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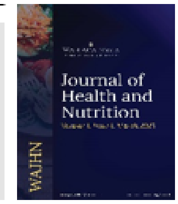

Original Article

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Proposing cost-effective alternatives of eggshell and pine cone to perlite for to use in hydroponic farming of iceberg lettuce: Evaluations under modified conditions for vegetative responses and antioxidant efficiency

Marul bitkisinin hidroponik yetiştiriciliğinde perlite düşük maliyetli alternatifler olarak yumurta kabuğu ve çam kozalağının önerilmesi: Vejetatif yanıtlar ve antioksidan etkinliği açısından değiştirilmiş koşullar altında değerlendirmeler

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Anahtar kelimeler: Topraksız tarım; Lactuca sativa; yetiştiricilik besi ortamları; DPPH; besin solüsyonları

Abstract / Özet

When considered within the context of resource efficiency in horticulture, perlite can be considered as tasking due to its extraction procedure and energy footprint despite its prominence as inert support medium for soilless lettuce production. We assessed whether two locally available organic residues, ground eggshell and shredded pine cone, can replace perlite in iceberg lettuce (*Lactuca sativa L.*) deep water culture. To conduct a factorial assessment, a commercial control nutrient solution coupled with three different support media were combined with nine modified solutions of potassium, calcium, and magnesium in concentrations of 5%, 10%, and 15% above the baseline for each modified solution. After 60 days, root length, leaf number, leaf length, marketable head weight, dry matter ratio, and DPPH radical scavenging capacity were recorded. Substrate did not significantly affect any vegetative trait when averaged across nutrient treatments ($p > 0.05$). In perlite, +5% and +10% potassium and +10% calcium reduced root length compared with the control ($p < 0.05$). Marketable head weight ranged from 325 to 440 g; the highest values were obtained on pine cone at +15% potassium. Dry matter ratio peaked at 4.2% under the same combination. DPPH EC50 lay between 18.97 and 59.72 μg mL–1, with the strongest antioxidant capacity in the control solution on perlite. Eggshell and pine cone produced outcomes statistically indistinguishable from perlite, suggesting that both materials are credible alternatives for low-input for plant hydroponic culturing systems.

Perlit, topraksız marul üretiminde baskın inert destek ortamıdır, ancak çıkarılması ve enerji ayak izi, kaynak bilincine sahip bahçeciliğin hedefleriyle pek bağdaşmamaktadır. Yerel olarak temin edilebilen iki organik kalıntı olan öğütülmüş yumurta kabuğu ve kalın öğütülmüş çam kozalağının, marul (*Lactuca sativa* L.) bitkisinin hydroponic su kültüründe perlitin yerini alıp alamayacağı bu çalışmada değerlendirilmiştir. Faktöriyel bir deneme oluşturularak, üç destek ortamı ve potasyum, kalsiyum veya magnezyum besin maddelerinin %5, %10 ve %15 konsantrasyonlarında kullanıldığı dokuz modifiye çözelti ile birleştirilmiştir. 60 gün sonra, kök uzunluğu, yaprak sayısı, yaprak uzunluğu, pazarlanabilir baş ağırlığı, kuru madde oranı ve DPPH radikal temizleme kapasitesi kaydedildi. Besin uygulamaları genelinde ortalama alındığında, substratın herhangi bir bitkisel özelliği önemli ölçüde etkilemediği görüldü ($p > 0{,}05$). Perlit içinde, +%5 ve +%10 potasyum ve +%10 kalsiyum, kontrol grubuna kıyasla kök uzunluğunu azalttı ($p < 0{,}05$). Pazarlanabilir baş ağırlığı 325 ila 440 g arasında değişti; en yüksek değerler +%15 potasyum içeren çam kozalağında elde edildi. Kuru madde oranı aynı kombinasyonda %4,2'ye ulaştı. DPPH EC50 değerleri 18,97 ile 59,72 μg mL–1 arasında değişti ve en güçlü antioksidan kapasitesi perlit üzerindeki kontrol çözeltisinde gözlendi. Yumurta kabuğu ve çam kozalakları, perlit ile istatistiksel olarak ayırt edilemeyen sonuçlar üretti; bu da her iki malzemenin de düşük girdi gerektiren hidroponik bitki yetiştirme sistemleri için güvenilir alternatifler olduğunu göstermektedir.

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Introduction

Global food demand is projected to rise sharply over the next two decades while arable land, freshwater, and mined inputs come under tightening environmental constraints [1,2]. Closed and semi-closed soilless systems offer one route through this pinch point: they decouple plant production from soil quality and reduce water use by 70 to 90% relative to open field cultivation [3]. Lettuce (*Lactuca sativa* L.) accounts for a large fraction of commercial soilless output and is the model species in most controlled-environment studies of nutrient and substrate management [4,5]. Deep water culture (DWC) keeps roots submerged in an aerated nutrient solution and tolerates simple, low-energy infrastructure [6,7], but it relies heavily on imported inert supports such as perlite, rockwool, or coconut coir. Perlite is mined, expanded at temperatures near 1,000 °C, and shipped over long distances; the resulting carbon footprint is increasingly cited as a barrier to sustainable horticulture [8]. Replacing this fraction with locally available residues would lower input costs and shorten the supply chain for small and medium growers.

Two candidate residues are readily available in Türkiye. Eggshell is produced at roughly 2.3 million metric tons per year worldwide and consists of about 94% calcium carbonate, with smaller amounts of magnesium and phosphorus [9]. Previous research indicated that pine bark can be used instead of perlite for lettuce farming by utilizing in coir-based blends, which had shown no noticeable decreases neither in yields nor in leaf nutrients, and in a similar vein, pine cone is also like pine bark that it is structurally stable and fibrous, yet lightweight forestry by-product [8]. However, examinations for using either crushed eggshell or shredded pine cone can be used as individual support medium in DWC are lacking.

Yet another point that requires clarification is the possible interactions between the substrate and macronutrient profile in a given solution. Of the known macronutrients, potassium is known to stimulate leaf expansion and regulation of osmotic balance; calcium is known to contributing to the integrity of cell-wall and limiting tipburn in head-forming cultivars; and magnesium is known as the essential constituent of chlorophyll [10,11]. While the optimum concentrations depend on cultivation, season, and system variables, reported ranges of these for lettuce farming reside in 150 and 300 mg L–1 for K, 150 and 250 mg L–1 for Ca, and 30 and 60 mg L–1 for Mg [12]. In commercial DWC production, it is common to see butterhead and looseleaf cultivars, yet it is also obvious that iceberg remains a rare sight in hydroponic research, may be due to it is being crisphead type.

This research primarily is aiming to evaluate to suitability of eggshell and pine cone in DWC instead of perlite for iceberg lettuce farming along with the evaluations of vegetative growth and antioxidant activity under modified macronutrient concentrations (K, Ca, Mg; 5%,10%, 15%) in support media.

Materials and methods

❖ Plant material and growth conditions

Experimental procedure was carried out in time frame between March 2022 to May 2022 in a commercial greenhouse that has passive ventilation located in Sivas province of Turkey (GEO: 39°45′ N, 37°01′ E; Altitude: 1,285 m). Research seedlings (*Lactuca sativa* L., crisphead type; Obtained from “Adana Fide ve Tohumculuk”, Türkiye) were in 3 to 4 true-leaf stage, and they were transplanted to plastic mesh pots having 5 cm in diameter, which were then placed in food-grade DWC tubes having 50 L volume and 40 × 62 × 27 cm dimensions. Dissolved oxygen level was kept above 5.5 mg L–1 by using diffuser stone equipped air pumps (3 W, 2.5 L min–1), which was above the reported lower threshold for lettuce [6]. Full-spectrum LEDs (220 µmol m–2 s–1 at canopy height) were used in 14 h photoperiod exposure. Mean air temperature was 22 ± 3 °C and relative humidity 60 ± 8%. Procedure was set to run for 60 days.

❖ Substrates and nutrient solutions

Three support media were used for comparison, which include coarse expanded perlite as control, 4 to 8 mm eggshell fragments, and shredded pine cone (*Pinus nigra* J.F. Arnold). Eggshell fragments and shredded pine cone were washed and dried in oven at 65 °C for 24 h prior to usage. Bulk densities for these media were 0.10 g cm–3 (Perlite), 0.83 g cm–3 (Eggshell), and 0.27 g cm–3 (pine cone).

A fertigation solution was prepared by using commercial fertigation mix obtained from “TARTES Tarım” (Turkey), and was used as control solution (EC 1.6 mS cm–1, pH 6.0, N 150, P 50, K 200, Ca 180, Mg 50 mg L–1). Nine single element modified experimentation solutions were prepared, which include 5%, 10% and 15% concentration increases relative to control solution in single elements of K, Ca, and Mg as seen in Table 1. Single element concentration increases were achieved by addition of KNO_3 for K, $Ca(NO_3)_2$ for Ca, and $MgSO_4$ for Mg. Solution renewals were carried out in 14 days periods along with daily dH2O toppings. EC and pH monitoring were conducted in two days periods with a 0.1 M KOH or HNO_3 correction in cases where more than 10% drifts occurred.

Table 1. Basics of Control and Experiment Solutions used in the study.

Factor	Code	Element added	Increment over control	Salt source
Control	CTL	Baseline (manufacturer)	0%	Commercial mix
Potassium	K5, K10, K15	K	+5, +10, +15%	KNO_3

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Calcium	Ca5, Ca10, Ca15	Ca	+5, +10, +15%	$Ca(NO_3)_2$
Magnesiu m	Mg5, Mg10, Mg15	Mg	+5, +10, +15%	$MgSO_4$

❖ Vegetative measurements

Twelve plants per treatment-substrate combination were harvested 60 days after transplanting. Root length was measured from the root collar to the tip of the longest primary root with a ruler (± 1 mm). Marketable leaves (firm, fully expanded, free of necrosis) were counted, and the longest leaf was measured from base to apex. Marketable head weight was determined on a top-loading balance (± 0.01 g) after removing the root collar. Dry matter ratio was obtained after oven-drying at 65 °C for 72 h and expressed as a percentage of fresh head weight.

❖ DPPH radical scavenging activity

Antioxidant activity was determined following Sánchez-Moreno et al. [13] with the modifications described by Sridhar and Charles [14]. Freeze-dried leaf tissue (50 mg) was extracted in 2 mL methanol (HPLC grade) for 24 h at 4 °C in darkness. Aliquots (10–100 µL) were combined with 1 mL of 0.1 mM DPPH (1,1-diphenyl-2-picrylhydrazyl) and 2.65 mL methanol, held in the dark for 30 min, and read at 517 nm on a microplate spectrophotometer (Thermo Multiskan FC). Percent inhibition was calculated against a DPPH blank, and EC_{50} values were derived from linear regression of percent inhibition against sample volume [15,16].

❖ Statistical analysis

Treatments were arranged in a randomized complete block design with three blocks and four replicate plants per block. One-way analysis of variance was used to test the effect of nutrient level within each substrate, with Dunnett's test for pairwise comparison against the unmodified control. Substrate effects on overall trait means were assessed by an additional one-way ANOVA across the ten nutrient treatments. Normality (Shapiro-Wilk) and homoscedasticity (Levene) were verified at $\alpha = 0.05$. All analyses were performed in IBM SPSS Statistics v.23 (IBM Corp., Armonk, NY, USA). Significance was set at $p < 0.05$.

Results

❖ Root length

Root length was the only vegetative trait that responded significantly to nutrient treatment, and the effect was confined to plants grown on perlite (Figure 1a). Within that substrate, the +5% and +10% potassium treatments reduced mean root length by 9.0 and 8.2 cm relative to the unmodified control (Dunnett, $p = 0.009$ and $p = 0.018$), and the +10% calcium treatment shortened roots by 7.75 cm ($p = 0.046$). On eggshell and pine cone, none of the modified solutions differed significantly from the control (Table 2). Mean values for root length measured in the range of 10.5 cm to 37.0 cm, which indicate possible biomass redistribution since root shortening in perlite did not result in head shrinkage or leaf decreases.

Table 2. Substrate to Element One-way ANOVA results for Root Length ($\alpha = 0.05$)

Trait	Substrate	Element	F	p
Root length	Perlite	Potassium	5.443	0.003
Root length	Perlite	Calcium	3.229	0.032
Root length	Perlite	Magnesium	2.096	0.116
Root length	Eggshell	Potassium	0.782	0.511
Root length	Eggshell	Calcium	1.052	0.380
Root length	Eggshell	Magnesium	0.429	0.733
Root length	Pine cone	Potassium	1.656	0.192
Root length	Pine cone	Calcium	1.388	0.260
Root length	Pine cone	Magnesium	0.655	0.585

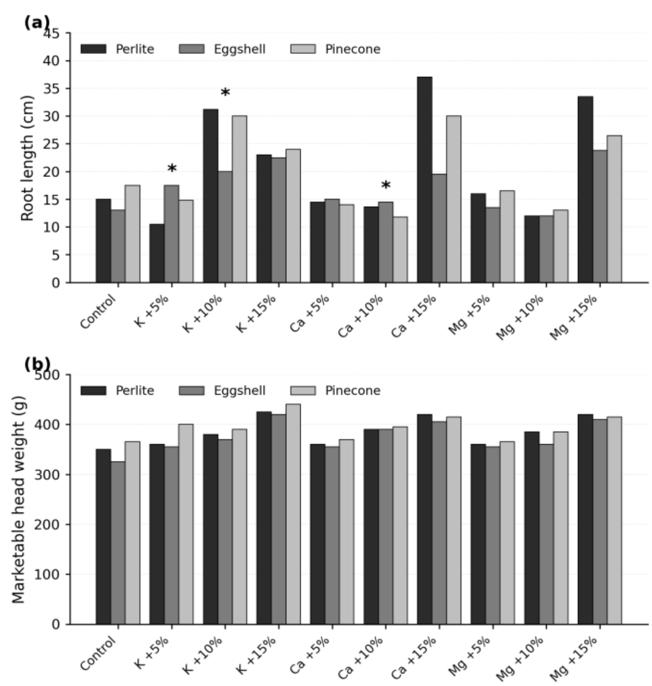

Figure 1. Root length (a) and marketable head weight (b) of iceberg lettuce after 60 days of hydroponic culture under three support media and ten nutrient solutions. Asterisks denote treatments that differed significantly from the unmodified control within the perlite substrate (Dunnett, $p < 0.05$). Bars represent means of 12 plants.

❖ Leaf number, leaf length, and head weight

Leaf number, leaf length, and marketable head weight did not respond significantly to nutrient treatment within any substrate (all $p > 0.05$). Leaf counts varied between 18 and 24 per plant, leaf length between 19.8 and 33.7 cm, and marketable head weight between 325 and 440 g (Figure 1b, Figure 2a). The +15% potassium treatment produced the heaviest heads on each substrate (perlite 425 g, eggshell 420 g, pine cone 440 g), but the differences against the corresponding controls were not significant. Averaged across all ten nutrient regimes, marketable head weight on pine cone (395 ± 23 g) was numerically higher than on perlite (388 ± 26 g) and eggshell (378 ± 27 g), without reaching significance (Table 3).

❖ Dry matter ratio

The obtained average mean for dry matter ratio was 3.69%, which has a variation between 3.02% in eggshell medium with control solution and 4.20% in pine cone medium with 15% increase in K concentration (Figure 2b). While comparisons to control solution groups were observed as insignificant, K concentration increases returned pronounced dry matter ratios across the media.

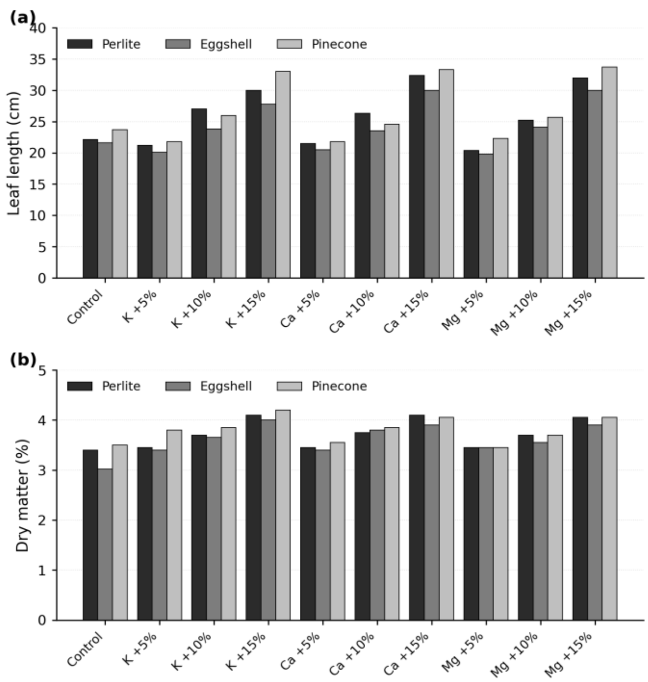

Figure 2. Parameters of Leaf length (a) and dry matter ratio (b) of 60 days cultured iceberg lettuces across different media and modified solutions. Bars are indicating mean values of the 12 plants used in the study.

❖ DPPH radical scavenging activity

DPPH EC50 values ranged from 18.97 to 59.72 µg mL–1 with an overall mean of 33.21 µg mL–1 (Figure 3). The lowest EC50 value, which is indicating the highest antioxidant scavenging, was obtained from control solution at 18.97 µg mL–1 and the highest EC50 value was obtained from eggshell medium with 15% increase in Ca concentration at 59.72 µg mL–1. The experimental concentration groups gave up a range of 20.2% and 56.4% inhibition ratio that is in line with linearity for EC50 estimation. Mg concentration increases returned weaker scavenging activities, particularly in organic media, compared to Ca concentration increases (Table 3).

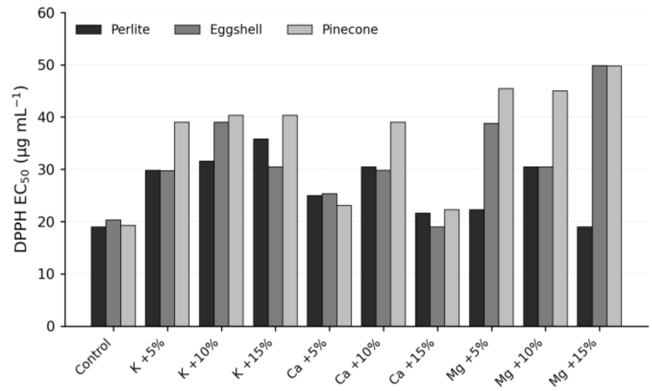

Figure 3. Methanolic extract DPPH EC_{50} values in experiment after 60 days of culturing. Lower values point to stronger antioxidant activity and bars are representatives of triplicates.

Table 3. Trait measurements in different media across nutrient solutions presented as mean ± SD.

Trait (unit)	Perlite	Eggshell	Pine cone
Marketable head weight (g)	388 ± 26	378 ± 27	395 ± 23
Leaf number (n/plant)	21.4 ± 1.6	19.5 ± 1.4	21.4 ± 1.8
Leaf length (cm)	25.8 ± 4.6	23.8 ± 3.7	26.6 ± 4.6
Dry matter ratio (%)	3.74 ± 0.27	3.59 ± 0.30	3.83 ± 0.24
DPPH EC50 (µg mL^{-1})	26.3 ± 6.0	30.9 ± 9.4	34.1 ± 12.0

Discussion

It can basically be said that both crushed eggshell and shredded pine cone are as effective as mainstream perlite in hydroponic iceberg lettuce farming. Across the three-support media and used solutions, there was <5% deviance, which indicates statistical insignificance, in parameters of marketable head weight, leaf number, and dry matter ratio. The experimental setup had the capacity to assess 15% reduction in head weight, yet no reduction observed was nearing to capacity. Obtained results were found to be consistent with those of reported by Dias et al. [8], where pine bark substitution for perlite in coir-based blends was conducted and provided no decreases in leaf macronutrient concentrations and fresh biomass levels as seen in two different electrical conductions.

In the context of K enrichment, root shortenings were observed with 5% and 10% concentration increases in perlite medium, only nearing to control values in case of 15% concentration increase. This observation can be attributed to ion balance disruption in root surface that was returning to normal under higher concentration due to the restoration of rhizasphere buffering. Such observations were not the case for eggshell and pine cone media, which can be attributed to the nature of the medium, where eggshell would release Ca ions,

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and pine cone might release phenolics and tannins to create a buffering for alterations in fertigation [9].

Tipburn symptoms, even in the highest Ca concentration enrichment solutions, were not observed in the research. The greenhouse environment in the experimental setup can be attributed to this outcome, where the passive ventilation and LED supplementation possibly prevented inner-leaf formations [11]. This outcome has limited the assessment of Ca-dependent protective effects in tipburn formation.

Across the experimental groups, antioxidant activity assessment results were observed as highly variable, where the highest antioxidant scavenging result was observed in perlite medium having control solution (18.97 µg mL−1), which practically emphasizes the sufficiency of baseline fertigation for antioxidant capacity. Single element concentration increases did not result in enhanced antioxidant scavenging, while in some resulted in weakening. Similar cases were reported by Moon et al. [16] and Materska et al. [17] previously, where compositional shifts in polyphenols were attributed to K and Ca concentrations rather than total ion compositions. The obtained mean value of 33.2 µg mL−1 can be considered as the expected antioxidant capacity for iceberg lettuce, and is within the range of antioxidant capacity from greenhouse production as reported by Liu et al. [18].

Overall, in the context of DWC, perlite can be changed with either eggshell or pine cone, at least in small to moderate operations. Eggshell is ubiquitous in food industry and incurs expense due to landfill disposal requirements [9,19], while pine cone is readily abundant due to forestry operations across the Mediterranean. Both of these cost-effective alternatives require only washing, oven-drying, and screening that can be achieved by fairly modest equipments. Nevertheless, the present study was included single geographical locale and had cycles of 60 days for singular trials along with single element concentration increases that might prevent the occurrences of ionic interactions between K, Ca, and Mg, which could be observable in cases where multiple element concentrations were the case [10]. It can be stated that differentiation of ion absorptions from biomass dilution would require direct ion analysis on leaf tissues.

Conclusion

The results provided ample support for the use of eggshell and pine cone as cost-effective alternatives to perlite in iceberg lettuce farming as seen from marketable head weights (325 and 440) and insignificance on vegetative traits. Aside from the obtained dry matter ratio in 15% concentration increase in K, other single element concentration increases did not result in improvements in marketable yields. Organic natures of eggshell and pine cone provided buffering of ion concentration alterations as seen in root length observations, which was a significant difference compared to perlite medium. DPPH antioxidant activity was strongest under the unmodified control. Locally available organic residues can therefore replace perlite in low-input DWC operations without sacrificing iceberg lettuce yield or quality.

Conflict of Interest: NIL

Funding Sources: NIL

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Declarations:

Authors' Contribution:

- All Authors Conceptualization, data collection, interpretation, drafting of the manuscript and intellectual revisions
- The authors agree to take responsibility for every facet of the work, making sure that any concerns about its integrity or veracity are thoroughly examined and addressed
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