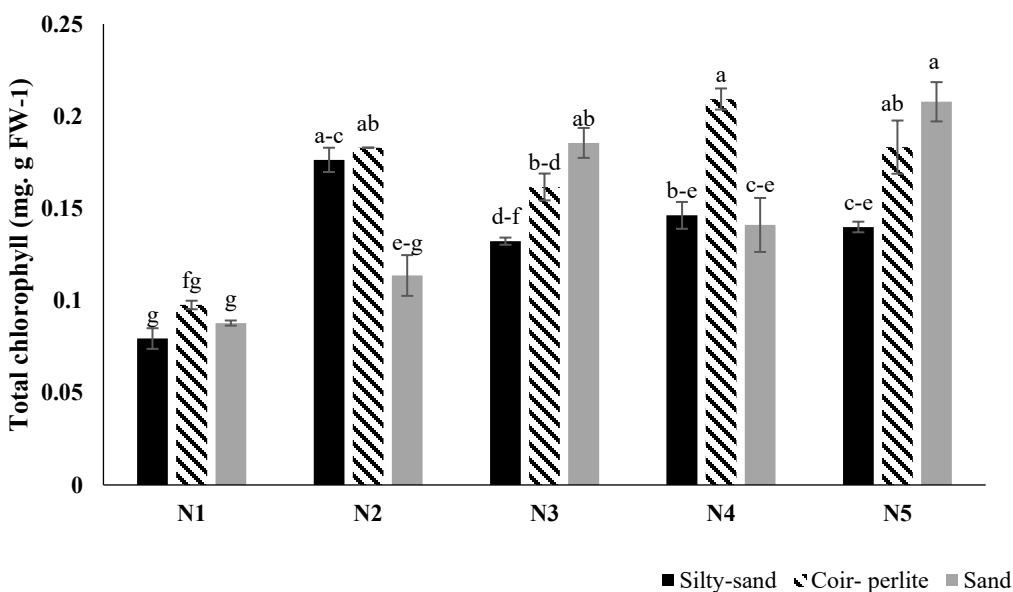


Fig 9. Leaf total chlorophyll content of broccoli as affected by medium and nutrient solutions interaction. Values are means $\pm$ SD (n=3). Columns with the same letter are not statistically different at the ($P < 0.05$, LSD test). N1- N5 represent different nutrient solution and growth stage of application (see material and methods for detail).

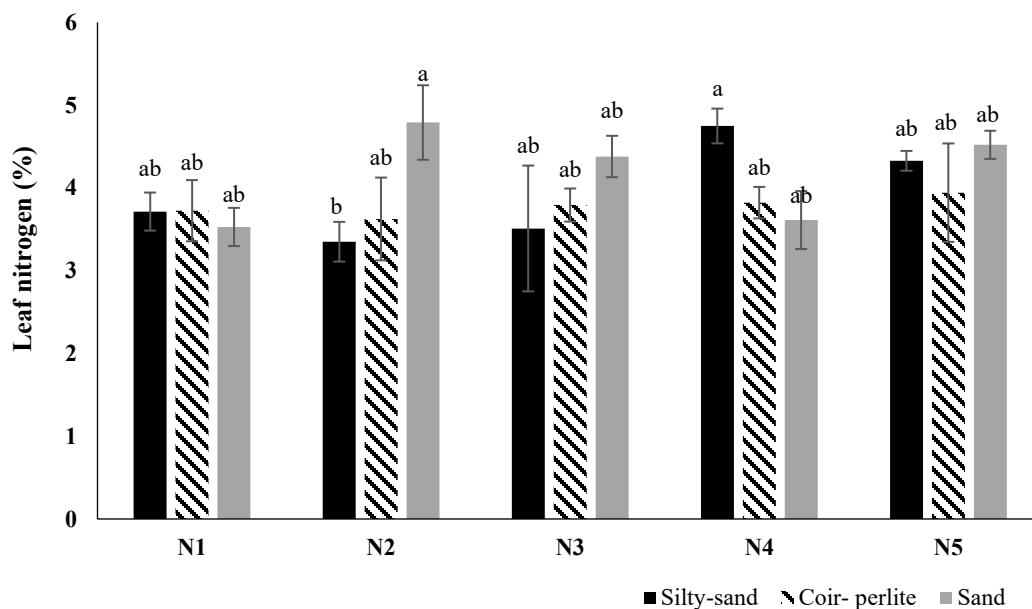


Fig 10. Leaf nitrogen content of broccoli as affected by medium and nutrient solutions interaction. Values are means $\pm$ SD (n=3). Columns with the same letter are not statistically different at the ($P < 0.05$, LSD test). N1- N5 represent different nutrient solution and growth stage of application (see material and methods for detail).

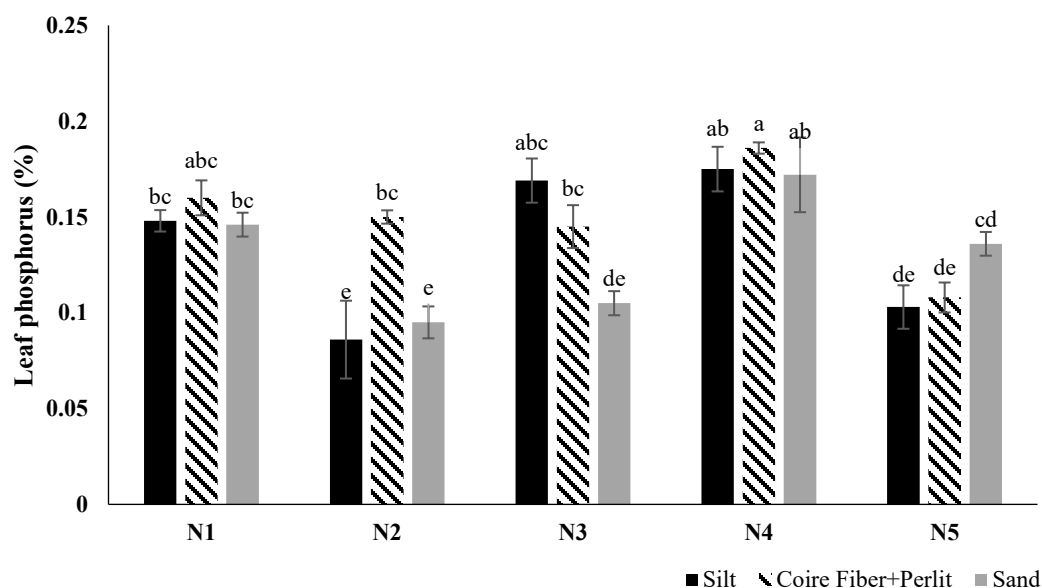


Fig 11. Leaf phosphorus content of broccoli as affected by medium and nutrient solutions interaction. Values are means $\pm$ SD (n=3). Columns with the same letter are not statistically different at the ($P < 0.05$, LSD test).

test). N1- N5 represent different nutrient solution and growth stage of application (see material and methods for detail).

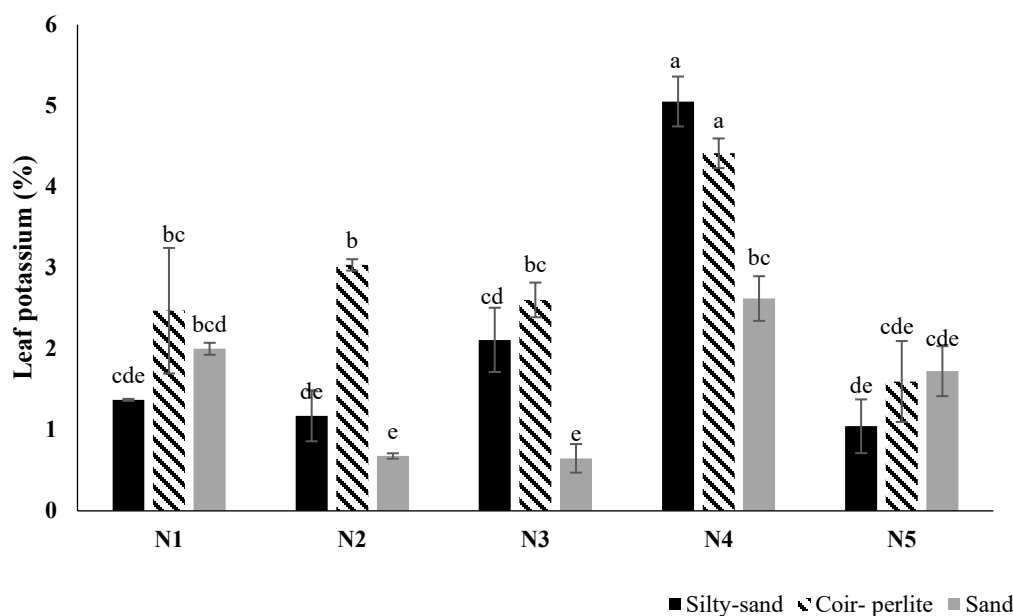


Fig 12. Leaf potassium content of broccoli as affected by medium and nutrient solutions interaction. Values are means $\pm$ SD (n=3). Columns with the same letter are not statistically different at the ($P < 0.05$, LSD test). N1- N5 represent different nutrient solution and growth stage of application (see material and methods for detail).

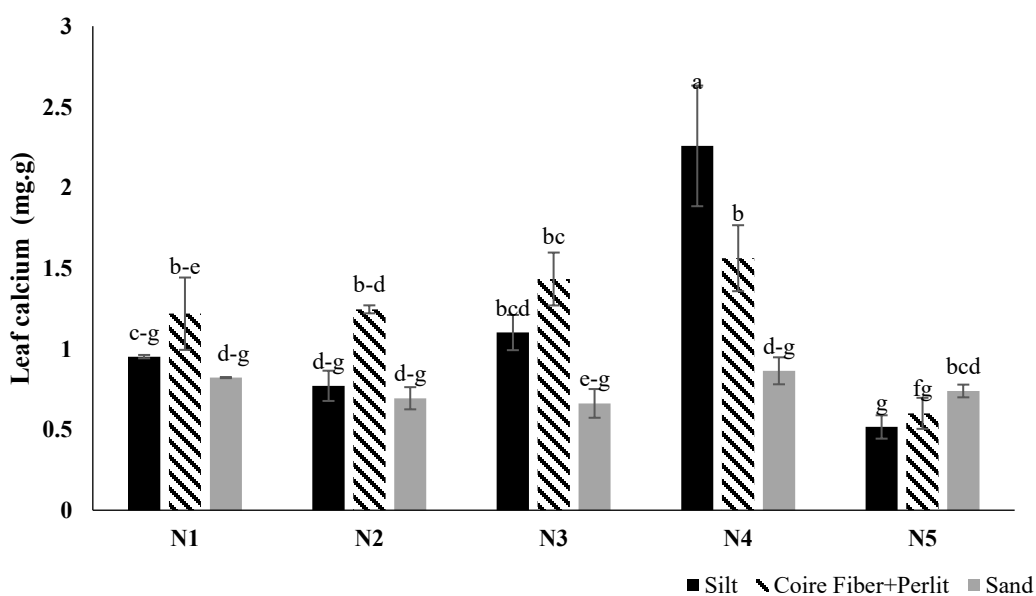


Fig 13. Leaf calcium content of broccoli as affected by medium and nutrient solutions interaction. Values are means $\pm$ SD (n=3). Columns with the same letter are not statistically different at the ($P < 0.05$, LSD test). N1- N5 represent different nutrient solution and growth stage of application (see material and methods for detail).

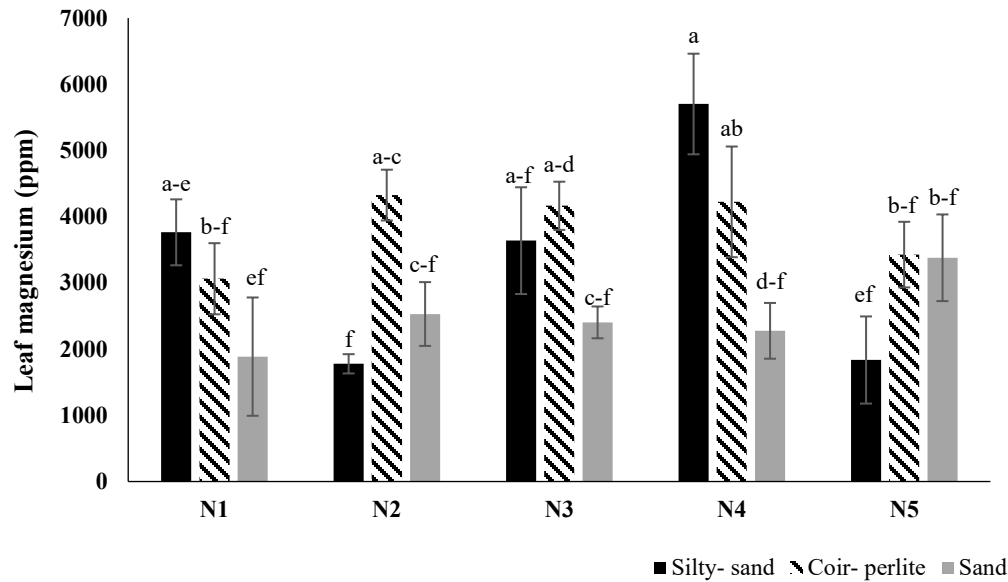
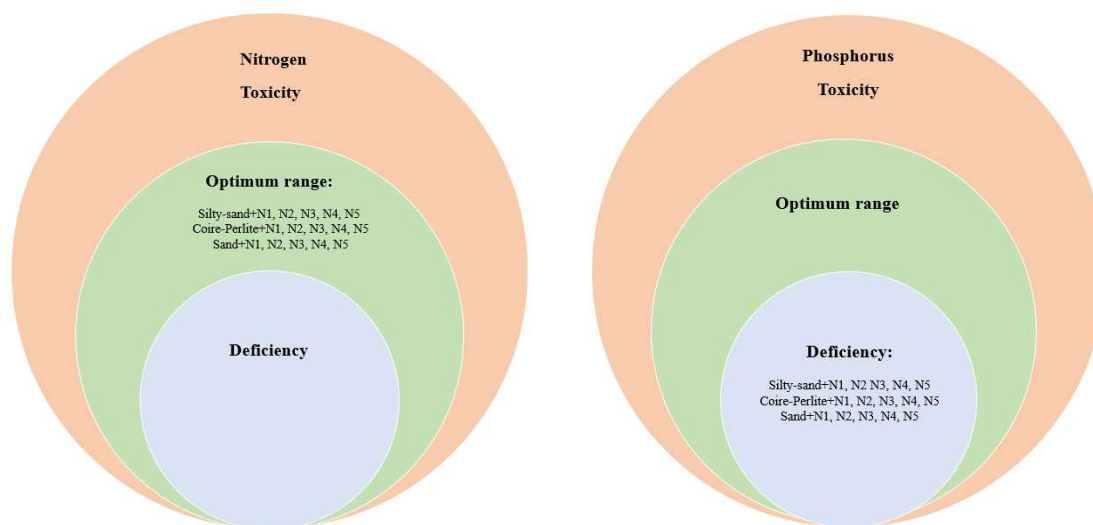


Fig 14. Leaf magnesium content of broccoli as affected by medium and nutrient solutions interaction. Values are means $\pm$ SD (n=3). Columns with the same letter are not statistically different at the ($P < 0.05$, LSD test). N1- N5 represent different nutrient solution and growth stage of application (see material and methods for detail).

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Fig 15. Optimum range of leaf nitrogen content (Left) and phosphorus (Right) deficiency and toxicity range obtained from different medium and nutrient solution in broccoli optimum range adopted from Uchida (2000).

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Fig 16. Optimum range of leaf potassium (Left), magnesium (Right), and calcium (Below) content deficiency and toxicity obtained from different media and nutrient solutions in broccoli optimum range adopted from Uchida (2000).

Supplementary Files

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