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Rice Hulls and Anaerobic Digestion Residues as Substrate Components for Potted Production of Geranium and Rose

Antonio Bassan, Stefano Bona , Carlo Nicoletto , Paolo Sambo  and Giampaolo Zanin * 

Department of Agronomy Food Natural Resources Animal and Environment (DAFNAE), University of Padova, Viale dell'Università 16, 35020 Legnaro, Padova, Italy; antoniobassan1@hotmail.com (A.B.); stefano.bona@unipd.it (S.B.); carlo.nicoletto@unipd.it (C.N.); paolo.sambo@unipd.it (P.S.)

* Correspondence: paolo.zanin@unipd.it; Tel.: +39-049-827-2902

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Abstract: Economic and environmental concerns limit peat use for substrate production, promoting interest in alternative materials. Hence, in this study, 16 substrates were obtained by mixing, in a factorial combination, eight substrates with different ratios of peat, rice hulls (RH), and anaerobic digestion residues (ADR) and two types of RH: whole (WRH) or ground RH (GRH). Substrates were physically and chemically characterized and then tested as potting substrates for *Pelargonium peltatum* 'Ville de Paris' and *Rosa × hybrida* 'La Sevillana' production. Physical characteristics worsened the increasing RH content. This problem was partly solved using GRH and adding ADR to the substrates. As for chemical characteristics, RH increased P and K, reducing cation exchange capacity, NO₃-N, and Ca, thus causing a possible nutritional imbalance. ADR addition increased all nutrients, restoring the nutritional balance. Geranium and rose plants were negatively affected by an increasing rate of RH. In both species, the use of GRH improved the considered parameters, whereas ADR improved some parameters but only in geranium. It was possible to partly substitute peat with 33% RH, but GRH plus ADR is necessary for geranium production, and facultative for rose. The multiple regression method and principal component analysis appear to be useful tools to understand which substrate parameters, and to what extent, influence the growth of ornamental plants.

Keywords: ground rice hulls; whole rice hulls; peat alternatives; multiple regression; PCA analysis

1. Introduction

Because of its high porosity, high water-holding capacity, and relatively high cation exchange capacity, peat is the most important ingredient for media production [1]. However, its wide exploitation has raised concerns about wetland conservation [2,3] and carbon dioxide emissions [4–6], hence European laws [7] have been passed to limit peat availability, increasing its price [8], especially in southern European countries that import peat. For these reasons, there has been an increasing interest in finding cheap and locally available alternative materials with a sustainable approach [9–11]. Reducing water and fertilizer use is also important for achieving a sustainable container production system [12]. The alternative materials should therefore have suitable physical and chemical properties, uniform quality, be available in large quantities, and be cost effective [13,14]. Since no universal substrate exists, many materials are mixed with peat to achieve desirable physical and chemical properties [15,16].

Rice hulls (RH) are an interesting rice byproduct that can be used as a component of substrates and are readily available in large quantities [17–19] as they represent 20% of the weight of harvested rice. They have been studied under different aspects since the 1970s as a potential material for growing media. The use of parboiled RH has been recommended to avoid soil-borne diseases, rice seed

germination, and the release of toxic levels of Mn [20]. Carbonized and composted RH improve the air porosity of substrates [21,22], reducing the water-holding capacity [22], whereas ground RH presents physical properties closer to peat [23–25]. Fresh and ground RH chemical properties have also been studied [21,26–29]. In cultivation experiments, good plant growth was obtained cultivating woody ornamentals [9,30] and herbaceous ornamentals [22,29], whereas different results depending on the cultivated species were obtained with vegetables and ornamental seedlings [18,28,31]. Rice hulls represent a good perlite alternative for growing ornamental foliage [32] and flower plants [33].

Anaerobic digestion is an interesting disposal method for fresh biomass waste that may bring numerous environmental and economic advantages [34] and it can be applied to a wide range of biomasses (e.g., plant residues, sewage sludge, the organic fraction of municipal wastes, agricultural byproducts) [35]. Because of their low C/N ratio, high nutrient levels, and organic matter, anaerobic digested residues can be used in agriculture as fertilizers or amendments [36–38] after separation by liquid fraction [35]. Anaerobic digestion biosolids of cattle manure (ADB) have also been demonstrated to have a potential application as a component of the growing substrate: geranium (*Pelargonium × hortorum* ‘Red Elite’) had a greater performance when grown in substrates containing ADB than in only peat substrates; orchids (*Cypripedium reginae* and *Cypripedium parviflorum* var. *pubescens*) had three to four times higher growth in substrates containing ADB, compared to those containing coir; and poinsettia (*Euphorbia pulcherrima* ‘Classic Red’) plants grown in peat-ADB-perlite showed higher performances than in peat-perlite substrate [39–41]. *Abelia* (*Abelia × grandiflora* ‘Prostata’) re-potted with substrate containing 20% (by volume) of anaerobic digestion residue of distillery wastes had better growth than in the only peat control [42].

Limited information is available about the use of RH and anaerobic digestion residues (ADR) of vegetal biomass in substrates for pot plant cultivation. Hence, this study aimed to evaluate the physical and chemical properties of 16 substrates containing fresh whole or ground RH and ADR, and their effect on two potted ornamental species. A further aim of this work was to try to define the specific contribution of each characteristic of the substrates on the production parameters of the two species using the multiple regression model after coupling the data with the bootstrap procedure. The purpose of the bootstrap methodology used in this work was to randomly associate all the parameters of the plants with the corresponding values of the substrate utilized for their cultivation. In this way, we overcame the classic approach of correlating the average values of the substrates with the average values of the plants, thus allowing an estimation of the variability associate to the regression. We used the multiple regression model in order to estimate the contribution of each single parameter of the substrate to the plant biometric traits. A similar objective was performed using the principal component analysis (PCA); this tool allowed to identify, for each substrate, which were the main parameters of the plant that were affected.

2. Materials and Methods

2.1. Treatments and Substrate Preparation

The materials selected for the experiment were a Baltic peat (AS Prelvex, Türi, Estonia), RH (La Pila Soc. Agr. Coop., Isola della Scala, VR, Italy), and ADR deriving from fruit and wine distillery stillage (Distilleria Marzari S.p.A., Sant’Agata sul Santerno, RA, Italy). Four substrates were prepared by mixing peat (P) with 0%, 33%, 67%, and 100%, respectively, of whole RH (WRH) by volume. Four more substrates were prepared by adding to the previous mixes ADR at the rate of 20% (by final volume). Eight other substrates were prepared in a similar manner but using ground RH (GRH). Compressively, 16 substrates were evaluated according to a two-way factorial design of an 8 component ratio (P:RH:ADR, % v:v:v) and two RH grinding (WRH and GRH). The ADR used in the experiment had the following properties: pH 7.27, electrical conductivity 1.3 dS m⁻¹, organic matter 364 g kg⁻¹, total Kjeldahl N 24.1 g kg⁻¹, C/N ration 8.5, NO₃-N 1420 g kg⁻¹, NH₄-N 3.7 g kg⁻¹, total P 5.4 g kg⁻¹,

and total K 2.2 g kg⁻¹. The volumetric percentages of the different components of the substrates are summarized in Table 1.

Table 1. Volumetric ratio of peat (P), rice hulls (RH), either whole (WRH), or ground (GRH), and anaerobic digestion residues (ADR) in the evaluated substrates.

Component Ratio (% v:v)	RH Grinding	Substrate ID	Peat	WRH	GRH	ADR
100:0:0	WRH	100:0:0-W	100.0	0.0	0.0	0.0
67:33:0	WRH	67:33:0-W	66.7	33.3	0.0	0.0
33:67:0	WRH	33:67:0-W	33.3	66.7	0.0	0.0
0:100:0	WRH	0:100:0-W	0.0	100.0	0.0	0.0
80:0:20	WRH	80:0:20-W	100	0.0	0.0	0.0
53:27:20	WRH	53:27:20-W	66.7	0.0	33.3	0.0
27:53:20	WRH	27:53:20-W	33.3	0.0	66.7	0.0
0:80:20	WRH	0:80:20-W	0.0	0.0	100	0.0
100:0:0	GRH	100:0:0-G	80.0	0.0	0.0	20.0
67:33:0	GRH	67:33:0-G	53.3	26.7	0.0	20.0
33:67:0	GRH	33:67:0-G	26.7	53.3	0.0	20.0
0:100:0	GRH	0:100:0-G	0.0	80.0	0.0	20.0
80:0:20	GRH	80:0:20-G	80.0	0.0	0.0	20.0
53:27:20	GRH	53:27:20-G	53.3	0.0	26.7	20.0
27:53:20	GRH	27:53:20-G	26.7	0.0	53.3	20.0
0:80:20	GRH	0:80:20-G	0.0	0.0	80.0	20.0

2.2. Physical and Chemical Properties of Substrates

Physical characteristics of the substrates evaluated included the bulk density (BD), total pore space (TPS), water-holding capacity (WHC), and air-filled porosity (AFP). The chemical characterization regarded the pH, electrical conductivity (EC), organic matter (OM), cation exchange capacity (CEC), and total nitrogen (TKN). With organic matter and TKN, the C:N ratio was determined. In order to obtain a measure of nutrients promptly available to plants, water extraction with deionized water (EN 13652) was preferred to other official methods (e.g., EN 13651) even if it underestimates the total nutrient content. Hence, the nitrate-nitrogen (NO₃-N), ammonium nitrogen (NH₄-N), P, K, Ca, and Mg concentrations in the water extract were determined. Each physical and chemical analysis was performed on 3 samples per substrate. In Table 2, the methodology and the instruments used are reported.

Table 2. Physical and chemical characteristics determined in the evaluated substrates.

Parameter	Methodology	Instrument	Literature
Bulk Density	EN 13040 (1999)		
Total pore space, air filled porosity, water holding capacity	NCSU porometer	NCSU Porometer, Horticultural Substrates Laboratory, North Carolina State University (NCSU), Raleigh, NC, USA	[43]
pH	EN 13037	HI 9813-5, Hanna Instruments, Padova Italy	
Electrical conductivity	EN 13038	HI 9813-5, Hanna Instruments, Padova Italy	
Organic matter	EN 13039		
Cation exchange capacity	BaCl ₂ -TEA		[44]
Total Kjeldahl nitrogen	Total Kjeldahl nitrogen		
NO ₃ -N, NH ₄ -N, P, K, Ca, Mg	EN 13652	ICS-900, Dionex, Sunnyvale, CA, USA	

2.3. Agronomic Evaluation of Substrates

Agronomic performances of the substrates were investigated using two species, geranium (*Pelargonium peltatum* (L.) L'Hér. ex Aiton) and rose (*Rosa × hybrida* L.). A choice was made among representative species of short and long production cycles.

Geranium. Substrates were used to grow rooted cuttings of geranium 'Ville de Paris'. Cuttings were transplanted in September and cultivated in a PE film greenhouse with openings in the roof and at the sides. A minimum temperature of 13 °C was ensured with a heating system. During the growing cycle, average temperatures ranged from 14.3 to 26.3 °C. Pots used in the experiment had a 14-cm diameter (1.2 L). Two rooted cuttings were transplanted for each of the 336 pots. Water was provided as reported below, whereas fertigation was applied once a week using an all-purpose fertilizer (Ferty 3 15N-4.4P-12.5K-2Mg + TE; Planta Düngemittel GmbH, Regenstauf, Germany) at a rate of 1.5 g L⁻¹ (100 mL per pot). The experiment was stopped at the 70th day of cultivation when the first plants reached marketable standard (plant height: pot height = 3:1; >3 inflorescence). Plant height (i.e., length of the longest shoot), number of flowers and main shoots, SPAD values (SPAD 502, Konica-Minolta, Japan), and the dry weight of the shoot (i.e., aboveground biomass) and roots (in a ventilated oven at 105 °C until constant weight) were determined.

Rose. The same PE film greenhouse was used for the rose 'La Sevillana' cultivation experiment. Cuttings were transplanted in September as for geranium. During the growing cycle, the average temperatures ranged from 3.5 to 26.3 °C, as no supplementary heating was used since the 70th day, when the geranium experiment ended. Here, 10-cm square pots (1.1 L) were used. One rooted cutting was transplanted per pot (336 pots in total). Substrates were previously fertilized with a controlled-release fertilizer (Osmocote Pro 16N-4.8P-8.3K-2Mg + TE 8–9 M, Scotts International B.V., Harderwijk, The Netherlands): this is a common practice for a species with a long growing cycle that have cultivation practices closer to ornamental shrubs than a floriculture crop. Plants were pruned at above the third leaf with 3 leaflets after 81 days from transplant to promote growth and branching. The experiment was stopped after 210 days of cultivation when plants reached marketable standard (plant height: pot height = 2:1; >3 inflorescence). Plant height, number of flower and main shoots, SPAD values, and shoot dry weight (i.e., above ground biomass) were determined. Root dry weight was not obtained in this species because the root system was too crowded to be accurately separated from the substrate.

For both species, the decision to irrigate was determined gravimetrically when the average weight of three pots per substrate and species dropped below the 40% of WHC, then water was applied to restore the WHC and the volume was recorded.

2.4. Statistical Analysis

Data were analyzed by analysis of variance (ANOVA) using Statgraphics 18 (Statpoint Technologies Inc., Warrenton, VA, USA) and means separated according to Tukey's honest significant difference (HSD) procedure ($p < 0.05$). Data on physical and chemical characterization were analyzed as an 8 × 2 factorial experiment in a completely randomized design with three replications. Data on both cultivation experiments were also analyzed as an 8 × 2 factorial experiment but in a randomized block design with four repetitions and six plants per replication as sub-samples. Values expressed as percentages were transformed prior to ANOVA analysis.

The effect of the substrate characteristics on geranium and rose performances were also evaluated by means of a bootstrap procedure [45,46] using Microsoft Excel Professional Plus 2010. It allowed random pairing, within the same treatment, of the replicates of the parameters measured on the plants with those of the substrates. A set of 96,000 data was created (3000 pairings per treatment). These data were used to execute a forward multiple regression analysis (Statgraphics 18) using the substrate parameters as independent variables and plant traits (one at time) as the dependent ones. The variables were included in the model if the increase of significance was greater than 5%.

Principal component analysis (PCA) on the plant parameters was performed (Statgraphics 18). All the plant traits measured were used in the PCA using, as classification factors, the 8 component ratios and WRH vs. GRH.

3. Results and Discussion

3.1. Physical Properties of Substrates

All physical properties were significantly affected by the main factors ($p < 0.001$) and their interaction ($p < 0.01$).

Higher BD was, in general, found in ADR-containing substrates. Values progressively decreased after increasing the WRH content, whereas it remained quite stable for the increasing GRH, in particular, in substrates without ADR (Figure 1A). The different values are due to the BD of the different materials constituting the substrates, but an important role is also played by the settling and packing of particles, which can give less predictable results when three components are mixed in a substrate rather than two [24]. Even if ADR addition worsened BD, all substrates had values below the limits for this parameter (i.e., $0.4 \text{ kg}\cdot\text{m}^{-3}$ [47]).

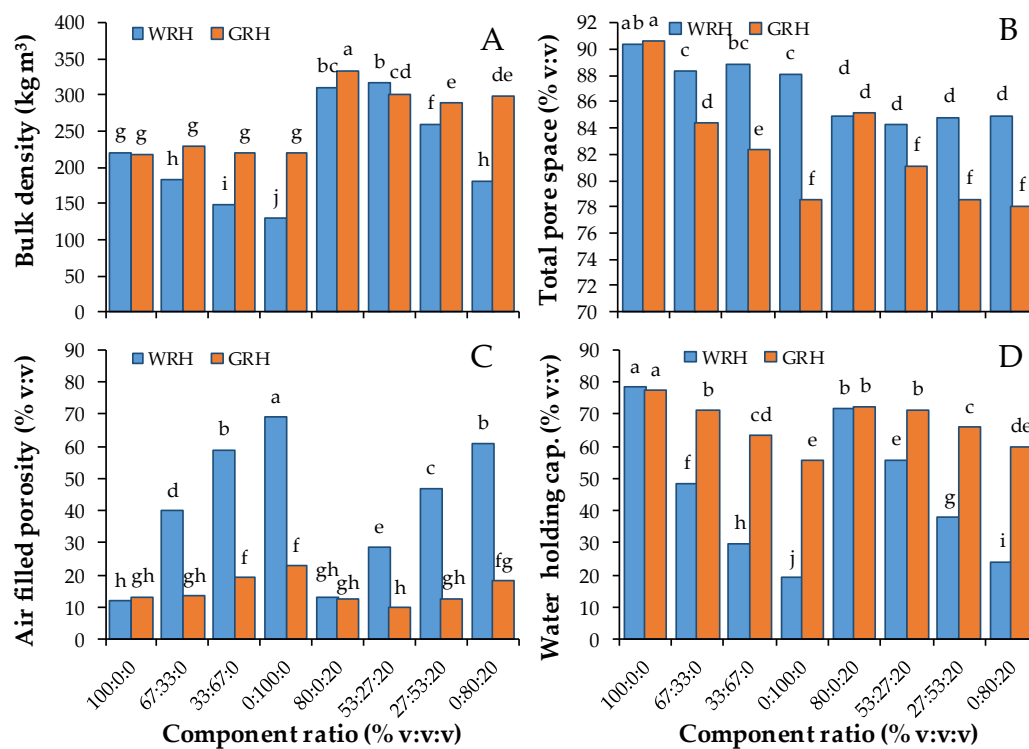


Figure 1. Interaction effects of the component ratio (P:RH:ADR % v:v) and rice hulls (RH) grinding (whole rice hulls and ground rice hulls, WRH and GRH) on: (A) bulk density; (B) total pore space; (C) air-filled porosity; and (D) water-holding capacity of the evaluated substrates. Significant differences among treatments are indicated by letter according to Tukey's HSD test ($p < 0.05$). P: peat; ADR: anaerobic digestion residues.

Total pore space was higher in WRH substrates. After increasing the WRH content in substrates, TPS varied very little in those without ADR and did not vary in ADR-containing substrates (Figure 1B). Differently, with the increase of the GRH rate, a strong reduction of TPS was observed, in particular in substrates that were ADR free. Bilderback et al. [48] suggested an optimal TPS ranging between 50% and 85%, whereas other authors recommended a minimum of 85% TPS [49,50]. As TPS values oscillated from 78.0% in 80:0:20-G to 90.6% in 0:80:0-G, all substrates were close to or within the recommended range.

The air-filled pore space of the studied substrates was very similar in substrates containing no RH (on average 12.8%) (Figure 1C). After increasing the content in WRH, AFP increased dramatically, in particular in ADR-free substrates. In substrates containing increasing rates of GRH, only a slight increase of AFP was observed, and also in this case, the increase was higher in ADR-free substrates. Several authors recommended an AFP of 10% to 20% [51,52]. De Boodt and Verdonck [53] suggested values ranging from 20 to 30% and Bilderback et al. [48] from 10% to 30%. According to these authors, substrates containing WRH had high AFP and, in accordance to Evans and Gachukia [54], substrates containing more than 33% WRH should not be used. Instead, all substrates containing GRH had values within the acceptable range.

The water-holding capacity was significantly reduced by an increasing amount of RH, so that substrates with 100% RH presented too low values despite the ADR addition slightly improving this parameter in substrates with 33% RH or more (Figure 1D). However, as seen for the increase of AFP values, the decrease WHC was less marked when GRH was used in place of WRH (on average, -22.8% and -71.2% , respectively). The optimal WHC has been reported to be between 45% [43,44] and 65% [49,50]. Hence, substrates containing more than 27% RH, both whole and ground, had high values, whereas substrates containing more than 53.3% WRH had very low values.

High AFP, and consequently low WHC, of WRH is already known [21,22,54]. GRH having physical properties closer to those of peat was also reported in other experiences [23,24]. If substrates with a higher AFP and a lower WHC are utilized, this requires a significant change in crop water management, i.e., more frequent water applications but with smaller amounts [55,56].

3.2. Chemical Properties of Substrates

Chemical properties were significantly affected by the main factors ($p < 0.001$) with the exception of RH grinding for pH and CEC. The interaction was not significant only for the CN ratio and significant at least at $p < 0.01$ for the other parameters (Table 2).

Substrate pH ranged from 6.1 to 6.8 (Figure 2A). ADR increased the pH in almost all substrates, whereas the RH content values increased but only up to 67% RH. Pure ADR pH was higher than peat (7.3 vs. 6.1) and this, of course, raised the values in ADR-containing substrates. As a pH range between 5.3 and 6.5 is recommended [47], substrates containing both RH and ADR presented values slightly higher than advisable. However, if we consider the wider range (5.5–7.0) proposed by other authors [57], all substrates were within limits. The peat used in the experiment had pH 6.1 due to liming; if a high pH is expected, a reduced amount of lime can be used.

Grinding of RH raised EC in ADR-free substrates so that values strongly increased along with the increase of the GRH content (Figure 2B). In ADR-containing substrates with only 80% WRH substrate, it had a lower EC than 0% WRH, while a slight decrease was noted as the GRH content raised from 0% to 67%. Some authors [47,58] suggested an EC level lower than $0.5 \text{ dS}\cdot\text{m}^{-1}$ whereas others [52] proposed a normal EC level range from 0.36 to $0.65 \text{ dS}\cdot\text{m}^{-1}$. Thus, the substrates' EC was lower than the maximum levels proposed by the different authors, but unfertilized substrates had, in general, lower values than advisable. The peat utilized in this experiment was not previously fertilized and this explains the low EC of substrates with no ADR and low RH content. As pure ADR presented $1.3 \text{ dS}\cdot\text{m}^{-1}$, it is expected that its use will increase the EC levels. The increase of EC values due to a higher rate of RH has already been observed [26,28] and that the grinding process increases the EC values is also already known [59].

Increasing the RH content caused the OM to decrease with no difference in ADR-free substrates. In general, the addition of ADR reduced substrates' OM but in a major extent in GRH substrate at intermediate RH rates (Figure 2C). According to Abad et al. [47], substrates without ADR presented an optimal OM content (more than 80%). The lower values observed in ADR-containing substrates are due to its lower OM content compared to peat. This lower value is caused by the industrial process that produced gas, losing organic matter [35]. The higher OM values in ADR-containing substrates

with GRH are probably due to their higher bulk density, compared to those with WRH, which results in a higher contribution of RH in terms of weight to the final mix.

Increasing the RH content from the minimum to the maximum resulted in a decreased CEC by 78.4% and 70.0% in ADR-free and ADR-containing substrates, respectively, on average of the two grinding treatments. At intermediate RH ratios, substrates with GRH without ADR had the highest values and those with ADR the lowest (Figure 2D). The CEC is considered one of the most important factors of the chemical fertility of a substrate as it indicates the nutrient storage capacity. High CEC values were reported for peat by other authors [56,58,60], and our 100 peat substrates has values within the normal range (150–250 cmol kg⁻¹ [58]). The decrease observed along with the increase of the RH rate is due to the lower level of OM of RH compared to peat. Desirable CEC values range from 50 to 200 cmol kg⁻¹ [52]; hence, only 80% and 100% RH substrates had too low values.

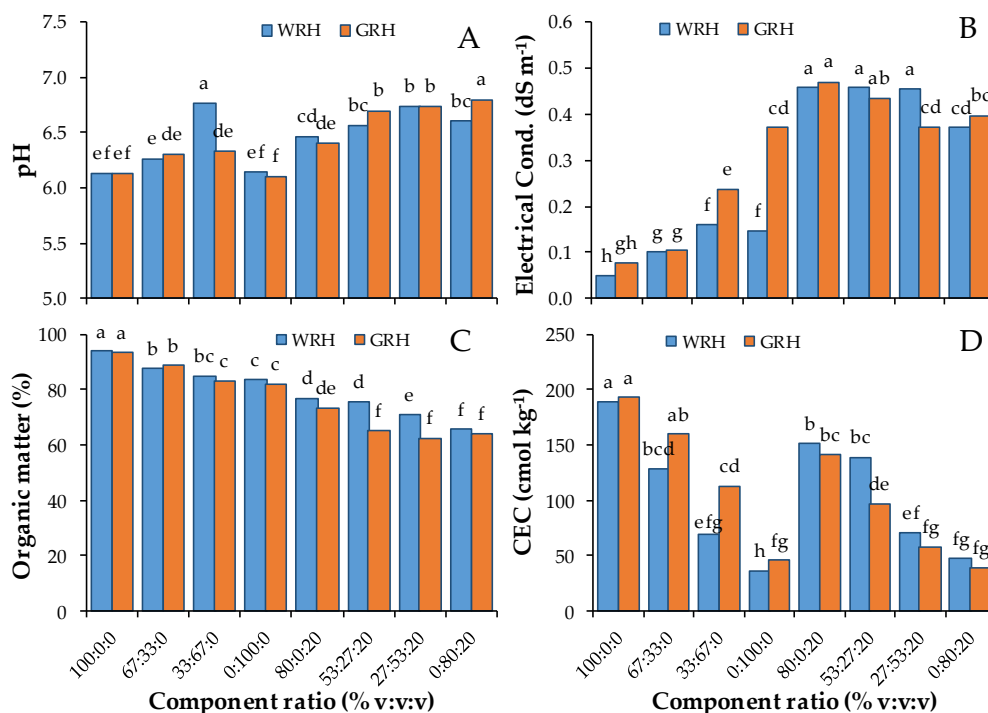


Figure 2. Interaction effects of the component ratio (P:RH:ADR % v:v:v) and rice hulls (RH) grinding (whole rice hulls and ground rice hulls, WRH and GRH) on: (A) pH; (B) electrical conductivity; (C) organic matter content; and (D) cation exchange capacity (CEC) of the evaluated substrates. Significant differences among treatments are indicated by different letters according to Tukey's HSD test ($p < 0.05$). P: peat; ADR: anaerobic digestion residues.

Substrates containing ADR resulted in a much lower C:N ratio compared to ADR-free substrates, and values increase radically along with the increase of the RH content, in particular in substrates containing GRH and ADR (+240%; Table 3). Desirable values of C:N in substrates are within 25 and 30 as lower values indicate a rapid loss of structure as a consequence of faster decomposition [61]; hence, here only ADR-containing substrates, and in particular those with WRH, are within advisable values.

The concentration of NO₃-N in the water extract of ADR-free substrates was close to zero regardless of the RH content or RH grinding (Table 3). In ADR-containing substrates, the higher values were observed at high peat rates and, in particular, when GRH was used; then values strongly decreased along with the increase of RH. Values of ADR-containing substrates were within the normal range (11–23 mg·L⁻¹ [62]).

Table 3. Interaction effect of component ratio and rice hulls (RH) grinding on the C/N ratio and concentration of macro- and meso-nutrients in the evaluated substrates.

Substrate ID	C:N	NO ₃ -N	NH ₄ -N	P	K	Ca	Mg
100:0:0-W	119.7 bc	0.52 e	0.87 b	0.01 g	1.01 i	6.46 def	0.87 bc
67:33:0-W	127.0 b	0.02 e	0.79 b	0.94 f	16.80 h	6.78 de	0.70 bc
33:67:0-W	106.0 bc	0.02 e	1.49 a	1.75 e	29.55 gh	3.91 ef	0.67 c
0:100:0-W	164.7 ab	0.12 e	1.65 a	4.13 c	35.69 fg	1.44 f	0.84 bc
80:0:20-W	26.1 d	17.92 bc	0.13 c	0.90 f	45.05 ef	27.42 a	1.89 ab
53:27:20-W	26.7 d	22.83 a	0.18 c	2.81 d	61.60 cd	26.04 a	2.22 a
27:53:20-W	32.7 cd	19.82 b	0.20 c	4.00 c	75.43 bc	19.12 ab	2.77 a
0:80:20-W	45.1cd	15.76 c	1.32 a	7.63 a	79.24 b	11.05 cd	2.72 a
100:0:0-G	117.0 bc	0.42 e	0.77 b	0.01 g	0.95 i	5.89 def	0.93 bc
67:33:0-G	141.9 b	0.01 e	0.42 bc	0.21 g	15.05 hi	5.65 ef	0.52 c
33:67:0-G	193.2 a	0.02 e	0.28 c	2.83 d	53.13 de	2.48 ef	0.52 c
0:100:0-G	200.4 a	0.85 e	0.54 bc	5.12 d	95.08 a	1.46 f	1.10 bc
80:0:20-G	28.7 d	23.99 a	0.26 c	0.93 f	43.40 efg	25.24 a	1.91 ab
53:27:20-G	42.8 cd	15.90 c	0.08 c	1.13 f	70.28 bc	15.19 bc	1.79 ab
27:53:20-G	69.0 c	4.17 d	0.14 c	3.06 d	69.66 bc	4.00 ef	0.83 bc
0:80:20-G	97.6 c	6.35 d	0.52 bc	5.55 b	101.71 a	2.57 ef	1.02 bc
Significance ¹							
Component ratio	***	***	***	***	***	***	***
RH grinding	***	***	**	***	***	***	***
Interaction	n.s.	***	**	***	***	***	***
Normal range ²		11–23	8–12	4.6–6.2	4–14	10–19	6–10

¹ significance according to ANOVA analysis. *** and ** significant at $p < 0.001$ and 0.01 , respectively. n.s. not significant. ² normal range of nutrients in the water extract [62]. Significant differences among treatments are indicated by different letters according to Tukey's HSD test ($p < 0.05$).

In ADR-free substrates, the increase of WRH led to an increase of the NH₄-N concentration (Table 3). The addition of ADR reduced values regardless of the rate of RH. Substrates containing GRH had, in general, lower values than those of substrates containing the corresponding relative percentage of WRH; furthermore, the concentration initially decreased with the increase of the relative RH rate and then increased. This apparently anomalous trend is due to the particle settling as previously discussed [24]. In all substrates, the NH₄-N values are very low [62].

Regardless of RH grinding, the P and K concentrations in the water extract rose dramatically with an increasing RH content (Table 3). ADR addition further increased the P and K concentrations. RH grinding produced a slight reduction in the P concentration in ADR-containing but not in ADR-free substrates, whereas it increased the K content in particular at high RH rates (Table 3). It is already known that RHs present higher levels of P [59] and K [28,60,63] than peat. While the P concentration values appeared lower than recommended, and the K concentration appeared too high [62].

The higher concentrations of Ca were found in ADR-containing substrates. After increasing the RH content in substrates, the Ca content in the water extract decreased in particular in GRH with ADR substrates (−89.9% in 0:80:20-G than in 80:0:20-G) whereas a lower decrease was observed in WRH with ADR (−59.7% in 0:80:20-W than in 80:0:20-W). The content of Ca is linked both to decreasing levels of CEC [60] due to the decreasing amount of peat, and also because Ca is a component of the peat-liming ingredient. Concentrations of Ca appeared lower than advisable in ADR-free substrates and too high in ADR-containing substrates without RH [62].

The concentration of Mg was similar in ADR-free substrates (Table 3). In ADR-containing substrates, the increase of the relative RH rate increased the values in WRH substrates, whereas it reduced the concentration in the GRH substrates to about the same extent (about 45%). Despite the ADR addition, the Mg values appeared lower than advisable [62].

3.3. Agronomic Evaluation of Substrates

Among the main factors, the component ratio in substrates affected all the considered plant parameters of geranium and all parameters except the stem number in rose (Tables 4 and 5), while three out of six and three out of five parameters were affected by RH grinding. The interactions were also often significant.

Table 4. Interaction effect of component ratio and rice hulls (RH) grinding on geranium plant traits.

Substrate ID	Height (cm)	SPAD Value	Flower Number	Stem Number	Shoot Dry Weight (g)	Root Dry Weight (g)
100:0:0-W	37.3 a	53.0 a	5.4 ab	10.0 ab	10.73 a	0.97 abc
67:33:0-W	36.6 ab	50.3 abcd	3.9 cdef	7.5 def	8.21 b	0.95 abc
33:67:0-W	30.5 de	53.0 a	3.1 efg	5.6 fg	6.34 cd	0.86 abcd
0:100:0-W	27.2 e	51.7 abc	3.4 defg	5.3 fg	5.10 de	0.93 abc
80:0:20-W	40.0 a	49.1 cde	5.3 ab	11.8 a	10.78 a	0.58 e
53:27:20-W	37.2 a	50.5 abcd	4.4 abcde	8.1 cde	8.82 b	0.78 cde
27:53:20-W	35.8 abc	48.4 def	4.5 abcd	7.4 def	8.39 b	0.82 bcde
0:80:20-W	31.3 cde	46.3 ef	2.5 g	4.6 g	5.05 e	0.84 bcd
100:0:0-G	38.4 a	52.1 ab	5.1 abc	10.5 ab	10.63 a	0.67 de
67:33:0-G	38.4 a	50.3 abcd	5.6 a	9.9 abc	10.33 a	0.86 abcd
33:67:0-G	32.3 bcde	49.6 bcd	3.1 efg	6.6 efg	6.78 c	1.02 ab
0:100:0-G	27.2 e	48.0 def	2.6 fg	5.0 g	5.01 e	0.96 abc
80:0:20-G	39.4 a	50.3 abcd	5.0 abc	10.5 ab	11.34 a	0.77 cde
53:27:20-G	39.2 a	50.8 abcd	3.8 cdefg	9.5 bcd	11.26 a	0.80 bcde
27:53:20-G	36.6 ab	48.6 def	4.2 bcde	9.1 bcd	8.76 b	0.77 cde
0:80:20-G	31.9 bcd	46.1 f	3.3 defg	6.5 efg	6.89 c	1.10 a
Sign ¹						
Component ratio	***	***	***	***	***	***
RH grinding	**	**	n.s.	***	n.s.	n.s.
Interaction	n.s.	***	***	***	***	***

¹ significance according to ANOVA analysis. *** and ** significant at $p < 0.001$ and 0.01 respectively. n.s. not significant. Significant differences among treatments are indicated by different letters according to Tukey's HSD test ($p < 0.05$).

Table 5. Interaction effect of component ratio and rice hulls (RH) grinding on rose plant traits.

Substrate ID	Height (cm)	SPAD Value	Flower Number	Stem Number	Shoot Dry Weight (g)
100:0:0-W	29.7 ab	41.9 abc	4.3 a	3.2	14.2 a
67:33:0-W	28.8 abc	39.7 abc	3.3 abc	3.3	13.3 abc
33:67:0-W	21.4 de	42.4 ab	2.3 cde	3.5	9.1 ef
0:100:0-W	16.5 e	39.1 abc	1.7 e	3.5	5.4 g
80:0:20-W	31.0 ab	37.3 bc	3.2 abc	3.5	12.7 abcd
53:27:20-W	26.3 abcd	40.5 abc	2.8 bcde	3.4	10.6 de
27:53:20-W	25.0 abcd	41.4 abc	2.7 bcde	3.4	9.3 ef
0:80:20-W	21.8 cde	39.0 abc	2.5 bcd	3.6	8.1 f
100:0:0-G	26.4 abcd	43.6 a	3.6 ab	3.3	13.7 ab
67:33:0-G	27.9 abcd	39.2 abc	3.2 abc	3.1	12.2 abcd
33:67:0-G	30.3 ab	37.0 c	2.4 cde	3.1	10.8 de
0:100:0-G	24.3 bcd	39.1 abc	2.0 de	3.0	9.1 ef
80:0:20-G	31.8 a	39.6 abc	3.4 abc	3.4	13.4 abc
53:27:20-G	29.5 ab	39.6 abc	3.3 abc	3.0	11.9 bcd
27:53:20-G	24.1 bcd	42.5 ab	2.9 bcd	3.1	11.3 cde
0:80:20-G	29.4 ab	39.0 abc	2.8 bcde	3.2	11.2 cde
Sign ¹					
Component ratio	***	***	***	n.s.	***
RH grinding	***	n.s.	n.s.	**	***
Interaction	***	*	n.s.	n.s.	***

¹ significance according to ANOVA analysis. ***, **, and * significant at $p < 0.001$, 0.01 , and 0.05 , respectively. n.s. not significant. Significant differences among treatments are indicated by different letters according to Tukey's HSD test ($p < 0.05$).

Geranium height (Table 4) was in general significantly higher when GRH was used. About the component ratio, height was negatively affected by the high rate of RH, in particular when ADR

was not added to the substrate. No significant differences were observed on the SPAD values after increasing the RH content up to 67%, or even at 100% when WRH was used without ADR; the lowest values were observed in 80% RH and 20% ADT, regardless of the RH grinding (Table 4). Increasing RH to the maximum ratio, either with or without ADR, progressively reduced the number of flowers, which about halved at the maximum rate (Table 4).

Stem number (Table 4) was, on average, higher in GRH compared to WRH and, either with or without ADR, was strongly reduced by the RH increasing rate (about 50% between the highest and lowest rates). An increase of the GRH rate in ADR-containing substrates resulted in a much lower reduction. The shoot dry weight was decreased by increasing the RH content, but the reduction was lower using both GRH and ADR (Table 4). The root dry weight response to the component ratio was different to all the other treatments (Table 4). After increasing WRH rates in ADR-free substrates, the root dry weight remained stable and even improved, increasing the WRH rate in ADR-containing substrates or increasing GRH either with or without ADR (e.g., by 43% using GRH in ADR-free substrates) (Table 4).

After increasing the WRH content, the rose plant height was reduced (Table 5). Using 67% GRH or more, the rose height was not reduced compared to 80% or 100% peat. Values of SPAD were not different in substrates containing WRH, whereas they were the highest in 100:0:0 and 27:53:0, and the lowest of 33:67:0 in GRH-containing substrates (Table 5). The flower number was affected only by the component ratio main factor: values decreased along with the increase of the RH rate that about halved in 100% RH compared to 0% RH in ADR-free substrates, whereas the reduction was not significant in ADR-containing substrates (Table 5). RH grinding was the only treatment that affected the main stem numbers with WRH that improved the branching by 8.25% compared to GRH (Table 5). With an increasing RH rate, shoot dry weight decreased in ADR-free substrates, and to a bigger extent when using WRH compared GRH; whereas in ADR-containing substrates using GRH, the observed reduction was not significant (Table 5).

The water volumes applied through irrigation of the two species are reported in Figure 3. The water consumption of geranium was similar in substrates with RH lower than 67% and the highest value was observed with 100% GRH (3656 L). Despite the longer growing cycle, rose had lower water consumption, which increased along with the increase of the RH rate in particular when WRH was used. Differences between species are due to the different growth rates and the lower average temperatures observed during rose growth compared to that of geranium, which lowered evapotranspiration. The different evapotranspiration also affected the differences in water values among substrates. In fact, the differences in rose reflect the differences in the WHC, whereas in geranium, the differences were much lower. The first behavior is in accordance with data in the literature that mentioned that higher irrigation events in substrates are partially counteracted by the lower water volume applied at each event so the cumulative irrigation water volume is not so affected [30].

All aerial parameters of geranium were reduced by increasing the RH content. Most of the parameters of rose were also negatively affected, even if it was to a lower extent. A similar response was observed by Evans and Gachukia [33], who reported a reduction in the shoot dry weight of geranium even though no specific test was performed to describe the dry weight reduction along with the increase of the RH content. This reduction was also noted in some other annual vegetables and ornamentals. A growth reduction in pepper seedlings was reported when plants were grown in substrates containing fresh RH [64,65] or an increasing rate of RH [28]. According to the latter reference, the decreasing values obtained for geranium's aerial parameters and rose appear to be linked to the inferior physical properties of substrates, which in high RH rate substrates showed a too high AFP to the detriment of WHC. About the chemical characteristics, instead, RH increased the K content, particularly if ground, and reduced CEC, but all other nutrients were not substantially modified by RH, not even at high rates, and were in general low also in the peat-only substrate. Weekly fertigation in geranium and basal dressing of rose with a controlled release fertilizer counteracted for this. The only parameter that improved was the root dry weight, which increased with the RH content in the substrates; this might

be related to water stress, which stimulates root growth to find moisture [66]. In this experiment, pots were watered daily as needed, but this does not exclude that substrates with higher AFP and lower WHC lost water faster than the others, resulting in lower shoot and higher root production. Use of GRH with respect to WRH improved the physical properties of substrates and this also permitted the production of geranium plants with better characteristics. The addition of ADR slightly improved the physical properties of substrates with high RH rates (e.g., reducing AFP and increasing WHC by 33.3% and 20.9% in substrates containing WRH) and provided a higher nutrient level (e.g., P and K increased by 170% and 220%, and N from a negligible amount to advisable values). This may explain why ADR addition generally improved the values of the considered parameters in geranium. Other authors using decomposed biomasses (composted agro-wastes, manure, or sewage sludge) observed higher parameters in ornamental plants and attributed this positive effect to a higher nutrient content [8,67]. However, different authors [28,68] concluded in their experiments that substrate physical characteristics were more useful than the chemical ones in explaining responses to cultivation substrates. In this experiment too, the better chemical profile of some substrates appeared insufficient to counteract the poorer physical properties.

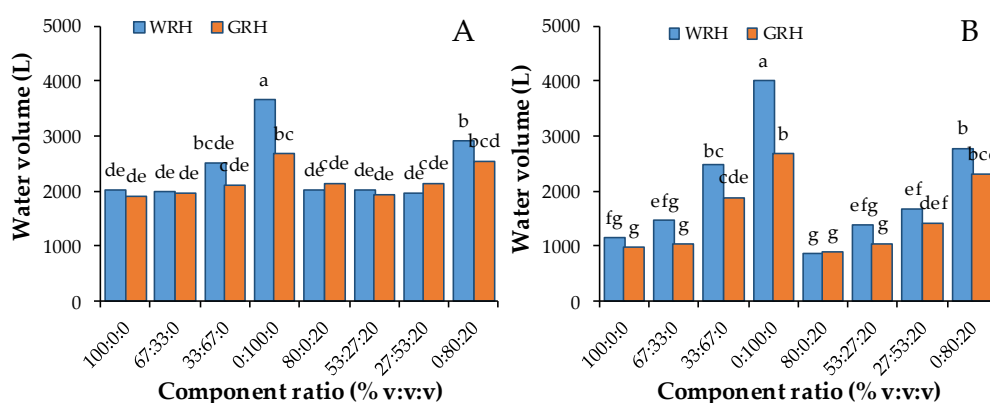


Figure 3. Interaction effects of the component ratio (P:RH:ADR % v:v:v) and RH grinding (whole rice hulls and ground rice hulls, WRH and GRH) on the volumes of irrigation water required by geranium (A) and rose (B) during cultivation in the evaluated substrates. Significant differences among treatments are indicated by different letters according to Tukey's HSD test ($p < 0.05$).

The rate of RH in the substrate also appeared to be the limiting factor in rose cultivation, but this ornamental tolerated the rice byproduct better than geranium. ADR addition to substrates had very little effect on the various parameters, confirming that nutritional support might be less important than physical properties [28,68].

Considering the results of the cultivation trials as a whole, it appeared that the complete substitution of peat with RH and ADR might be possible, as at harvest, all plants appeared marketable, even if of inferior grade, having a pleasant appearance and no symptoms of nutrient or physiological disorders were evident. Plants just appeared smaller or younger, particularly those grown in 100% RH. However, if growth reduction is not tolerable or the growing cycle gets longer, this research highlighted that about 50% (i.e., 46.7%) of peat can be suitably replaced by 26.7% of RH, preferably ground, and 20% of ADR, which basically did not affect rose growth or even improve that of geranium.

3.4. Multiple Regression and PCA Analysis

Table 6 presents the results of the multiple regression analysis; the parameters were normalized, thus allowing the comparison of their effects on plant morphological traits. The reported coefficients indicate the magnitude of variation of the selected plant features due to the variation of each substrate parameter. Interestingly, the model excluded, for each plant trait, at least one of the hydrological parameters. This is not surprising as the three parameters (i.e., total porosity, air-filled porosity, and

water-holding capacity) are linked together and they are correlated; the inclusion in the model of a substrate parameter produced a lowering of the significance of the others. Furthermore, the absolute value of the coefficient of these parameters are also high, confirming the relevance of the physical characteristics of a substrate in determining plant structure. The EC was demonstrated to be one of the most important substrate characteristics, showing, often, a high absolute coefficient value. In general, EC negatively affected the plant parameters. Salinity is known to reduce plant growth but also leaf extension [69], while increasing the mesophyll thickness and chlorophyll concentration [70]. This explains the increase of SPAD values observed in the literature [69,71], and, despite the low R^2 , also in this experiment. It is also interesting to note that the coefficient for EC was very high even if the substrates were all within the recommended values. Apart from EC, geranium appeared to be sensitive to P and K concentrations, and for dry weight to the $\text{NO}_3\text{-N}$ concentration. Concerning rose, the $\text{NO}_3\text{-N}$ concentration had a high coefficient for the plant height, flower number, and SPAD values; the air-filled porosity and water-holding capacity, instead, appeared to particularly affect the shoot and flower number (Table 6). Plants, in the present experiment, were fertilized through fertigation or slow-release fertilizer application. Nevertheless, the high coefficient values often observed for $\text{NO}_3\text{-N}$, P, and K contents in the substrates highlight how the basal nutrient level of a substrate is of great relevance for the overall plant nutrition and growth, and endorse the positive contribution that alternative organic materials can add to a growing substrate [72,73].

Table 6. Constant, regression coefficients, and R^2 obtained by multiple regression analysis of selected geranium and rose traits.

	Geranium					Rose				
	PLH [†]	SN	FN	SPAD	SDW	PLH	SN	FN	SPAD	SDW
Constant (*10 ⁶)	0	-0.234	0	0	0	0.149	-0.02	0.124	0	0.272
BD	0.042	0.067	0.663	-0.191	0.068	0.132	0.063	0.251	0.323	0.408
TPS	-0.274	-	-0.165	0.385	-	-	-	-	0.345	0.051
AIF	-0.118	-	0.139	-	-0.318	-0.209	0.593	0.316	-	-
WHC	-	0.575	-	-	0.113	-	0.501	0.513	-0.229	0.209
pH	0.024	0.060	-0.068	0.067	0.108	-0.052	0.018	-	0.069	-0.070
EC	-0.147	-0.344	-0.620	0.506	-0.366	-0.635	-	-0.431	1.024	-0.217
CEC	0.118	-0.240	-0.149	-	-0.139	0.113	-	-0.083	0.104	0.097
OM	0.126	-0.221	0.091	0.079	0.188	-0.090	-0.105	0.353	0.424	0.223
C:N	-0.174	-0.105	-0.066	0.064	-0.092	0.088	-0.024	-0.115	-0.186	-0.122
$\text{NO}_3\text{-N}$	0.158	-	0.080	-0.123	0.636	0.606	-0.104	0.464	-0.821	0.084
$\text{NH}_4\text{-N}$	-0.013	-0.058	-0.084	-0.040	-0.045	0.073	0.033	0.044	-	0.124
P	-0.241	-0.040	-	-0.440	-0.475	-0.243	0.159	-0.083	-	-0.183
K	-0.152	-0.315	-0.382	-	-0.058	0.548	-0.115	0.128	-0.327	0.188
Ca	0.086	0.121	0.115	-0.069	-0.122	-0.141	0.083	-0.065	-	-
Mg	0.140	0.099	0.159	-0.054	0.121	-0.111	-0.029	0.041	0.144	-0.034
R^2	0.365	0.454	0.300	0.217	0.712	0.189	0.032	0.204	0.075	0.447

PLH[†] = plant height, SN = stem number, FN = flower number, SDW = shoot dry weight, BD = bulk density, TPS = total pore space, AIF = air-filled porosity, WHC = water-holding capacity, EC = electrical conductivity, CEC = cation exchange capacity, OM = organic matter. - = parameter not included in the model.

The whole analysis confirms that geranium is more responsive to the substrates than rose as the model explains more than 70% of the plant response in terms of the dry weight, and the SPAD value is less affected with 21.7% of R^2 . The traits of rose plants, such as the shoot number and SPAD values, had an R^2 of only 0.032 and 0.075. The model, however, explained the plant dry weight by almost 45% (Table 6).

In Figure 4, the effects of the principal components on the classification of the different factors under control in geranium are reported. All the parameters but the SPAD value showed similar responses as classification factors (Figure 4A); SPAD was orthogonal compared to the others and it showed the highest values (length of the segment). All other factors had a similar amplitude (length). In Figure 4A (weights for principal components), it is also interesting to note that the shoot dry weight

and flower number act in a divergent way while the height and number of sprouts acted similarly and in an intermediate manner. In other terms, the plants seemed to produce either flowers or stems.

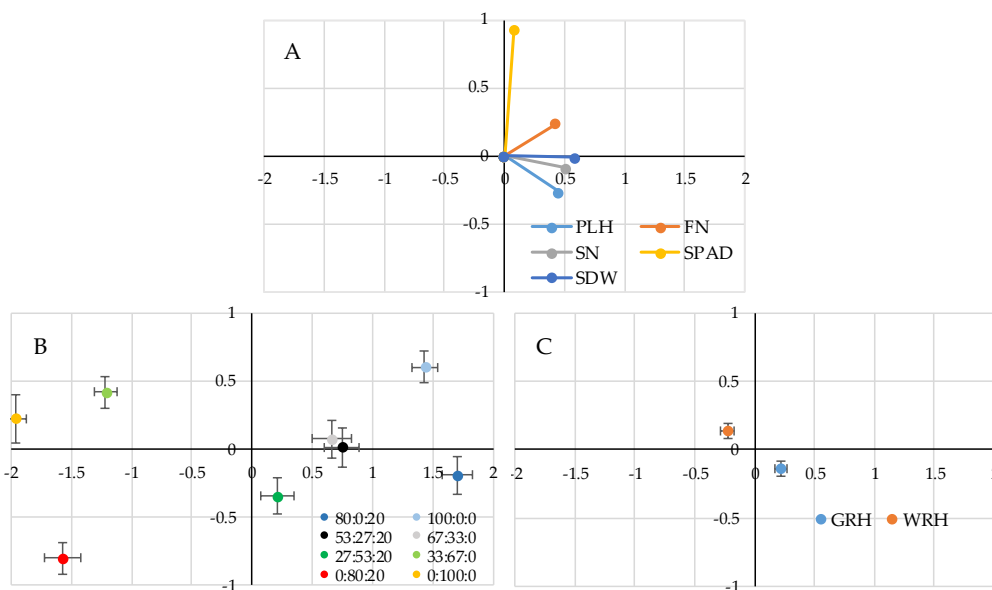


Figure 4. The plot (A) shows the weights for principal components of the analysis for the 5 parameters measured for each geranium plant. In the other plots, the centroids and their standard errors in the two dimensions for each classification factor are plotted. (B) classified using the P:RH:ADR ratio (% v:v:v); (C) whole or grinded RH (WGH and GRH, respectively) PLH, FN, SN, SPAD, and SDW = plant height, flower number, shoot number, SPAD value, and shoot dry weight, respectively.

The classification based on the component ratio followed the direction of the four morphological components; the higher RH concentration led to a decrease of the vegetative parameters and on the number of flowers. On average, comparing Figure 4B with the trajectories of Figure 4A, the presence of ADR was able to determine an increase of all the vegetative parameters but decreased the number of flowers and the values of SPAD. This was true for all the treatments but the 53:27:20 vs. 67:33:0, in which the values were very similar. The grinding of the RH positively influenced plant height, shoot number and shoot dry weight (comparing Figure 4C with Figure 4A) but negatively affected SPAD and the number of flowers.

As for the effects of the principal components on the classification of the different plant parameters under control in rose (Figure 5A), there was only a little contribution of the SPAD on classifying the factor, and the most relevant was shoot number; the other factors resulted in a similar amplitude. The flower number and the shoot dry weight followed the same trajectory and showed a similar amplitude, whereas the trajectory of the stem number was orthogonal to the others. This is in accordance with the ANOVA analysis as SPAD, for instance, was not affected by the RH relative rate whereas all the others decrease along with the increase of the RH rate. In Figure 5A, it appears clearly that the PLH and SN acted in opposite ways. In other terms, a plant with a lot of stems does not show vertical growth.

The classification based on the substrate component ratio followed the direction of the two components (flower number and the shoot dry weight). For the lower concentration of RH, the response of the plant was an increase of FN and SDW while the presence of a less fertile substrate (high RH rate) led to a dramatic decrease in the plant biomass and in the number of flowers. This was particularly true for the treatment 0:100:0 (comparing Figure 5B to Figure 5A).

The grinding of the RH positively influenced the height of the plant and negatively affected the stem number (Figure 5C).

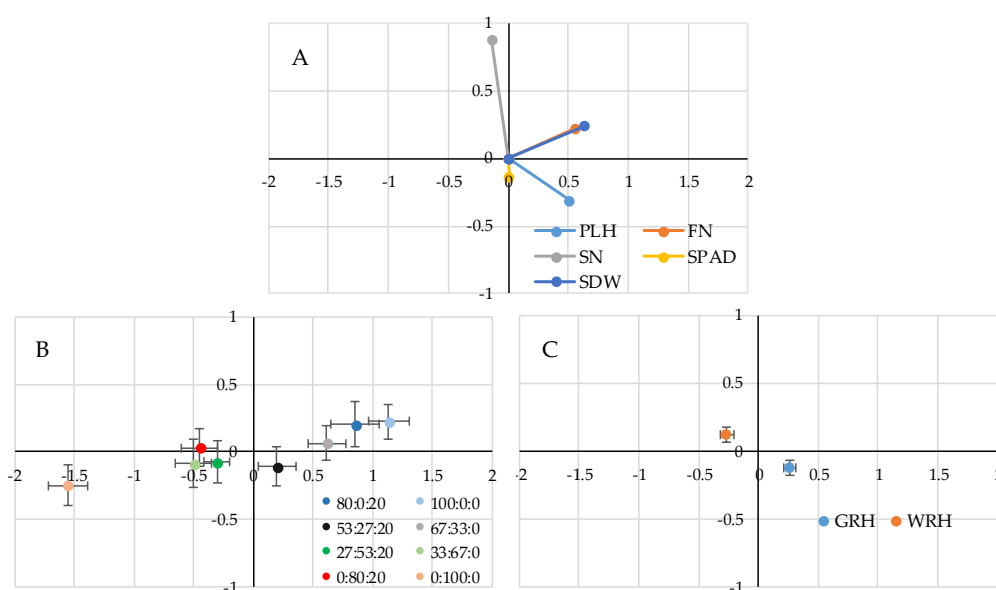


Figure 5. The plot (A) shows the weights for the principal components of the analysis for the 5 parameters measured for each rose plant. In the other plots, the centroids and their standard errors in the two dimensions for each classification factor are plotted. (B) classified using the P:RH:ADR ratio (% v:v:v); (C) whole or grinded RH (WRH and GRH, respectively). PLH, FN, SN, SPAD, and SDW = plant height, flower number, shoot number, SPAD value, and shoot dry weight, respectively.

To our knowledge, an evaluation of the agronomic performances of growing substrates was never performed considering the different plant traits as a whole by means of the PCA analysis. Our results are very interesting because they summarize the results of the ANOVA analysis, confirming that geranium is more sensitive to the substrates than rose, high RH relative rate is negative for both species, the addition of ADR was positive only for geranium, and RH grinding was positive for both species.

4. Conclusions

The rate of RH in substrates modified their physical properties, strongly increasing AFP and consequently reducing WHC. The use of GRH permitted better values to be obtained, generally within the proposed optimal range. The use of ADR showed a positive effect, similar to but smaller than the use of GRH.

The studied factors also modified the chemical characteristics. An increasing RH rate implied a rise of some nutrient contents and a drop of others that may cause a nutritional imbalance, but the problem was solved with the addition of ADR that increased all nutrient levels. Grinding of RH slightly modified the chemical characteristics.

In the cultivation experiments, both ANOVA and PCA analysis demonstrated that geranium is more sensitive to the substrates used than rose. Both species were negatively affected by increasing the rate of RH, which involved all parameters considered for geranium and three out of five parameters for rose. Both ADR addition to the substrates and the use GRH, instead of WRH, improved many parameters in geranium, whereas only RH grinding affected those of rose. Hence, in general, it is possible to substitute up to about 50% of peat, by using 33% GRH and 20% of ADR without compromising plant growth and quality. This does not allow the substrate to fulfil EU-Ecolabel requirements [74]; however, it surely goes toward a reduction of the use of peat in professional substrates embracing the principle of wise use of peat by increasing the proportion of by-product organic materials [75,76]. Hence, this achieves both environmental and economic benefits due both to reduced peatland exploitation and the use of by-products of rice cultivation and distillery.

The multiple regression methods and the PCA analysis, applied to the evaluation of the performances of different substrates, appear to be useful tools for understanding which parameters of

the substrate, and to what extent, are able to influence the growth of potted ornamental plants. In other terms, these statistical analyses give the possibility of understanding in a multidimensional approach the performances of the plants under test, giving the scientist some tools for a better interpretation of data.

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