Soilless greenhouse cultivation: growth and yield of ginger in response to the pot size and culture media

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Abstract: The increasing shortage of arable land and adverse weather conditions have caused a significant part of crops, especially medicinal plants, to be cultivated through soilless cultivation methods. In this research, the soilless greenhouse cultivation of ginger was studied. The experiment was laid out in a 2×3 factorial scheme based on a completely randomized design with 3 replications. Treatments consisted of two different sizes of pots (10 l and 20 l), and three types of culture media, including sand, perlite, and an equal (1:1) mixture of sand and perlite. Based on the results, except for leaf area and rhizome sodium content, the interaction effect was significant for most of the traits. The highest rhizome dry mass (58.13 \pm 2.05), rhizome dry material content (43.68 \pm 1.19), were recorded in larger pots containing the mixture medium. There were positive and significant correlations between leaf area and some of important traits such as rhizome dry mass (0.48) and rhizome dry matter content (0.58). The results of this research confirmed that by taking into account the most suitable pot size and cultivation medium (i.e. 20 l pot and mixed culture medium), the maximum yield of rhizome fresh mass can be obtained up to 133.5 ± 7.94 g.

Key words: culture medium, perlite, sand

Odziv rasti in pridelka na velikost lonca in substrat pri gojenju ingverja v rastlinjaku

Izvleček: Naraščajoče pomanjkanje orne zemlje in neugodne vremenske razmere so povzročile, da se številne gojene rastline, še posebej zdravilne zdravilne rastline, gojijo v različnih načinih brez tal. V tej raziskavi je bilo preučevano gojenje ingverja v rastlinjaku v loncih. Poskus je bil zasnovan kot popolni naključni 2 × 3 faktorski poskus s tremi ponovitvami. Obravnavanja so obsegala dve velikosti loncev (10 l in 20 l) in tri vrste substrata in sicer pesek, perlit in mešanico peska in perlite v razmerju 1:1. Rezultati so pokazali, da je bila interakcija med obravnavanji z izjemo listne površine in vsebnosti natrija v koreniki značilna za večino lastnosti. Največja suha masa korenike $(58,13 \pm 2,05)$ in vsebnost suhe snovi v njej $(43,68 \pm 1,19)$ sta bili ugotovljeni v večjih loncih, ki so vsebovali mešanico substratov. Ugotovljena je bila tudi značilna pozitivna povezava med listno površino in nekaterimi pomembnimi lastnostmi kot sta suha masa korenike (0,48) in vsebnostjo suhe snovi v njej (0,58). Rezultati raziskave so potrdili, da je potrebno upoštevati najprimernejšo velikost loncev in sestavo substrata (20 l-lonci in mešanica substrata), če hočemo doseči največji pridelek sveže mase korenik, to je do 133,5 \pm 7,94 g.

Ključne besede: gojitveni medij, perlit, pesek

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1 INTRODUCTION

Ginger, (*Zingiber officinale* Roscoe), belongs to the Zingiberaceae family with numerous medicinal, nutritional, and ethnomedical values. This plant is widely used as a spice, flavoring, and herbal medicine all over the world (Zhang et al., 2021). Traditionally, ginger has been used in many medicinal systems to treat a variety of ailments including nausea and vomiting, indigestion and constipation, asthma and cough, heart palpitations, inflammation, loss of appetite, etc. (Shahrajabian et al., 2019). Ginger is cultivated mainly in tropical regions up to 1500 m above sea level, both in dry and irrigated conditions (Guo et al., 2023).

The expansion of urbanization, industrialization of societies, and rise of the sea level have caused an increasing shortage of arable land. In addition, conventional soil cultivation faces increasing problems such as successive droughts, the unpredictability of climatic patterns, inefficient management of water resources, etc (Nerlich & Dannehl, 2021). As a result, today, a considerable part of crops are cultivated using soilless methods. Besides, such methods have a unique ability to optimally use inputs as well as dense agriculture (Ragaveena et al., 2021). In comparison with field cultivation, the volume of the culture medium used for each plant in soilless cultivation is considerably reduced and thus plant growth is significantly affected by the physical and chemical characteristics of the growing medium. A suitable substrate directly affects the growth, development, and maintenance of a functional root system. Moreover, the substrate acts as a reservoir of nutrients and water and is directly responsible for the diffusion of oxygen into the roots and other gas exchanges, so choosing a suitable culture medium is of particular importance. Various inorganic compounds are used as a cultivation medium in soilless agriculture, for example, pumice (Uzun et al., 2021), light expanded clay aggregates (LECA) (Mlih et al., 2020), prockwool (Nerlich et al., 2022), pearlite (Asaduzzaman et al., 2018), and sand (Robinson et al., 2013). Expanded perlite is produced by roasting perlite ore at temperatures up to 1000 °C. Due to its unique properties, such as low mass, and the ability to retain water, perlite is used as a substrate (Son, 2000). A suitable culture medium should be readily available, relatively inexpensive, stable, and easy to make it easy to handle and inexpensive to transport (Nemati et al., 2015).

Cultivation and processing of plants in cultivation containers have become popular as a prominent strategy in greenhouse industries. Studies show that plants grown in containers have generally different characteristics than field crops (Balliu et al., 2021). A meta-analysis revealed that by halving the pot size, biomass production decreased by an average of 43 % (Poorter et al., 2012). Logically, an ideal pot size depends on the size of the plant to be grown in it. Also, an incorrect choice of pot size may lead to changes in the results of the experiment. Therefore, this study focuses on the effect of the type of cultivation media and pot size on the growth and rhizome yield of ginger under greenhouse conditions.

2 MATERIALS AND METHODS

2.1 SITE SPECIFICATIONS

This study was carried out in the research greenhouses of the Faculty of Agriculture of Shahed University ($35^{\circ} 33' 15'' \text{ N} / 51^{\circ} 20' 24'' \text{ E}$), Tehran, Iran. The average temperature and humidity of the greenhouse were 28 °C and 70 %, respectively.

2.2 STATISTICAL DESIGN AND STUDIED TREAT-MENTS

The research was conducted in the form of a 2×3 factorial experiment based on a completely randomized statistical design with 3 replications. The treatments were: two different sizes of the pot (including 35×30 cm (10 l) and 45×35 cm (20 l)) and three different types of media, including sand, perlite, and an equal mixture (1:1) of sand and perlite.

2.3 PREPARATION OF SEEDS, GROWING MEDIA, POTS, AND PLANTING

Chinese ginger rhizomes were cut into pieces with an approximate weight of 30 ± 10 g and immediately disinfected using fungicide-bactericide (2 % Brodofix, www.baghbantak.com). To prepare the sand substrate, some beach sand was prepared and washed to remove its salinity and then dried in the open air. Perlite was also purchased from Madankavan Co. (https://madankavan. com). The mixed medium was also prepared by mixing equal parts of sand and perlite (1:1). Subsequently, pots and media were disinfected using a 2 % Brodofix solution. The pots were then filled with the appropriate amount of growing media. Inside each pot, 3 to 4 seeds were planted at a depth of 8 cm and were watered immediately. All the seeds were planted on February 4, 2022.

After planting, during the first two months, the pots were irrigated daily with 100 ml of distilled water. After these two months, and for 30 days, 100 ml of 50 % of Hoagland's nutrient solution (Table 1) was added to each pot Soilless greenhouse cultivation: growth and yield of ginger in response to the pot size and culture media

Macronutrients		Micronutrients		
Component	Quantities in solution (g l ⁻¹)	Component	Quantities in solution (g l-1)	
2M KNO ₃	202	MnCl ₂ •4H ₂ O	1.81	
2M Ca(NO ₃)2•4H2O	472	$ZnSO_4 \bullet_7 H_2O$	0.22	
2M MgSO ₄ •7H2O	493	CuSO ₄ •5H ₂ O	0.08	
1M KH ₂ PO ₄	136	Na ₂ MoO ₄ • ₂ H ₂ O	0.12	
Iron Chelate	15			

Table 1: Components of Hoagland solution

(Hoagland & Arnon, 1950). Subsequently, for the next 30 days, 100 ml of 70 % Hoagland's nutrient solution was added to each pot. Finally, rhizomes were harvested in July 2022.

2.4 STUDIED TRAITS

Before emptying the pots, leaf number (LN) was counted and the leaf area (LA) in cm² was determined using a leaf area meter (Delta-T, Cambridge, UK). Additionally, leaf fresh mass (LFM) and leaf dry mass (LDM) were also measured.

The yield was determined as rhizome fresh mass (RFM) at the end of the experiment. Also, root number (RN), root length (RL), and root fresh mass (RFM) were measured. Root dry mass (RDM) and rhizome dry mass (RDM) were determined after air-drying. The rhizome dry matter content (RDMC) was calculated by the ratio of the rhizome dry mass to the rhizome fresh mass and expressed in percent.

2.5 RHIZOME SODIUM AND POTASSIUM CON-TENT

The sodium and potassium content of the rhizomes were measured using the standard method for determining Na and K (Vdlufa, 2012). 2 g of powdered rhizome was ashed at 700 °C for 15 hours. The obtained ash was then dissolved in hydrochloric acid (25 %), heated in a water bath, and filtered to obtain the extract. Then, Na and K content was measured by a flame photometer device (Model 410, Sherwood Scientific Ltd, Cambridge, UK).

2.6 SUBSTRATE SOLUTION CHARACTERISTICS

The properties of the substrate solution that were studied included EC, and pH values. To obtain the substrate solution, the substrates were completely dried in the oven for 14 hours at 110 °C. Then, 5 g of each of the dried substrates was weighed and dissolved in 50 ml of distilled water for 2 hours in a shaker.

2.7 STATISTICAL ANALYSIS

Data were statistically analysed using SAS software (SAS Institute Inc., Cary, NC, USA). The data were subjected to the Kolmogorov-Smirnov normality test before the analysis of variance (ANOVA). A *post hoc* analysis was performed using Duncan's Multiple Range Test. Pearson correlations between traits were calculated using IBM SPSS software (version 22). Principal components analysis was performed using Minitab software (version 19).

3 RESULTS AND DISCUSSION

3.1 ANALYSIS OF VARIANCE

The results obtained from the analysis of the variance of the effects due to pot size and type of culture medium on some growth indices of ginger in soilless greenhouse cultivation conditions have been shown in Table 2. As can be seen in this table, the interaction effect was significant for most of the traits, except for leaf area and rhizome sodium content. Generally, main effects are not interpreted when the interaction term is significant

	Mean squares				
Traits	Culture medium (DF = 2)	Pot size $(DF = 1)$	Interaction (DF = 2)	Error (DF = 12)	
Leaf number	7.17n.s	88.89**	17.39**	2.5	
Leaf area pot ⁻¹	17116.67**	5477.56**	748.22n.s	436.39	
Leaf area	57.07**	1.03n.s	0.11n.s	1.45	
Leaf fresh mass	18.92*	71.16**	28.49*	4.11	
Leaf dry mass	8.41*	27.38**	14.86**	1.72	
Root number	0.5n.s	4.5**	3.5**	0.33	
Root length pot ⁻¹	188.39**	1250**	225.17**	17.39	
Root length	20.11*	18.97n.s	17.22*	4.11	
Root fresh mass	13.99**	55.3**	5.58**	0.62	
Root dry mass	3.84**	16.65**	1.42*	0.33	
Rhizome fresh mass	3889.35**	5742.35**	1405.01**	119.75	
Rhizome dry mass	1082.67**	1907.56**	491.76**	27.6	
Rhizome dry matter	252.49**	375.65**	85.76*	12.44	
Rhizome sodium content	665.26*	196.68n.s	266.68n.s	121.68	
Rhizome potassium content	41.76*	0.13n.s	46.54*	7.54	
Substrate pH	0.44**	0.01n.s	0.07*	0.01	
Substrate EC	310722.67n.s	732050*	606980.67*	128280.28	

Table 2: Analysis of variance of the effect of pot size and type of culture medium on the medicinal properties of ginger in soilless greenhouse cultivation conditions

*, and ** mean significance at the probability level of 0.05 and 0.01 respectively, and n.s means non-significant.

in the ANOVA table. Hence, in most traits, the focus is mainly on the interpretation of the interaction effect and the main effects were interpreted only for leaf area and rhizome sodium content.

3.2 CORRELATION COEFFICIENTS BETWEEN TRAITS

Pearson's correlation coefficients between the traits studied have been shown in Table 3. In this Table, only significant correlations ($p \le 0.05$) are shown. As can be seen, the negative correlations were not significant and therefore have been removed from the Table. Since investigating the ginger rhizome yield was one of the important goals of this research, the correlation between rhizome mass and other studied traits was of high importance. Rhizome mass showed a positive and significant correlation with root number, root length, root fresh mass, root dry mass, rhizome dry mass, rhizome dry matter percent, leaf dry mass, leaf number, leaf area, and substrate electrical conductivity (Table 3). Also, leaf area pot⁻¹ was positively and significantly correlated with, root fresh mass, root dry mass, rhizome fresh mass, rhizome dry mass, rhizome dry matter percent, leaf dry mass, root

length pot⁻¹, and substrate electrical conductivity (Table 2). Similar correlations were reported by Islam et al. (2008), and Jatoi and Watanabe (2013).

3.3 LENGTH, NUMBER, AND MASS OF ROOTS

Figure 1 demonstrates the comparisons of mean traits related to ginger root in soilless greenhouse cultivation as affected by substrate type and pot size. The highest root fresh mass of 9.03 g was observed in 20 l pots and mixed culture medium, which was not statistically different from that recorded in 20 l pots and perlite culture medium while, the minimum root fresh mass (2.65 g) was observed in 10 l pots and substrate. A similar trend was observed for root dry mass. Likewise, the maximum root length (12 cm) was found in the mixed substrate and larger pots (Fig. 1A). As shown in figure 1B, the highest number of roots (4.33) belonged to larger pots containing mixed culture medium, which showed more than 85 % growth compared to that recorded in smaller pots.

Figure 1 shows that in each of the three substrates, root number, root mass, as well as root length increased with pot size. This result suggests that root physical ap-

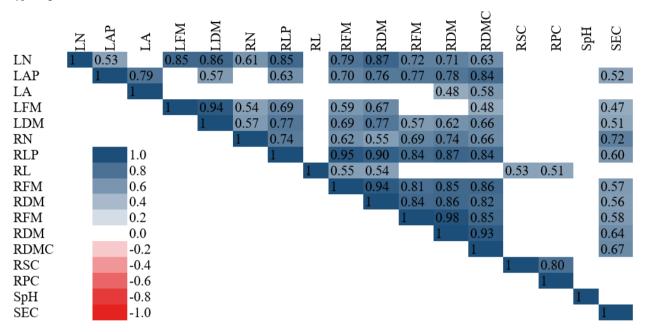


Table 3: Pearson's correlation coefficients between studied traits in ginger medicinal plant under the effect of pot size and substrate type in greenhouse conditions.

LN: leaf number, LAP: leaf area pot⁻¹, LA: leaf area, LFM: leaf fresh mass, LDM: leaf dry mass, RN: root number, RLP: root length pot⁻¹, RL: root length, RFM: root fresh mass, RDM: root dry mass, RFM: rhizome fresh mass, RDM: rhizome dry mass, RDMC: rhizome dry material content, RSC: rhizome sodium content, RPC: rhizome potassium content, SPH: substrate acidity, SEC: substrate electrical conductivity

pearance was a function of pot size independent of growing media. In line with this result, Hess and De Kroon (2007) reported that regardless of the type of culture medium or the concentration of nutrients, increasing the pot volume will lead to an increase in the size of the roots (i.e. root mass is a function of available rooting volume independent of nutrients). Unlike the Hess and De kroon hypothesis, Murphy et al. (2013) reported that root mass increased with pot size only in the treatment with high water-soluble fertilizer where plant size increased greatly with pot size. In this research, Murphy's report could not be confirmed or rejected since only a Hoagland solution was used to feed the plants. Figure 1 also shows that in the larger pot size, the root growth of plants in mixed media or perlite was significantly higher than that found in sand. This result suggests that a suitable pot size along with a suitable substrate led to the expansion of the root system of the ginger plant.

3.4 MASS, NUMBER, AND AREA OF LEAVES

The effects due to pot size and the type of culture medium on ginger plant leaf attributes have been demonstrated in Figures 2 and 3. The results showed that in similar substrates, leaf attributes (mass, number, area) were significantly higher in larger pots, which seems to be due to the improvement in root growth. As previously shown, larger pots were found to have greater root length and mass, which would logically lead to improved water and nutrient uptake and thus increased plant growth. The significant positive correlation between leaf growth indices (i.e. number, area, and mass), and root attributes (i.e. length and mass) listed in Table 1 confirms this hypothesis. Also, the significant positive correlation between the leaf area and root growth indices suggests that decreased root growth indices observed in smaller pots were mainly due to the decrease in the photosynthetic area of the plant (Table 1). In line with this suggestion, it has been reported that the growth of plants in the pot is a function of photosynthesis per unit of leaf area (Poorter et al., 2012).

According to Figure 3A, the leaf area in the pots containing perlite as well as mixed substrates was significantly higher than in the pots containing sand. In line with this result, Vanaei et al. (2008) by studying the effects of pot size and the type of culture medium on potato seedlings observed that the highest number and mass of small tubers found in the pearlite culture medium.

The physical characteristics of the cultivation substrate have an effective role in root growth and water absorption. By definition, the water holding capacity (WHC) of a substrate is the volume of water retained by a saturated growing medium after it is allowed to drain,

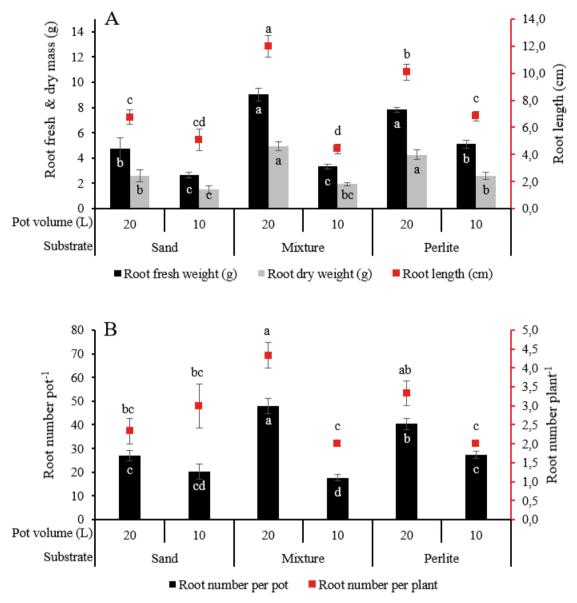


Figure 1: Comparisons of mean traits related to the ginger root (*Zingiber officinale*) in soilless greenhouse cultivation as affected by substrate type (A) and pot size (B). Within each trait, the difference between the means that have common letters is not statistically significant according to Duncan's Multiple Range Test ($\alpha = 0.05$)

which is greatly influenced by the size of the particles. Therefore, in this research, it seems that compared to sand mediums, perlite, as well as the mixture substrates, had a higher WHC, which resulted in more appropriate growth indices of ginger plants. This conclusion is supported by Kakoei and Salehi (2013) who reported that the WHC of perlite is much higher than that of sand.

3.5 YIELD AND DRY MATTER CONTENT OF RHIZOMES

Figure 4 shows the comparison of the average rhizome yield of the ginger plant under the influence of pot size and culture medium. The maximum rhizome yield (fresh and dry mass) was obtained in larger pots and

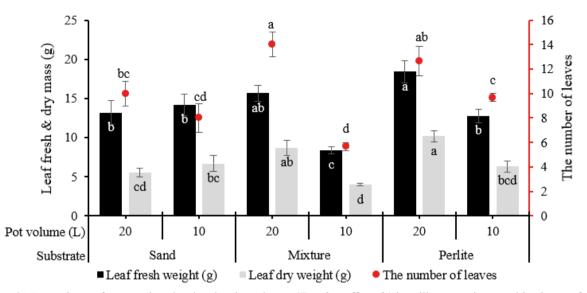


Figure 2: Comparisons of mean traits related to the ginger leaves (*Zingiber officinale*) in soilless greenhouse cultivation as affected by substrate type and pot size. Within each trait, the difference between the means that have common letters is not statistically significant according to Duncan's Multiple Range Test ($\alpha = 0.05$)

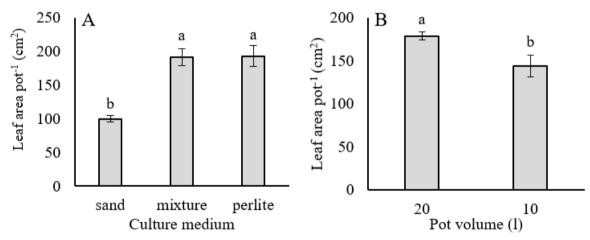


Figure 3: Comparisons of the mean area of ginger leaves (*Zingiber officinale*) under the effect of culture medium (A) and pot size (B) in soilless greenhouse cultivation. The difference between the means that have common letters is not statistically significant according to Duncan's Multiple Range Test ($\alpha = 0.05$)

mixed culture medium, while the lowest rhizome yield belonged to smaller pots and sand culture medium (Figure 4). Similarly, the larger pots containing either perlite or mixture substrates were found to have the highest percentage of rhizome dry matter content (Figure 4), while the lowest percentage was found for sand substrates in both pot sizes. These results show that the better allocation of photosynthetic products within the rhizomes has occurred in the large pots containing mixed media or perlite, which can be caused by the improvement of the leaf area and as a result, the increase in photosynthetic products. In agreement with the results of this study, Hemmat et al. (2014) reported that larger pots (3 l) containing an equal mixture of peat moss and sand significantly improved the number and dry matter of minitubers.

3.6 SODIUM, AND POTASSIUM CONTENT OF RHIZOMES

The average rhizome sodium content fluctuated from 44.50 mg g⁻¹ for the sand medium to 65.33 mg g⁻¹ for the perlite medium (Figure 5).

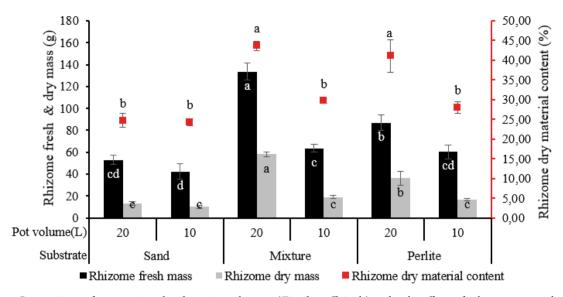


Figure 4: Comparisons of mean traits related to ginger rhizome (*Zingiber officinale*) under the effects of substrate type and pot size in soilless greenhouse cultivation. Within each trait, the difference between the means that have common letters is not statistically significant according to Duncan's Multiple Range Test ($\alpha = 0.05$)

The absorption of metal ions by the plant root system is a function of the concentration of these ions in the culture medium. In this study, compared to the perlite, the lower water-holding capacity of pots containing sand caused the nutrients to be removed from the pots during the leaching process, as a result, the plant's access to nutrients decreased. Consequently, the sodium content of the rhizome showed a significant decrease in the sand culture medium. On the contrary, in perlite culture medium (or mixture), due to the higher capacity of holding water (i.e. reduction in the leaching rate of nutrition solution), the absorbable sodium ions increased and as a

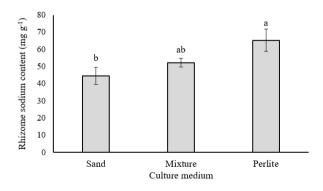


Figure 5: Comparisons of mean sodium content in ginger rhizome tissue (*Zingiber officinale*) under the effects of substrate type and pot size in soilless greenhouse cultivation. The difference between the means that have common letters is not statistically significant according to Duncan's Multiple Range Test ($\alpha = 0.05$)

result, the sodium content of the rhizome was observed at the highest level (Figure 5).

The effects of substrate type and pot size on potassium content of ginger rhizome tissue in soilless greenhouse cultivation have been shown in Fig. 6. On average, the potassium content measured in rhizome tissue ranged from 3.00 mg g⁻¹ in smaller pots and sand medium to 13.67 mg g⁻¹ in smaller pots and perlite medium (Fig. 6).

As discussed the sodium content of the rhizome, due to the lower WHC of the pot, the concentration of potassium in the pots containing sand was lower compared to other culture media, which led to a decrease in potassium absorption and therefore, in the sand substrate, the potassium content of the rhizome showed a significant decrease. On the contrary, in the perlite culture medium, after increasing the WHC and reducing nutrient leaching, the absorbable potassium ions increased and as a result, the rhizome potassium content was at the highest level (Figure 6). The pattern governing the changes of this trait among the experimental treatments showed that in any culture medium, the larger pots had more rhizome potassium content.

3.7 ACIDITY AND ELECTRICAL CONDUCTIVITY OF SUBSTRATES

The comparison of the mean acidity of culture medium in pots of different sizes has been presented in Figure 7. In general, the acidity fluctuated from 6.62

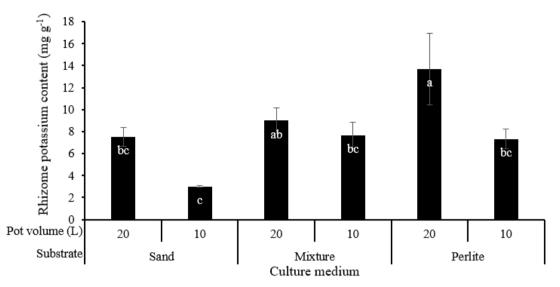


Figure 6: Comparisons of mean potassium content in ginger rhizome tissue (*Zingiber officinale*) under the effects of substrate type and pot size in soilless greenhouse cultivation. The difference between the means that have common letters is not statistically significant according to Duncan's Multiple Range Test ($\alpha = 0.05$)

(for the treatment combination of mixed substrate-10 l pots) to 7.34 (for the treatment combination of perlite substrate-10 l pots). It has been reported that perlite is neutral with an acidity between 7 and 7.5, which is consistent with the results of this research (Bar-Tal et al., 2019). Also, it has been reported that the acidity of sand is around 7 (Chen et al., 2022). In this research, the acidity of each sand and perlite culture medium was around 7, but when perlite was mixed with sand, the pH value decreased significantly. The unusually high pH when treating perlite 10 l pots was probably more related to the use of the nutrient solution itself than to the substrate. Figure 7 also shows the comparison of the mean electrical conductivity (EC) of the substrates. The pattern governing the changes of EC among the experimental treatments showed that, in general, the pots containing sand substrate had lower EC. Also, for the sand substrate, smaller pots had more EC, while for the other two substrates (i.e. perlite and mixture), larger pots showed more EC. The highest EC (1.43 dS m⁻¹) was observed for larger

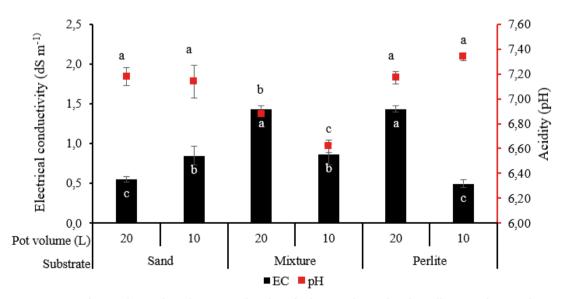


Figure 7: Comparison of mean electrical conductivity and acidity of culture media used in the soilless greenhouse cultivation of ginger (*Zingiber officinale*). Within each trait, the difference between the means that have common letters is not statistically significant according to Duncan's Multiple Range Test ($\alpha = 0.05$)

pots containing perlite as well as mixed substrates while the lowest EC (0.49 dS m⁻¹) belonged to the smaller pots containing the perlite substrate, which was not statistically different from that recorded for sand substrate and larger pot (0.55 dS m⁻¹).

In this study, based on the Wilcox diagram, the EC of the culture media was in class c1, which was not salty at all. However, there were significant differences between the substrates EC. Since perlite and sand are neutral (Bush, 2001; Chen et al., 2022), the observed increase in the EC of the substrates seems to be due to the elements in the nutrient solution (Hoogland's solution) added to the pots. Considering that the volume and concentration of the nutrient solution added to all the pots were the same, therefore, the change in EC should logically be caused by the difference in leaching rate. The WHC of perlite is higher than that of sand, which means that compared to sand, the leaching rate in perlite (as well as the mixed substrate) is lower which naturally leads to more accumulation of nutrient solution and subsequently, increased concentration of ions in the substrate. Consequently, the increase of ions will rise the EC of the substrate. Moreover, by increasing the size of the pot, the leaching rate will decrease and as a result, more nutrients will accumulate in the substrate. Consequently, the larger the size of the pot, the greater the EC.

4 CONCLUSIONS

The results obtained from this research confirmed the possibility of economically growing ginger in a greenhouse. In general, 20 l pots significantly increased rhizome yield and were therefore more suitable for greenhouse cultivation. Since most rhizome-related traits were recorded in the sand-perlite mixture medium, this type of substrate was recognized as the most suitable substrate for potted ginger plant cultivation. The results of this research confirmed that by taking into account the most suitable pot size and cultivation medium (20 l pot and a mixture medium) the maximum yield of rhizome fresh mass is 133.5 g. Examining Pearson's correlations between traits showed that the leaf area was positively significantly correlated with important traits such as rhizome dry mass and rhizome dry matter content.

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