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# Suitability of walnut and pistachio shells as soilless substrates for producing Genovese basil in aquaponic systems

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**Introduction:** The use of agricultural by-products, such as walnut and pistachio shells, is emerging as a sustainable substrate alternative to conventional substrates in soilless farming systems like hydroponics and aquaponics. These materials offer a promising solution for agri-waste management while advancing circular economy objectives in sustainable agriculture.

**Methods:** The present study evaluates the performance of crushed walnut shell (CWS) and line-waste pistachio shell (LWPS) as partial to complete replacements for coconut coir (CC) in decoupled aquaponic systems, using Genovese basil (*Ocimum basilicum*) as a model crop. Two independent experiments were conducted concurrently, one for each nutshell substrate (n=6 replicate trays). Each experiment included nine treatments: a control group (50% peat moss/50% perlite), four nutshell-based blends (10%, 25%, 33%, and 50% of either CWS or LWPS), and four CC-based blends at the same inclusion rates. Plant growth metrics, including height, number of nodes and leaves, SPAD chlorophyll index, fresh and dry shoot weight, and root development were measured to assess treatment performance.

**Results:** Overall, basil grown in lower inclusion rates of CWS and LWPS media performed comparably to those grown in CC-based media. However, growth suppression was observed in the 33% and 50% CWS treatments, likely due to juglone toxicity. In contrast, LWPS exhibited a modest growth benefit at 25%, before declining at higher levels, which may be attributed to reduced water holding capacity.

**Discussion:** These findings suggest that walnut and pistachio shells can serve as effective components of soilless growing media when used at moderate inclusion rates. While higher concentrations presented limitations, low to

moderate inclusion levels yielded growth performance comparable to that of CC. These findings support the potential of regionally sourced nutshell by-products as viable alternatives to imported growing media, contributing to a more diversified and resilient horticultural supply chain.

#### KEYWORDS

agricultural by products, aquaponics, circular economy, hydroponics, *Ocimum basilicum*, soilless production

## Introduction

The increasing scarcity of arable land, the progressive shift in land utilization toward urban infrastructure, and the multifaceted impacts of climate change place significant stress on agricultural productivity (Gruda, 2019). Soilless cultivation systems, such as hydroponics and aquaponics, are viable alternatives that mitigate these challenges by facilitating greater control over water and nutrient delivery to plants. Compared to traditional soil-based agriculture, soilless growing systems, when paired with controlled environmental agriculture, facilitate significant savings of input requirements (Massa et al., 2020; Trejo-Téllez and Gómez-Merino, 2012), reduce the prevalence of soil-borne pests and diseases, and protect crops from adverse weather conditions (Kwon et al., 2021). Soilless growing systems also allow for plant production in areas unsuitable for conventional farming, either where arable land is not available or when the soil is contaminated (Vinci and Rapa, 2019). However, the sourcing and disposal of horticultural substrates with minimal environmental impact remains a global challenge. The valorization of agricultural by-products, such as nutshells, is emerging as a sustainable approach (Atzori et al., 2021; Xue and Farrell, 2020).

Walnut and pistachio production represents the most important nut crops in the world, with an estimated production of about 2.6 million and 782,000 tons in-shell, respectively, for the year 2022–2023 (United States Department of Agriculture - Foreign Agricultural Service, 2023). The United States is the second-largest walnut producer in the world, with a production of 752,000 tons of in-shell walnuts for the year 2022, following China (California Department of Food and Agriculture, 2022), and it is the largest pistachio producer in the world with a production of 441,000 tons of in-shell pistachio for the year 2022/23 (United States Department of Agriculture - Foreign Agricultural Service, 2023). Shell by-product related to nut production during its processing for consumption account for more than 50% of the total weight generated (Dufoo-Hurtado et al., 2021), and is usually disposed of as agricultural waste. Farmers harvesting cereals usually burn agricultural waste such as shells and husks, leading to environmental problems (Pirayesh et al., 2012). However, the walnut and pistachio shells could be used in whole or in part as sustainable alternative substrates in soilless food production. The

utilization of locally produced bioresources could foster a waste management strategy that aligns with the principles of reducing, reusing, and recycling in soilless culture systems, while simultaneously minimizing transportation-related emissions and supporting regional agricultural economies—thereby advancing circular economy practices. The use of nutshell by-product could mitigate environmental impacts (Wystalska and Kwarciak-Kozłowska, 2021) and in parallel could contribute to sustainable agriculture by providing an available, affordable and sustainable substrate option, that can represent a second source of income for farmers (Çöpür et al., 2007; Pirayesh et al., 2012).

Walnut shell fibers are a rich source of lignin (50.3%), cellulose (23.9%), hemicellulose (22.4%), and ash (3.4%) (Jahanban-Esfahlan et al., 2019). These components closely align with the composition of coconut coir (CC); lignin (40–45%), cellulose (35–43%), hemicellulose (24%), pectin (3–4%), and ash (2–3%), which is considered an ideal substrate for hydroponic production (Mishra and Basu, 2020; Park et al., 2010) and is a widely adopted (Aththanayake et al., 2021; Salam et al., 2020). Both substrates offer excellent water-holding capacity, sufficient air-filled porosity, and a slow release of essential nutrients.

The use of walnut shells as a substrate alternative must also consider the potential allelopathic effects due to the presence of juglone. Although Persian walnut (*Juglans regia*) shells contain juglone in considerably lower quantities compared to black walnut (*Juglans nigra*) (Bhamini and Kumar, 2019) their impact on plant growth warrants investigations. Several studies on juglone have revealed its status as a naturally occurring chemical compound with properties conducive to serving as an environmentally friendly pesticide and herbicide (Liu et al., 2022). Another study showed that juglone application to promote callus formation, inhibit rooting and promote shoot formation of tomato plants (Bamel and Gupta, 2022) and juglone has exhibited inhibitory effects on both seed germination and radicle elongation (Rietveld, 1983). However, at lower concentrations, juglone demonstrated stimulatory properties. In a separate study, it was deduced that hydroponically cultivated corn and soybean manifest sensitivity to juglone, with soybean exhibiting a higher susceptibility to the compound (Jose and Gillespie, 1998).

Conversely, there is limited information on the use of pistachio shells as an alternative substrate. The composition of pistachio differs significantly from walnut and CC, with a reported range of

12–38% lignin, 30–55% cellulose and 20–32% hemicellulose (Marett et al., 2017; Robles et al., 2021). Pistachio shells are rich in phenols which can become phytotoxic (Karimi et al., 2013). There are few studies assessing the use of pistachio shells as a soilless substrate at varying concentrations in vegetable production. Xue and Farrell (2020) found that using pistachio shells as substrate inhibits plant development. Nevertheless, Karimi et al. (2013) observed that pistachio shells can be used as a component of a growth medium, if mixed with materials that inhibit the activity of their phenolic compounds, such as rice husk or activated charcoal.

Nutshells possess significant potential as a growing medium; however, since they are not specifically produced for horticultural applications, they may exhibit certain limitations. These shortcomings can be addressed by combining them with conventional materials to enhance their properties (Barrett et al., 2016). Consequently, it is essential to determine the most appropriate proportions for mixing these substrates with other materials to optimize their functionality. In this way, the objective of this research is to explore the potential of walnut and pistachio shells against a commercial CC and conventional soilless substrates in a decoupled aquaponic system, using Genovese basil (*Ocimum basilicum* 'Genovese') as a model.

## Materials and methods

Two experiments were carried out with basil in a 280 m<sup>2</sup> polycarbonate greenhouse located at UC Davis Vegetable Crops Greenhouses Complex (University of California, Davis, CA, USA). Each experiment was transplanted and harvested on different dates; thus, these were not comparable. In one experiment, basil production was evaluated in growing media with crushed walnut

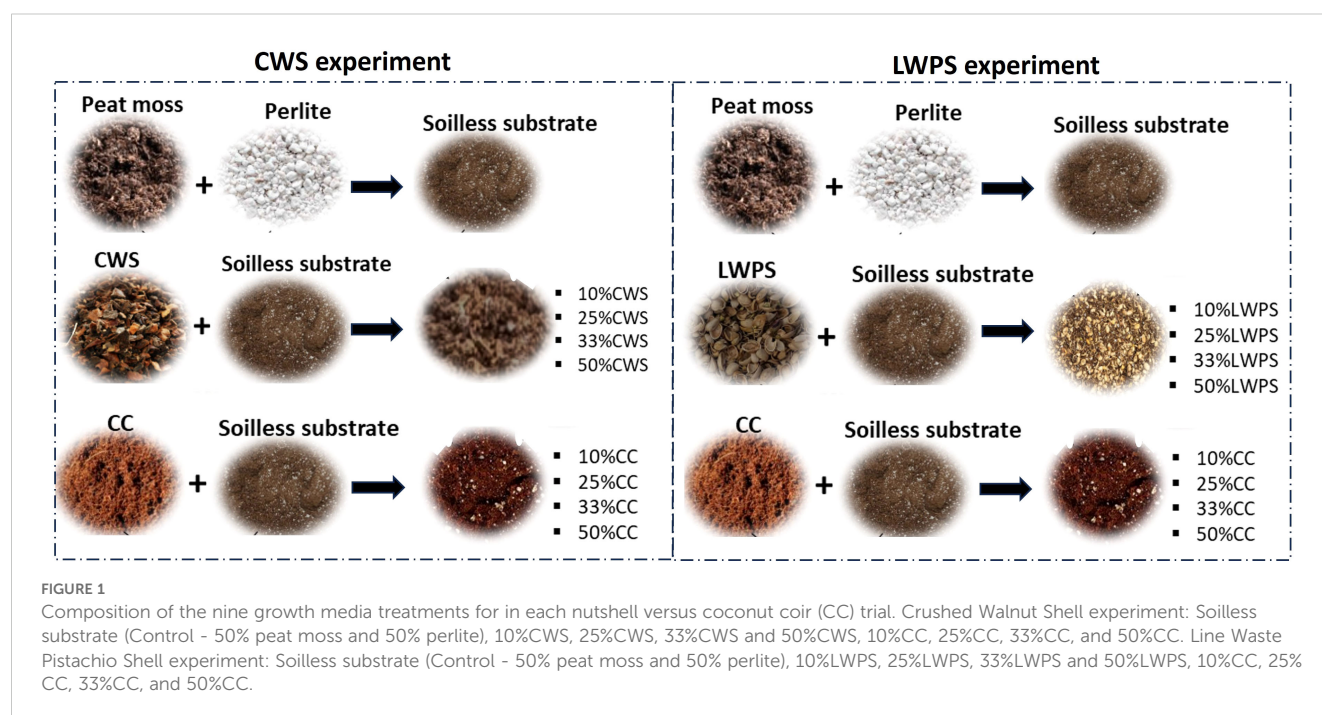
shells (CWS) from July 06 until August 19, 2023, and in the other experiment, line-waste pistachio shell (LWPS) was used from July 10 until August 24, 2023.

## Preparation of the substrate's treatments

The growth media tested were prepared by manually blending materials using a shovel to achieve specific volumetric proportions of the components under investigation (Figure 1).

For the walnut shell experiment, nine treatments were evaluated, including a control. The control base substrate consisted of a 50:50 volumetric blend of Canadian sphagnum peat moss (Black Gold<sup>®</sup>; Sun Gro Horticulture Distribution Inc., Agawam, Massachusetts, USA) and perlite (Therm-O-Rock West Inc., Chandler, Arizona, USA). Four treatments were prepared by incorporating crushed walnut shell (CWS) at 2–4 mm particle size (Mariani Nut Company, Winters, California, USA) into the base peat moss/perlite substrate at 10%, 25%, 33%, and 50% by volume. An additional four treatments were prepared using commercially available CC (Cyclo Coco Brick, S.J. Enterprises, Vancouver, Washington, USA) at identical volumetric inclusion rates. These CC treatments served as comparative benchmarks for evaluating the performance of CWS relative to an established, agriculturally derived soilless substrate.

Line waste pistachio shell (LWPS) treatments followed the same formulation and experimental design. Nine total treatments were prepared: one control (50% peat moss/50% perlite), four LWPS mixtures, and four CC mixtures at 10%, 25%, 33%, and 50% by volume. LWPS was used whole, without any mechanical processing, and had an average particle size between 2.0–2.5 cm. Pistachio shell material was sourced from The Wonderful Company: Almonds and Pistachios (Lost Hills, California, USA).





## Description of hydroponic plant production systems

Each nutshell media experiment consisted of six independent hydroponic plant production systems ( $n = 6$  replicate trays with 54 plants) arranged in two parallel rows of 3 trays (Figure 2). In both experiments, all 6 plants for each of the 9 treatments were randomly distributed by row within each tray to eliminate the potential effect of row and location. Plants were grown in 4 inch plastic nursery pots ( $0.102 \times 0.102 \times 0.089 \text{ m}^3$ ) (Figure 2), and were flood irrigated with aqueous aquaculture effluent 3 times a day for 30 min (07:00 h–07:30 h; 12:00 h–12:30 h and 16:00 h–16:30 h) to a depth of 1.27 cm.

Each hydroponic system consisted of an independent flood and drain table (1.22m wide; 2.44m long; 0.15m high, Botanicare, Chandler, AZ, USA), a food grade HDPE sump (473 L) filled with unfiltered aqueous aquaculture effluent, and a water pump ( $4164 \text{ L h}^{-1}$  ActiveAqua 1100 GPH, Hydrofarm, Petaluma, CA, USA).

## Production and monitoring of plants

Genovese basil (*Ocimum basilicum*), also known as sweet basil and Italian basil, was selected for this study because it is a common container-grown herb in commercial hydroponic plant production. In the USA it is regularly sold in-pot as a living plant, in fresh-cut bunches or by the pound, and dried as an herb and in spice-mixes. On June 16, 2023, vegetable propagation flats (338S67S, Proptek, Watsonville, CA, USA) were prepared with stabilized rooting media (0.22 mm Q plug® “PT338 Star”, International Horticulture Technologies, Hollister, CA, USA) and individually seeded with 800 pelleted basil seeds (Johnny’s Selected Seeds, Inc., Winslow, ME, USA). Propagation flats were watered overhead with a Damm Watering Wand (Manitowoc, WI,

USA) and municipal irrigation water 2 times a day (07:00 h–07:15 h and 16:00 h–16:30 h) for twenty-one days with ambient light. Plants were transplanted on July 6, 2023, for the CWS growth trial, and four days later on July 10, 2023, for the LWPS growth trial.

Sumps were topped-up with aquaculture effluent solution every Friday to replenish water loss due to evapo-transpiration. Before filling the sumps, the concentrations of  $\text{NH}_4^+$ ,  $\text{NO}_2^-$ , and  $\text{NO}_3^-$  of the incoming solution was measured with a Freshwater Master Test Kit (API®) by colorimetric methods, and temperature, electrical conductivity (EC) and pH were measured with a Bluelab® Guardian Monitor Connect (BLUELAB Tuaranga NZ). Aquaculture solution had an average pH of 8.6 and average concentration of 140 ppm nitrate ( $\text{NO}_3^-$ ) throughout the experimental period. A combination of phosphoric and citric acid (pH Down; General Hydroponics, Sebastopol, CA, USA) lowered pH to the desired range.

In addition, the temperature, EC, and pH of the sumps of each system was measured using a Bluelab® pen, before adding 0.2 g of chelated iron diethylene triamine Penta acetic acid (DTPA; 11% Fe) (Table 1). Foliar applications of  $\text{KNO}_3$  (1%) were performed twice a week in the morning with an electric fogger backpack sprayer (Fogger, Petratools®). These nutrients were added to each system to compensate for the deficiency of these nutrients regularly found in aquaponic systems and from previously conducted basil studies (Fernández-Cabanás et al., 2020). In both trials, the average concentration of  $\text{NO}_3^-$  was 80 ppm for each hydroponic sump.

Indoor air temperature and relative humidity within the greenhouse were monitored using a HOBO Pro Temp-HR U23–001 sensor (Onset Computer Corp., Bourne, MA, USA), with data recorded at 15-minute intervals (Figure 3). The observed temperature fluctuated from 15.8 to 41.0°C, while the mean relative humidity was reported as 71.7% (Figure 3A) for the CWS trial probe versus a corresponding mean relative humidity of 68.4% in the LWPS trial probe (Figure 3B).

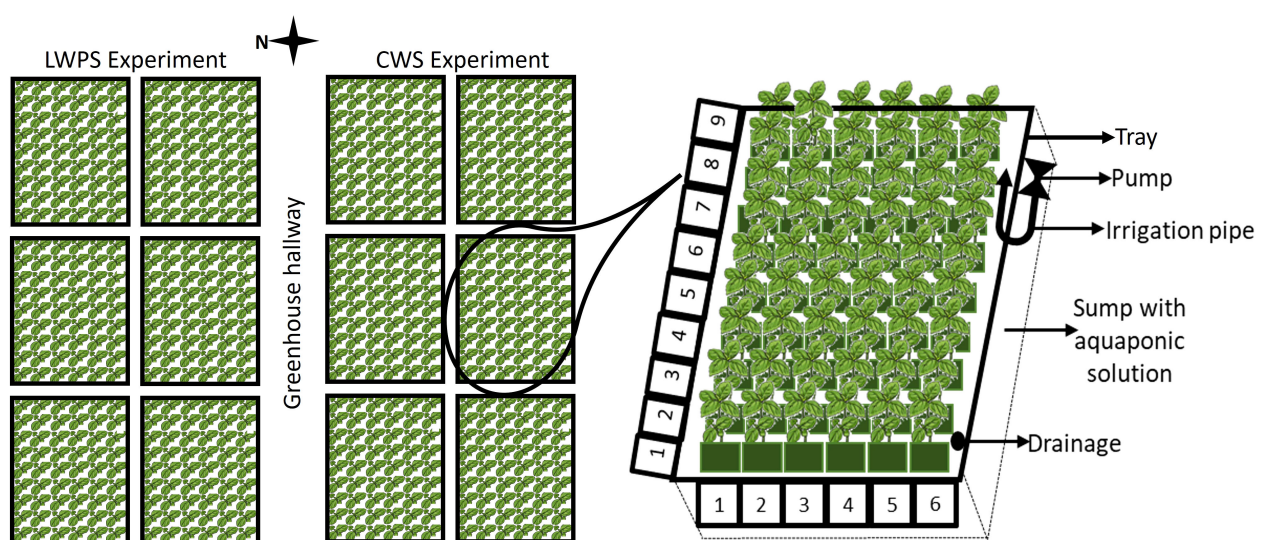
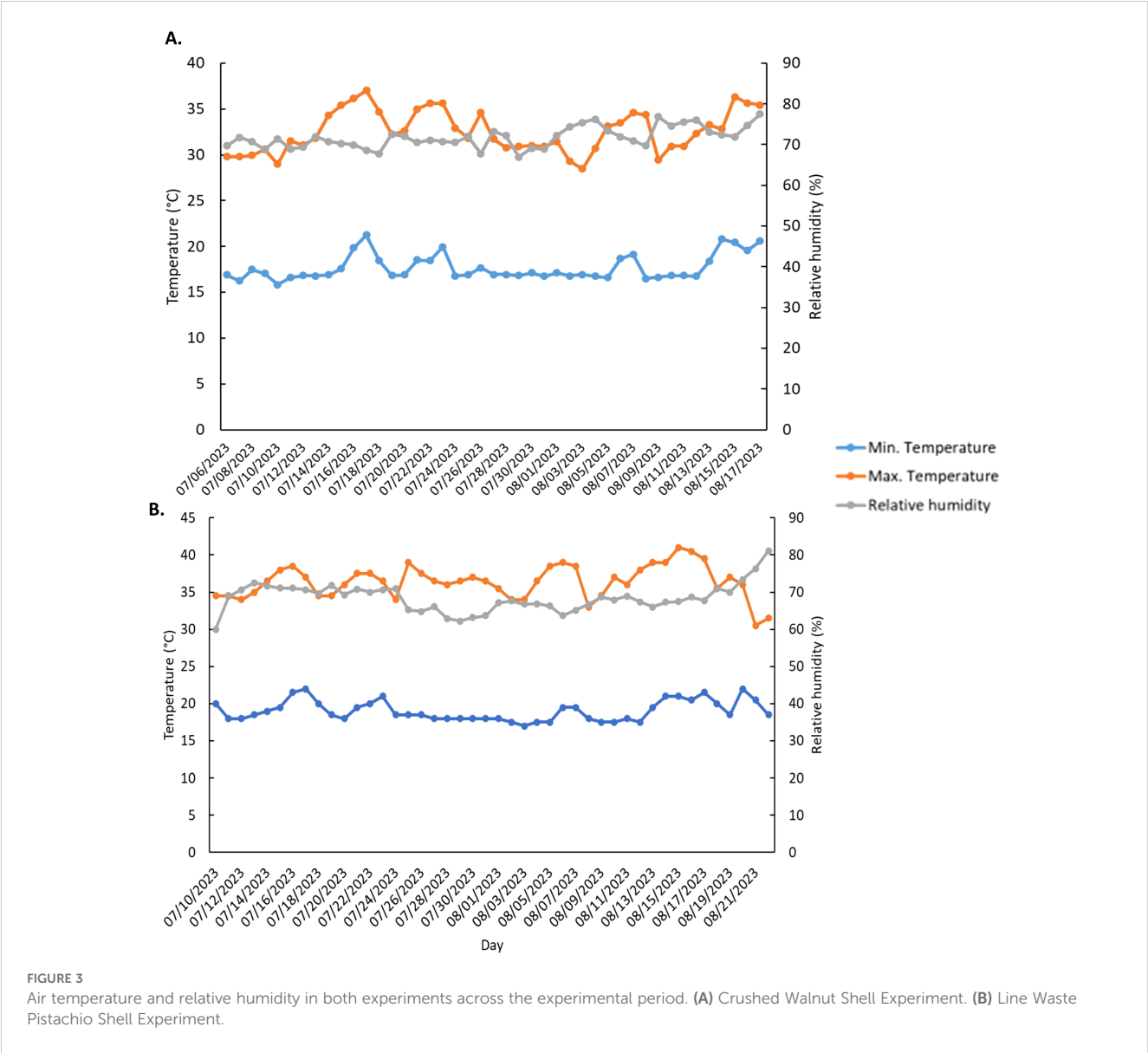


FIGURE 2

Experimental design and plant distribution in the hydroponic systems. Each growth trial consisted of six independent systems containing nine treatments each ( $n = 6$  replicate trays per growing media treatment with 6 plants per row). Each of the nine treatments were randomly distributed by row in each tray.

TABLE 1 Water quality parameters (mean ± SD) of the aquaponic solution in the sumps for CWS and LWPS trials.

CWS experiment				LWPS experiment			
Sump	Temperature (°C)	pH	EC (mS/cm)	Sump	Temperature (°C)	pH	EC (mS/cm)
1	22.7 ± 0.4	5.9 ± 0.2	0.7 ± 0.2	1	23.1 ± 0.3	5.4 ± 0.2	0.5 ± 0.1
2	22.6 ± 0.4	6.1 ± 0.1	0.9 ± 0.2	2	22.8 ± 0.3	5.2 ± 0.3	0.7 ± 0.2
3	22.5 ± 0.4	5.9 ± 0.1	0.8 ± 0.2	3	22.7 ± 0.3	5.4 ± 0.2	0.7 ± 0.2
4	22.7 ± 0.3	6.5 ± 0.1	0.7 ± 0.1	4	22.4 ± 0.8	5.7 ± 0.2	0.6 ± 0.1
5	22.1 ± 0.4	6.4 ± 0.1	0.8 ± 0.2	5	21.6 ± 0.3	5.6 ± 0.2	0.6 ± 0.1
6	22.4 ± 0.4	5.8 ± 0.3	0.5 ± 0.1	6	21.6 ± 0.3	5.2 ± 0.2	0.6 ± 0.1



For plant data collection, each plant from each tray was monitored weekly for height, the number of nodes, and leaves for 6 weeks. Prior to harvest, chlorophyll density was averaged by taking 1 measurement from 3 individual leaves from the canopy of each plant using a chlorophyll SPAD meter (Soil Plant Analysis Development CCM-200 device, Opti-Sciences, Hudson, NH, USA).

Plants were harvested on August 17, 2023, for the CWS experiment and on August 22, 2023, for the LWPS experiment by separating the above ground biomass from the root mass (Figure 4). Fresh vegetable biomass and dry biomass was measured by scale (Bonvoisin 3000 g x 0.01 g). Plants were placed individually in a paper bag and weighed immediately for fresh biomass and for dry biomass, after 48h in a 70°C drying oven with standard electronic temperature controls (Johnson Controls, A421 PENN®)

## Analysis of root surface coverage

At harvest, the stems were cut at the soil line, and the growing medium, along with the roots, was placed on a contrasting white

surface and background for visibility. Photographs of the growing medium containing the roots were taken from a randomly selected representative plant for each treatment across both experiments. These photos were captured from two different perspectives: a bottom view and a side view as shown on the left in Figure 5. The surface area coverage of the roots was determined using Image J 1.54d (Wayne Rasband and contributors National Institutes of Health, USA). Each image was converted to an 8-bit grayscale format, and subsequent threshold adjustments were made based on specific requirements to highlight the white areas representing the roots while excluding white areas representative of light-colored particles (perlite) in the mixed medium. The standardized threshold used for side profile images of all treatments had a lower boundary of 63 and an upper boundary of 255 while the threshold for the bottom view included a lower boundary of 30 and an upper boundary of 255. After the threshold was adjusted, the growing medium containing the roots was traced and the measurement tool was used to analyze the area fraction (%AF), which is a percentage representing how much white there is compared to black. In this case, it represents the extent of surface root coverage for a specific treatment.

**A.**



**B.**



**FIGURE 4**

Basil plant growth from transplantation (left image) to harvest (right image) for each nutshell experiment (A) Crushed Walnut Shell, (B) Line Waste Pistachio Shell).



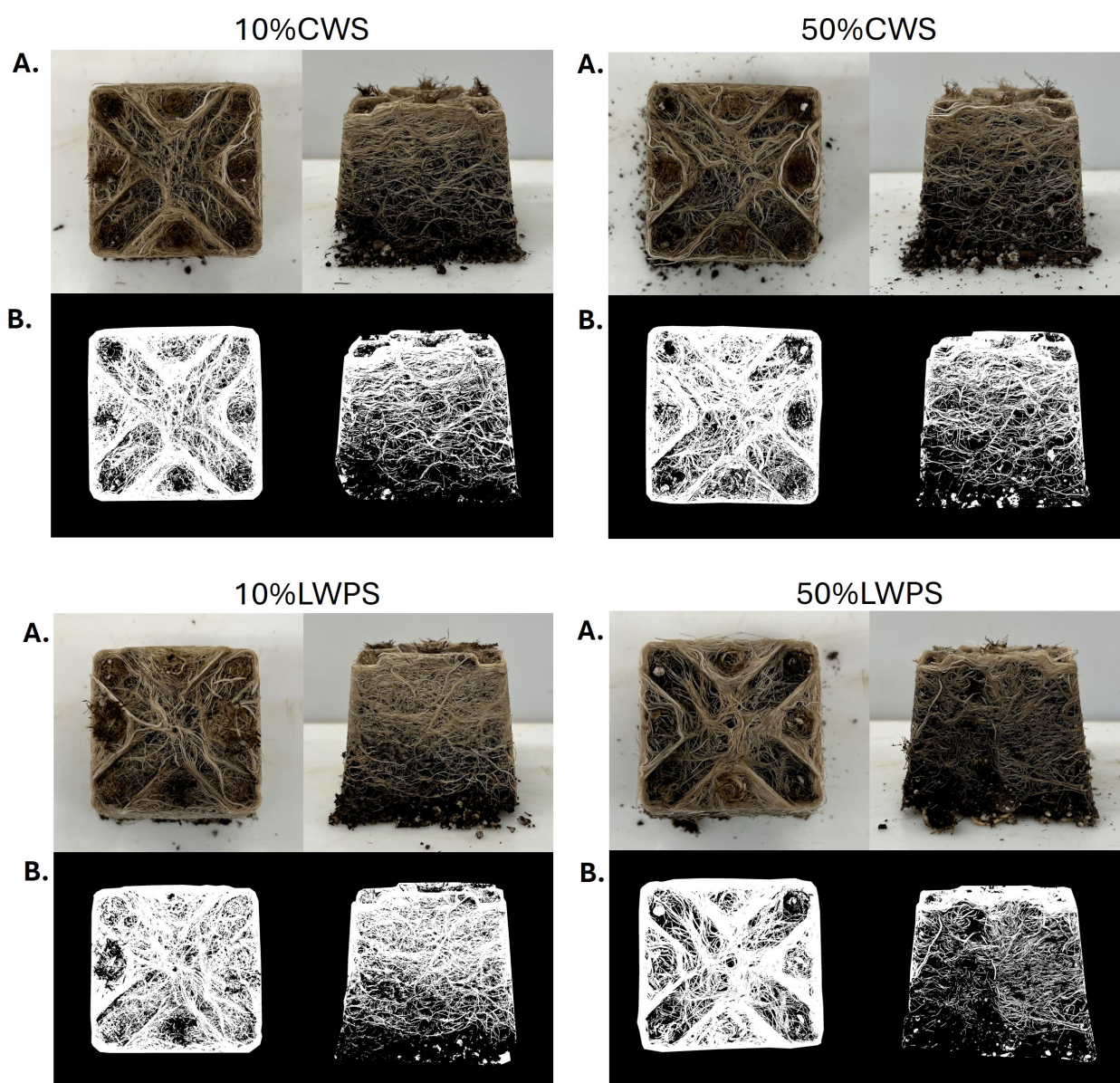


FIGURE 5

Examples of root coverage of Basil plants grown in the lowest (10%) and highest (50%) percent inclusion for CWS and LWPS experiments. (A) Images of root coverage before processing with ImageJ. (B) Images of root coverage after processing with ImageJ. Bottom view (left) and side view (right).

## Description and monitoring of recirculating aquaculture system

The aquaponic solution for this study was derived from wastewater collected from hybrid carp (*Cyprinus carpio* X *Carassius auratus*) maintained in 6000L recirculating aquaculture systems (RAS) located at the UC Davis Center for Aquatic Biology and Aquaculture (CABA). The RAS utilizes two circular fish tanks, a radial flow filter, and three IBC totes that served as 1) secondary clarifier using Matala filter pads, 2) a moving bed bioreactor filled with k1 media, and 3) a sump. Each fish tank (1893 L) contained 9 individuals, a total 18 fish. The total biomass and total length of the fish in tank 1 and tank 2 were 8.0 kg and 9.7 kg, and 33.8 ± 4.8 cm and

34.5 ± 2.5 cm respectively. Each tank was fed once a day on Mondays, Wednesdays and Fridays, with 200g of sinking complete feed for trout and steelhead (Skretting, Tooele, UT, USA). The trout feed consisted of 40% crude protein, 12% crude fat, 3% crude fiber, and 1% phosphorous. Once a week, 2200L of filtered water was pumped from the RAS and transported to the greenhouse to refill the plant sumps.

## Statistical analyses

Six trays (experimental unit) were utilized for the CWS and LWPS trials. Each tray contained 54 total plants with six plants for each of the nine treatments that were studied. In the CWS experiment, the

treatments comprised 10% CWS, 25% CWS, 33% CWS and 50% CWS, 10% CC, 25% CC, 33% CC and 50% CC, in addition to the 100% peatmoss/perlite control. A similar configuration was adopted in the LWPS experiment, wherein the treatments comprised 10% LWPS, 25% LWPS, 33% LWPS, 50% LWPS, 10% CC, 25% CC, 33% CC and 50% CC, in addition to the peatmoss/perlite control.

The weekly delta of basil height was computed for both the CWS and LWPS experiments. This involved determining the growth in centimeters for each week by subtracting the height recorded in week two from that of week one, and iteratively for subsequent weeks. Additionally, trend lines were calculated for each treatment utilizing Excel® to illustrate the growth patterns over the course of the experiments.

An Analysis of Variance (ANOVA) was conducted to assess the discrepancies in the number of basil leaves (recorded on the final day, day 42, prior to harvest) among distinct treatments within each experiment. Specifically, the treatments included in the analysis were those employed in the CWS experiment (control, coir coconut, and crushed walnut shells) and the LWPS experiment (control, coir coconut, and pistachio shells). Furthermore, comparisons were made among treatments with respect to the absolute height growth rate (AHGR), relative height growth rate (RHGR), number of nodes (ND), number of leaves (NL), SPAD values, fresh weight (FW), and dry weight (DW). In addition, a separate Analysis of Variance was performed to compare the %AF of the roots of a representative plant by the different treatments within both the CWS and LWPS experiments.

In the event of significant effects, *post hoc* mean comparisons were conducted utilizing the Tukey test, with a significance threshold set at a p-value less than 0.05. The statistical analyses were performed using Statgraphics Centurion 18 Version 18.1.12. All the variables complied with these assumptions of normality and homoscedasticity.

## Results

### Production and monitoring of plants

Weekly measurements of basil height revealed notable growth patterns across treatments in both the CWS and LWPS experiments (Figure 5). In the CWS trial, significant differences in basil height emerged by days 14 and 21, with the control and CC treatments demonstrating the greatest plant heights. However, by day 28, height differences among treatments began to converge, suggesting a leveling of growth across substrate types (Figure 6A). Growth trends in each treatment followed a polynomial regression model with a strong fit ( $R^2 > 97\%$ ), indicating consistent growth dynamics across replicates.

In the LWPS trial, the highest basil plants were observed in the 10%, 25%, and 33% LWPS treatments. Similar to the CWS results, basil growth in the CC treatments remained consistently high throughout the experiment (Figure 6B). Polynomial regression analysis also yielded strong model fits across treatments in the

LWPS trial, with  $R^2$  values exceeding 91% (Table 2), further supporting the robustness of the observed growth trends.

In the CWS trial, the absolute height growth rate (AHGR) exhibited similar growth patterns in the plants cultivated in CC substrate, 10% CWS, and 25% CWS. Notably, no significant differences were found at equal levels of inclusion of CC and CWS, excluding the 33% treatment. The relative height growth rate (RHGR) was higher and with similar performance for control and 10% CC. However, for the same percentage of inclusion, the values for CC were significantly higher than those for CWS. Plants with control, 25% CC and 50% CC substrate tended to have similar performance, and it is clearly observed that plants with 33% CWS and 50% CWS substrate had the lowest RHGR with a significant difference in relation to the other treatments. In relation to the number of nodes, treatments corresponding to control, 10% CWS, 25% CC, 33% CC and 50% CC substrates showed similar performance (Table 3).

The plants with the control, CC and CWS substrates tended to have a similar mean number of leaves and SPAD level. No significant differences for same inclusion level of CC and CWS, except for 33% (Table 3).

In relation to the LWPS trial, the AHGR was higher in the 25% LWPS and 10% LWPS treatments, with similar performance between them. No significant differences were observed for the 10%, 33% and 50% inclusion of CC and LWPS. According to RHGR, the highest values were found in the control, 10% LWPS and 25% LWPS treatments, which showed similar performance. No significant differences were observed for the 50% inclusion of CC and LWPS. Other LWPS inclusion levels showed better results. For the number of nodes and leaves, no significant differences were found at equal inclusion levels of CC and LWPS, excluding the 50% treatment. Plants grown in a 50% LWPS substrate had the lowest number of nodes and leaves (Table 3).

According to SPAD, no significant differences were found at equal levels of inclusion of CC and LWPS. Additionally, there were only significant differences between the plants with 10% LWPS and 50% LWPS, the second being the one with the lowest value.

In terms of fresh and dry weight, it was observed that increasing the concentration of CC in the substrate did not have a significant effect. In relation to fresh weight, no significant differences were found at equal levels of inclusion of CC and CWS, excluding the 33 and 50% treatments (lowest value). Furthermore, significant differences were only found with the control for 33 and 50% CWS. In the LWPS trial, significant differences in fresh weight with CC were only found for 50% LWPS, and this value was also significantly lower than the value obtained for the control.

Finally, the dry weight of the plants was not significantly different at the same levels of inclusion of CC and shells, except at 33% CWS and 25 and 50% LWPS. Compared to the control, the 33% and 50% CWS mixtures showed significantly lower values. In contrast, plants with 25% LWPS gave higher DW than CC and control (Table 3). Based on these results, it could be recommended to use 25% inclusion of both shells to maximize FW and DW production.



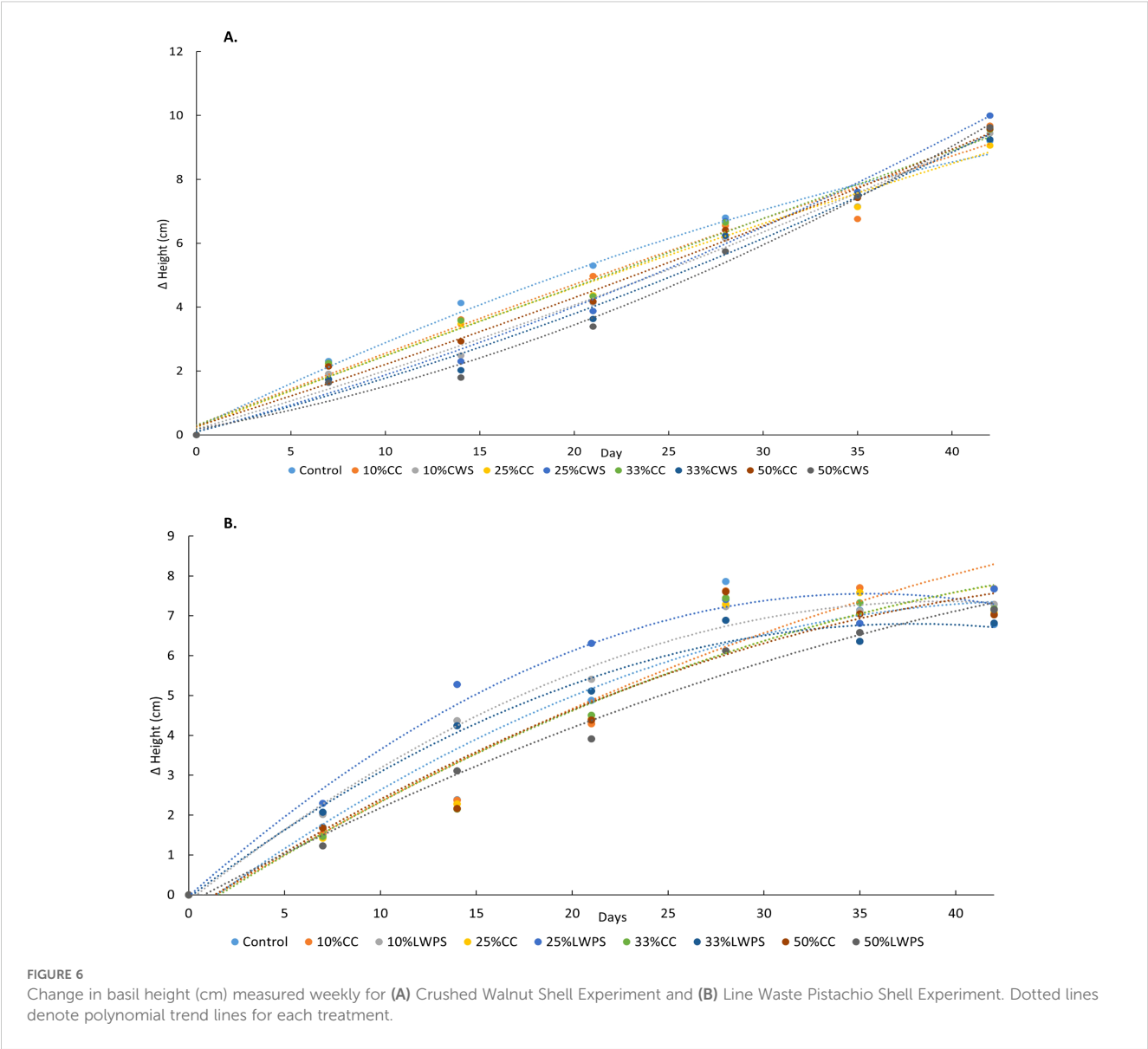


TABLE 2 Polynomial regressions to estimate plant height increments over time for each nutshell treatment by experiment.

CWS experiment		LWPS experiment	
Treatment	Polynomial regression	Treatment	Polynomial regression
Control	$Y = -0.0019x^2 + 0.2852x + 0.2278 \quad R^2 = 0.986$	Control	$Y = -0.004X^2 + 0.3543x - 0.5134 \quad R^2 = 0.9141$
10%CC	$Y = -0.0005x^2 + 0.2302x + 0.3009 \quad R^2 = 0.9745$	10%CC	$Y = -0.0021x^2 + 0.2967x - 0.4188 \quad R^2 = 0.9393$
10%CWS	$Y = 0.0011x^2 + 0.1711x + 0.1817 \quad R^2 = 0.9927$	10%LWPS	$Y = -0.0049x^2 + 0.3822x - 0.1569 \quad R^2 = 0.9901$
25%CC	$Y = -0.0006x^2 + 0.2309x + 0.2375 \quad R^2 = 0.9862$	25%CC	$Y = -0.0027x^2 + 0.3088x - 0.4892 \quad R^2 = 0.9356$
25%CWS	$Y = 0.0018x^2 + 0.1592x + 0.1003 \quad R^2 = 0.9865$	25%LWPS	$Y = -0.0061x^2 + 0.4287x - 0.0356 \quad R^2 = 0.9777$
33%CC	$Y = -7E-05x^2 + 0.2178x + 0.3019 \quad R^2 = 0.986$	33%CC	$Y = -0.0027x^2 + 0.3081x - 0.4862 \quad R^2 = 0.9311$
33%CWS	$Y = 0.0022x^2 + 0.1247x + 0.3997 \quad R^2 = 0.9786$	33%LWPS	$Y = -0.0048x^2 + 0.3636x - 0.0728 \quad R^2 = 0.9843$
50%CC	$Y = 0.0017x^2 + 0.1512x + 0.0952 \quad R^2 = 0.9848$	50%CC	$Y = -0.0029x^2 + 0.3098x - 0.4174 \quad R^2 = 0.9172$
50%CWS	$Y = 0.0029x^2 + 0.105x + 0.1796 \quad R^2 = 0.9897$	50%LWPS	$Y = -0.0018x^2 + 0.2567x - 0.2052 \quad R^2 = 0.9843$

X: Elapsed time (days). Y: ΔHeight (cm). Height increments for each week are calculated by subtracting the recorded height from the previous week's height.

**TABLE 3** Results for CWS and LWPS experiments. Mean and Standard Error (SE) of the absolute height growth rate (AHGR), relative height growth rate (RHGR), number of nodes (ND), number of leaves (NL), SPAD, fresh weight (FW) and dry weight (DW).

CWS experiment	AHGR (cm day <sup>-1</sup> )	RHGR (cm day <sup>-1</sup> )	ND	NL	SPAD	FW (g)	DW (g)
Treatment	Mean+SE	Mean+SE	Mean+SE	Mean+SE	Mean+SE	Mean+SE	Mean+SE
Control	0.83 ± 0.02 a	0.0425 ± 0.0003 ab	10.0 ± 0.8 ab	135.6 ± 10.7 a	25.1 ± 0.9 a	67.2 ± 7.8 ab	9.2 ± 0.8 a
10%CC	0.80 ± 0.02 ab	0.0442 ± 0.0009 a	9.6 ± 0.6 bcd	126.4 ± 10.6 ab	23.5 ± 0.8 abc	63.1 ± 4.1 abc	8.4 ± 0.4 abc
10%CWS	0.75 ± 0.03 bcd	0.0377 ± 0.0010 cde	10.2 ± 0.6 a	116.6 ± 7.2 abc	20.6 ± 0.8 cde	55.4 ± 4.3 abc	7.3 ± 0.5 bcd
25%CC	0.78 ± 0.03 abc	0.0406 ± 0.0009 bc	9.8 ± 0.8 abc	124.2 ± 16.5 ab	22.4 ± 1.0 abcd	64.8 ± 6.6 abc	9.0 ± 0.9 ab
25%CWS	0.77 ± 0.03 abcd	0.0376 ± 0.0014 de	9.7 ± 0.6 bcd	101.4 ± 7.3 bc	20.5 ± 1.2 de	56.4 ± 4.9 abc	7.5 ± 0.6 abcd
33%CC	0.81 ± 0.02 ab	0.0390 ± 0.0013 cde	9.8 ± 0.7 abc	131.4 ± 8.2 a	23.8 ± 1.7 ab	68.2 ± 4.2 a	9.2 ± 0.5 a
33%CWS	0.72 ± 0.03 cd	0.0371 ± 0.0008 e	9.3 ± 0.7 d	101.5 ± 5.3 bc	19.3 ± 0.6 e	52.8 ± 4.4 bc	6.9 ± 0.4 cd
50%CC	0.78 ± 0.04 abcd	0.0403 ± 0.0015 bcd	9.8 ± 0.8 abc	117.8 ± 6.3 abc	21.9 ± 1.1 bcde	61.6 ± 3.4 abc	8.0 ± 0.4 abcd
50%CWS	0.71 ± 0.02 d	0.0361 ± 0.0011 e	9.6 ± 0.6 cd	96.3 ± 6.7 c	19.4 ± 1.1 de	51.4 ± 6.1 c	6.7 ± 0.5 d
LWPS experiment	AHGR (cm day <sup>-1</sup> )	RHGR (cm day <sup>-1</sup> )	ND	NL	SPAD	FW (g)	DW (g)
Treatment	Mean+SE	Mean+SE	Mean+SE	Mean+SE	Mean+SE	Mean+SE	Mean+SE
Control	0.73 ± 0.04 bc	0.0339 ± 0.0010 abc	10.0 ± 0.4 ab	96.8 ± 7.3 bc	14.95 ± 0.60 ab	43.4 ± 4.2 ab	6.5 ± 0.5 c
10%CC	0.74 ± 0.03 bc	0.0317 ± 0.0006 cd	10.0 ± 0.3 ab	103.4 ± 6.4 abc	14.49 ± 0.65 ab	46.0 ± 3.1 ab	7.1 ± 0.6 bc
10%LWPS	0.80 ± 0.03 ab	0.0344 ± 0.0008 ab	10.4 ± 0.2 ab	109.2 ± 6.2 ab	15.43 ± 0.50 a	48.4 ± 3.1 ab	8.1 ± 0.4 ab
25%CC	0.72 ± 0.02 bc	0.0309 ± 0.0005 de	10.4 ± 0.3 ab	100.9 ± 8.0 abc	14.50 ± 0.33 ab	42.4 ± 3.0 ab	6.9 ± 0.5 bc
25%LWPS	0.85 ± 0.02 a	0.0356 ± 0.0007 a	10.3 ± 0.3 ab	119.3 ± 6.4 a	14.86 ± 0.93 ab	51.6 ± 4.4 a	8.6 ± 0.6 a
33%CC	0.72 ± 0.04 bc	0.0289 ± 0.0008 e	9.8 ± 0.5 abc	96.3 ± 11.6 bc	14.57 ± 0.67 ab	40.6 ± 3.6 b	7.1 ± 0.6 bc
33%LWPS	0.75 ± 0.02 bc	0.0326 ± 0.0006 bcd	9.6 ± 0.3 bc	85.0 ± 4.4 cd	15.26 ± 0.63 ab	38.8 ± 2.7 b	6.4 ± 0.4 c
50%CC	0.71 ± 0.03 bc	0.0327 ± 0.0009 bcd	10.6 ± 0.2 a	101.0 ± 7.1 abc	14.71 ± 0.67 ab	46.4 ± 5.9 ab	7.1 ± 0.6 bc
50%LWPS	0.67 ± 0.05 c	0.0313 ± 0.0015 de	9.1 ± 0.3 c	66.5 ± 4.5 d	13.46 ± 0.87 b	24.8 ± 3.0 c	4.3 ± 0.5 d

AHGR =  $\text{Height}_{\text{final}} - \text{Height}_{\text{initial}} / \text{time}_{\text{final}} - \text{time}_{\text{initial}}$ ; RHGR =  $\ln \text{Height}_{\text{final}} - \ln \text{Height}_{\text{initial}} / \text{time}_{\text{final}} - \text{time}_{\text{initial}}$ ; ND, NL and SPAD: Values reported for the last measurement (42d). Values without the same letters denote that there are no significant statistical differences ( $p$ -value < 0.05).

## Analysis of root surface coverage

It was observed that there was no significant difference in the %AF of the bottom part of the roots between treatments ( $p$ -value = 0.9766), the same occurred with the side part in the case of the CWS experiment ( $p$ -value = 0.4966). Respect to the LWPS experiment, it was observed no significant difference in the %AF of the bottom part of the roots between treatments ( $p$ -value = 0.5650). Conversely, there was significant difference in the %AF of the side part of the roots between treatments ( $p$ -value = 0.0031). The roots from the 50%LWPS substrate were the only one that had significant differences in comparison with the other treatments, having the lowest values of %AF (Table 4).

## Discussion

In our experiments with crushed walnut shells (CWS), basil plants initially exhibited reduced growth compared to those grown in substrates containing coconut coir (CC) and the control mixture. However, by day 28, growth rates had largely converged across all nine treatments. The different concentrations of CC in the substrate did not significantly impact fresh or dry weight, whereas increasing concentrations of CWS led to a noticeable decline in these parameters. This reduction in biomass may be attributed to the presence of allelopathic compounds such as juglone, known to inhibit plant growth at higher concentrations.

The observed early growth suppression in CWS substrates is consistent with the phytotoxic nature of juglone, which can have

TABLE 4 Mean percent area fraction (% AF) of the roots by the CWS experiment and the LWPS experiment.

Experiment	Treatment	Bottom part	Side part	Experiment	Treatment	Bottom part	Side part
<b>CWS</b>				<b>LWPS</b>			
	Control	74.06+1.81 a	41.74+2.72 a		Control	77.96+1.28 a	46.33+2.06 a
	10%CC	72.99+2.38 a	41.93+2.21 a		10%CC	78.85+1.35 a	47.58+2.86 a
	10%CWS	72.63+1.65 a	43.00+2.02 a		10%LWPS	76.91+2.93 a	46.89+2.03 a
	25%CC	72.25+2.86 a	47.12+2.05 a		25%CC	79.52+0.88 a	49.21+1.41 a
	25%CWS	72.02+1.39 a	41.59+1.9 a		25%LWPS	77.15+1.99 a	46.34+4.55 a
	33%CC	70.40+2.80 a	41.81+1.87 a		33%CC	79.02+1.77 a	47.30+1.75 a
	33%CWS	72.25+0.60 a	44.43+2.42 a		33%LWPS	80.08+3.38 a	45.65+2.70 a
	50%CC	73.02+2.17 a	43.35+2.79 a		50%CC	78.93+1.54 a	50.92+2.04 a
	50%CWS	73.48+1.83 a	42.06+0.92 a		50%LWPS	73.90+3.23 a	34.51+1.07 b

Values without the same letters denote that there are no significant statistical differences (Test *post hoc* – Tukey test,  $p$ -value<0.05).

both inhibitory and stimulatory effects depending on dosage and environmental conditions (Bhamini and Kumar, 2019). Over time, microbial activity in the aquaponic effluent and substrate likely degraded or transformed juglone, reducing its phytotoxicity. Adequate drainage may have further supported this process, eventually allowing plant growth to normalize across treatments (Schmidt, 1988).

In contrast, line waste pistachio shells (LWPS) used at moderate inclusion rates ( $\leq 33\%$ ) produced growth rates comparable to those in CC treatments. However, at 50% inclusion, a marked decline in growth was observed, particularly in dry weight. This reduction may be due to the increased presence of phenolic compounds known for their phytotoxic effects (Karimi et al., 2013). Additionally, the physical size and rigidity of uncrushed LWPS appeared to restrict root development and reduce water-holding capacity, further impairing plant performance (J. Gross, personal observation).

While physical properties such as air porosity, bulk density, and water holding capacity were not directly measured in this study, qualitative observations suggest these characteristics had a notable influence on plant growth. This was particularly evident in the LWPS treatments, where the surface of the root zone often remained visibly dry and failed to wick moisture effectively. In contrast, CWS substrates absorbed and retained water more readily, with their moisture-holding capacity improving over time. This difference is likely due to the relatively lower density and higher surface area of the walnut shells compared to pistachio shells, which are more dense and less porous. The improved hydration observed in CWS treatments may have contributed to the slightly greater plant height and biomass in basil grown in walnut substrates versus pistachio. Based on these findings, we recommend limiting the inclusion of CWS and LWPS to  $\leq 33\%$  of the total substrate volume unless additional processing steps are implemented. For LWPS, mechanical crushing could enhance water retention and reduce root impedance, while both substrates may benefit from chemical or microbial treatments to degrade phytotoxic compounds such as juglone (in walnut) and phenolics (in pistachio). Future studies

should include direct measurement of substrate physical properties to better characterize the effects of nutshell-based materials on irrigation dynamics, root zone moisture availability, and overall plant performance.

Although CC remains popular due to its water retention and nutrient-holding properties, it has notable environmental and agronomic drawbacks (Carlsson-Kanyama and González, 2009). Its high cation exchange capacity (CEC), while advantageous for nutrient retention, can cause nutrient lock-up, requiring extensive washing and chemical buffering to reduce sodium content (Domeño et al., 2011; Salah and Romanova, 2017). These practices are water-intensive and add to production costs. Moreover, the environmental burden of importing CC from tropical regions—including Indonesia, the Philippines, Sri Lanka, India, Thailand, and Malaysia, which account for  $\sim 90\%$  of global production (FAO, 2002)—raises concerns over its long-term sustainability (Atzori et al., 2021; Vinci and Rapa, 2019).

The use of walnut and pistachio shells in hydroponic systems offers a promising alternative to imported materials like CC. Beyond the U.S., this approach may also benefit other major nut-producing regions seeking local, sustainable, and cost-effective growing media options aligned with circular economy goals.

Replacing imported growing media with locally sourced nutshells can significantly reduce both the ecological footprint and the resource demands associated with substrate production. These agricultural byproducts require less processing than conventional media, helping to lower energy use and environmental impact while advancing sustainability goals. Additionally, utilizing nutshell waste supports circular economy principles in nut production systems, which are typically intensive in water, nutrient inputs, and biomass waste. Transforming this waste into a functional horticultural input repositions a by-product as a resource, enabling more efficient, climate-smart agricultural practices (Marvinney et al., 2014).

Future research should focus on optimizing the processing of these nutshells to further enhance their suitability as a complete



replacement for CC, especially considering the observed reduction in plant growth after transplanting at higher inclusion rates. Particular attention should be given to mitigating the potential initial presence of juglone in walnut shells and the phytotoxic effects of high concentrations of pistachio shells. Additionally, treatments that reduce phytotoxicity without relying on extensive water use would improve the practicality of these substrates for commercial-scale operations. Investigations into improving water holding capacity associated with higher nutshell use and reducing nutrient lock, issues associated with conventional media like coconut coir, could enhance the performance of nutshell-based blends and expand their application across a wider range of horticultural systems.

## Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

## Author contributions

GS-C: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Methodology, Investigation, Data curation, Formal analysis, Project administration. SL: Writing – review & editing, Writing – original draft, Validation, Methodology, Investigation, Data curation. NK: Writing – review & editing, Writing – original draft, Investigation, Conceptualization, Project administration, Supervision. AA: Writing – review & editing, Writing – original draft, Investigation, Data curation, Methodology, Visualization. MH: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Data curation. ID-G: Writing – review & editing, Writing – original draft, Funding acquisition, Resources, Supervision. VF-C: Writing – review & editing,

Writing – original draft, Supervision, Conceptualization, Formal analysis. JG: Writing – review & editing, Writing – original draft, Supervision, Conceptualization, Data curation, Funding acquisition, Investigation, Methodology, Resources, Validation, Visualization.

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## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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