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Oliviculture and Viticulture Crop Byproducts Use for Peat Partial Substitution for Carnation Production

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Abstract: The intensive cultivation of olive trees and grapevines in the Mediterranean region not only results in large yields but also generate wastes, with high restrictions on their impact on people's well-being and the environment. The current study sought to investigate the potential use of olive-mill waste (OW), grape-mill waste (GW) and their mixtures (OW + GW) at different levels (0%, 5%, 10% and 20% *v/v*) for partial peat substitution in the production of carnation (*Dianthus caryophyllus* L.) plants. The presence of OW, GW and OW + GW wastes raised the pH, the electrical conductivity, the content of organic matter and mineral content in substrate mixtures, while they decreased the total porosity and the available free air. The use of OW had more negative impacts than GW, while the OW + GW mixture alleviated, to some extent, the negative OW impacts. The use of high levels of residues decreased plant growth, chlorophyll content and mineral accumulation in plant tissue due to inappropriate growing media properties. The increased OW presence caused oxidative stress to the plants, as verified by the increased malondialdehyde and hydrogen peroxide content. This resulted in an upsurge in the total phenolics. However, GW presence did not impact any oxidative stress. It can be suggested that 10% OW, 10% GW or 20% OW + GW can be used in growing media, as they resulted in suitable plant growth. To ensure sufficient yields, nevertheless, the growing media's characteristics also need to be enhanced.

Keywords: *Dianthus caryophyllus*; potting plants; peat; antioxidants; minerals; plant residues

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1. Introduction

Olive and grapevine cultivation comprise two of the foremost important crops produced in the Mediterranean basin and across the globe, with both contributing significantly to the country's financial and social industrial activities [1]. In the last 60 years, the world's olive oil production has tripled to about 3.27 million tons in the 2019/20 crop year [2]. The Mediterranean region accounts for 95% of global olive oil production; however, the crop has now expanded to other regions, i.e., China. The largest part of 90% of global oliviculture is for olive oil production, with the remaining 10% for table olives [3]. In 2022, the global vineyard total area was estimated to be 7.3 million hectares (mha), with the European Union (EU) accounting for 3.3 million hectares (45%) [4], and Mediterranean countries accounting for 40% of the world's vineyard area, supporting millions of growers and wine employees in the industry [5]. Even though both olive and grapevine crops are perennial and often reach to 70–100 years old (sometimes centuries old) in the same field, the harvesting and preparing of olive oil or grape juice for wine production occurs over a short period of time in both crops, within couple of months every year. As a result, intensive crop production generates a significant amount of residues following processing, which generate environmental and human health constraints and must be treated in a timely manner [6].

Residues derived from olive-mill industries are various organic material produced from the tree's leaves and stems, fruit partially crushed stones, pulp and skin, and olive-mill wastewater. The type and load of residues, however, are dependent on the olive oil extraction systems (three-phase and two-phase), where the three-phase systems produce 20% olive oil, 50% wastewater and 30% semi-solid waste [7], and the two-phase system produce 20% olive oil and 80% wet pomace [8]. Olive-mill residues contribute significantly to environmental contamination due to their high levels of organic matter and phenolics, which are harmful to people if consumed or breathed. Furthermore, the residues may be toxic to plants and living organisms; yet, they contain considerable quantities of nutrients, and their fertility potential can be investigated thoroughly by farmers [7].

In grapevine industry, racemes or stalks, berry peel, pulp and seeds, as well as wine sediments after fermentation, are examples of wine industry residues [9]. Residues derived by the wine industry sector also have high organic acid and phenol content, which causes plant toxicity [10]. On the other side, grape-mill residues include significant amounts of minerals such as nitrogen-N, phosphorus-P, and potassium-K, and may help to reduce the production and use of chemical fertilizers for agricultural purposes [11].

Exploiting the 3-Rs (reduce-reuse-recycle) of agroindustry wastes is generating major attention in the research and utilization of the required landfill sites for waste materials' disposal. As a result, olive-mill wastes can be utilized for energy, to make soap, as feed for livestock, as well as for herbicides, pesticides, mulching for oliviculture, biofertilizer, and biosorbent for heavy metals [12,13], organic compound recovery and food additives [14]. Grape-mill wastes can be utilized in mushroom cultivation, as biofertilizer, for peat substitution in growing media, feed for livestock, energy generation [11,15,16], alcohols production and enhancing vegetable oils to improve their antioxidant capacity [17].

Agroindustry residues may be used under composting processes or applied directly to the soil as raw substances or as supplements that improve soil organic content and physicochemical characteristics, increase soil microbiology and contribute to fertility. Only some countries, like Spain, Italy and Portugal, have developed specific laws on the disposal of OW on agricultural land, whereas the majority of olive oil-producing countries do not, and instead adhere to some guidelines [13], such as a maximum annual spreading rate of 3.5 ton/ha in Cyprus, which must be a minimum of 300 m far from peoples' living areas [18]. The incorporation of OW or GW straight into soil as an organic fertilizer has led to beneficial (increase K content, soil ion mobilization) and unfavorable (increased salt and polyphenol content) effects, based on the waste source, the quantity and method of application, and the crop and environmental conditions [19,20]. Even if composting is the most popular waste management process for producing a stable material for use in agriculture [6,21,22], it requires a significant amount of energy, time, area and expertise, and it is occasionally a less attractive waste management process [23].

Nowadays, floriculture is a dynamic, global and fast-growing industry [24], and at the wholesale market, carnation (*Dianthus caryophyllus* L.) is one of the most popular cut flowers, with a rich flower color and form, ranking next only to rose [25]. A large breeding research implemented to date, as indicated by the increased number of carnation cultivars worldwide, has demonstrated the carnation's appealing and unique qualities, including different flower colors, sizes and shapes; enhanced fragrance; resistance to susceptibility; increased yield; and extended vase life [26]. The increased needs for carnation production mirrored the high research interest for the evaluation of different growing media for carnation production [27–29]. Growing media of high salt levels may affect negatively the root development and flower yield by interfering with plant nutrition, osmoregulation and photosynthetic capacity [30].

Peat is the most commonly used growing medium component in the agricultural sector due to its favorable agronomic properties; therefore, an estimated 14–20% of extracted peat is routinely provided to the agricultural sector [31]. Peatlands, on the other hand, are considered endangered places under Council Directive 92/43/EEC because they are natural habitats for undisturbed species and plants, and many environmental limits are in place to resist peat extraction. Various agroindustry residues have been proposed for substitutes of peat in numerous studies on substrate- or pot-grown ornamental and horticultural crops, with encouraging uses in regard to plant development under nursery, greenhouse and field conditions [32–35]. The current study looked at how wastes from the olive (olive-mill wastes) and winery (grape-mill wastes) industries, as well as their mixtures, may partially substitute peat in the production of carnation plants. The additional objective of the present study was to figure out how the investigated byproducts influenced the nutritional value, chemical content and bioactive constitutes of carnation in order to figure out the most effective growing conditions that could improve plant growth attributes and improve the overall quality of products.

2. Materials and Methods

2.1. Plant Material and Growing Media Preparation

The current study implemented at the soilless culture greenhouse infrastructures of the Cyprus University of Technology, at Limassol, Cyprus. The multi-spam greenhouse had north–south orientation and was covered by transparent polyethylene sheets material. The greenhouse was equipped with an automated climate control system (ventilation, shading, and cooling premises). Peat (professional-grade peat from Gebr. Brill Substrate GmbH & Co. KG, Georgsdorf, Germany) was used as a common growing media under nursery conditions. Commercial fertilizers (Novatec, simple superphosphate and potassium sulfate) were used to enrich the peat and achieve the levels of 75 mg N/L, 50 mg P_2O_5/L and 125 mg K_2O/L (*w*/*v*), respectively, close to the adequate mineral levels for growing media. The peat and fertilizers were then mixed thoroughly.

Olive-mill (olive stones and pulp) waste (OW) was provided by a local olive-mill factory of Limassol, Cyprus. It was produced after a 3-phase centrifugal oil extraction after fruit harvesting from olive trees (*Olea europaea* L. cv. Koroneiki). Standard cultivation practices were applied on the oliviculture plantation, including tillage (once a year), pruning, applications of fertilizer or manure and crop protection applications with pesticides (primarily against *Bractrocera oleae*). After that, the OW was kept in an open airfield, exposed to natural conditions (rain and sunlight) for approximately 5.5 months. The produced OW had a moisture content of around 10% and a dark brown to black appearance.

Grape-mill (winery) waste (GW) was provided by a local winery in the same region of Limassol, Cyprus. Following the harvesting of grapevines (*Vitis vinifera* L. cv Mavro, a native variety of Cyprus), grapes were milled to extract the grape juice (used to make red wines). Standard cultivation practices were used, including yearly tillage and pruning, no irrigation and several pesticides applications (mostly targeting *Plasmopara viticola*). The produced wastes were kept outside under natural conditions (rain and sunlight) for approximately 5.0 months. The GW material had a dark/brown appearance and around 12% moisture. Section 2.2 details the analysis of the physicochemical parameters of the OW 100% and GW 100% material.

The primary ingredient in all growth media preparations was peat (P). Residues from OW, GW or their mixture were proportionately substitute P at various ratios (5%, 10% and 20%, based on volume), to create the following 10 media mixtures (*v*/*v*): for the olive-mill waste, (1) peat at 100% (P; control), (2) P:OW 95:5 (OW 5%), (3) P:OW 90:10 (OW 10%), (4) P:OW 80:20 (OW 20%); for the grape-mill wastes, (1) peat at 100% (P; control), (5) P:GW 95:5 (GW 5%), (6) P:GW 90:10 (GW 10%), (7) P:GW 80:20 (GW 20%); for the mixtures with both wastes, (1) peat at 100% (P; control), (8) P:OW:GW 95:2.5:2.5 (OW + GW 5%), (9) P:OW:GW 90:5:5 (OW + GW 10%), (10) P:OW:GW 80:10:10 (OW + GW 20%) (Figure S1). After the preparation of each growing media, 10% of perlite was added to enhance media aeration and drainage. Before the seedling transplanting, the raw material of the media was sampled and analyzed for its physicochemical characteristics. The selected levels of the OW and the GW were based on preliminary trials and/or previous studies with plant residues incorporated in growing media [16,32].

Dianthus caryophyllus (carnation) seedlings were purchased at the 2-true leaves growing stage, from a commercial nursery, in black plastic trays with peat. The 10 different growing media under investigation were put in 0.5 L plastic pots before the seedling's transplantation in them. A single seedling was placed in each pot, and 12 replicate pots were used in each examined growing media. To retain the drained solution after every watering, the pots were set on plastic trays. Seedlings were watered every other day and were not treated with fertilizers, pesticides or other plant protection chemicals during the plant growth. Air temperature, air humidity and light conditions were recorded during the cultivation period. The average values were 19.2 °C, 59.1% and 1419.1 Lux, respectively.

Chemical reagents used in the present study were purchased from Sigma-Aldrich (Larnaca, Cyprus) unless otherwise specified.

2.2. Growing Media Properties

The physicochemical properties of the examined growing media and those of the raw materials were analyzed. The growing medias' total pore space (TPS), air-filled porosity (AFP), available water-holding capacity (AWHC) and bulk density (BD) by volume were determined according to the European Standard EN 13041 [36], according to previous reports [32]. Growing media was mixed with distilled water (1:5 *v*/*v*), and the extracts were used to measure the pH and the electrical conductivity (EC) (Jenway 430 pH/conductivity meter, Keison Products, Essex, UK) of each growing media. Organic matter content (%) and organic C (%) were determined when the media were burned to ash at 550 °C in a furnace (AAF 1100, Carbolite GERO, Neuhausen, Germany) [37]. For mineral analysis, the ash samples were then acid digested (2 N HCl), and the extract was used for the macronutrient's determination. Potassium (K) and sodium (Na) were measured with a flame photometer (Lasany Model 1832, Lasany International, Panchkula, India), phosphorus (P) was measured with a spectrophotometer (Multiskan GO, Thermo Fisher Scientific, Waltham, MA, USA), while magnesium (Mg) and calcium (Ca) were measured with an atomic absorption spectrophotometer (PG Instruments AA500FG, Leicestershire, UK). Nitrogen (N) was determined by the Kjeldahl method (BUCHI, Digest Automat K-439 and Distillation Kjelflex K-360, Flawil, Switzerland) following the method of Chrysargyris et al. [32]. Data are presented in g/kg of dry weight. The C/N ratio was also computed, indicating the decomposition level of the organic material.

2.3. Plant Growth, Physiology and Mineral Analysis

Carnation plants were grown for 35 days, and then several growth-related parameters were measured, with six seedlings per treatment. Leaf number and plant height were recorded. Plants were harvested, and the above-ground fresh biomass was weighed (g) and dried; then, the total dry weight (in g) was measured, and the dry matter content (%) was calculated.

Leaf chlorophyll fluorescence was used to estimate the efficiency of photosystem II to determine if environmental factors were affecting photosynthesis. The fluorescence of leaf chlorophyll was observed on two fully developed leaves per plant (Opti-Sciences fluorometer OS-30p, Hertfordshire, UK). The contents of the leaf chlorophylls/pigments were also determined (6 replicates/treatment) after dimethyl sulfoxide (DMSO) extraction, and chlorophyll a (Chl a), chlorophyll b (Chl b), total chlorophylls (total Chl) and carotenoid contents were expressed as mg of chlorophyll (or carotenoids) per g of fresh weight (mg Chl/g Fw or mg Car/g Fw) [37].

Mineral content in carnation leaves was assessed on four replicates for each growing media (2 pooled plants per replicate). The plant tissue was oven-free air-dried at 65 °C; then, the dried tissue (~0.5 g) was ashed at 550 °C in a furnace (AAF 1100, Carbolite GERO, Neuhausen, Germany) and acid-digested with hydrochloric acid (2 N HCl) [37]. The obtained extracts contained all the inorganic minerals. Phosphorus content was assessed via spectrophotometry (Multiskan GO, Thermo Fischer Scientific, Waltham, MA, USA), and K, Na, Mg and Ca were measured by Ion Chromatography (ICS-3000, Dionex Aquion, Sunnyvale, CA, USA) and an IonPac CS19 analytical column (4 × 250 mm, Dionex Corporation). N was assessed with the Kjeldahl method (BUCHI, Digest automat K-439 and Distillation Kjelflex K-360, Flawil, Switzerland) following the method used by Chrysargyris et al. [37]. Data are expressed in g/kg of dry weight.

2.4. Total Phenolics, Total Flavonoids and Antioxidant Activity

In each treatment, six leaf samples (pooled by two separate plants/sample) from the freshly cut plants (0.5 g) were milled with 10 mL of 50% (*v*/*v*) methanol, and extraction was enabled by ultrasonication [38]. The extracts were centrifuged (Sigma 3–18K, Sigma Laboratory Centrifuge, Osterode, Germany) for 30 min at 4000× *g* at 4 °C. The supernatant was stored at 4 °C until analysis (within 48 h) for the antioxidant activity employing three assays (ferric-reducing antioxidant power- FRAP; 2,2-diphenyl-1-picrylhydrazyl -DPPH; and 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid -ABTS), as well as to determine the total phenolics and flavonoids content.

Total phenols were quantified using the methanolic extracts with the Folin–Ciocalteu method (at 755 nm; using a microplate spectrophotometer Thermo Scientific, Multiskan GO), as described previously [38]. Results were expressed in gallic acid equivalents (mg GAE/g of Fw) [39]. The content of total flavonoids was determined based on the aluminum chloride colorimetric method [40] as modified in Chrysargyris et al. [37]. The absorbance was measured at 510 nm. The total flavonoid content is expressed in rutin equivalents (mg rutin/g of Fw).

Free radical-scavenging activity was determined as descripted previously [38]. In brief, DPPH radical scavenging activity of the plant methanolic extracts was measured at 517 nm, and FRAP activity was measured at 593 nm as described previously [38]. The ABTS assay was implemented according to the methodology described by Woidjylo et al. [41]. Results are expressed as Trolox ((±)-6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid) equivalents (mg trolox/g of Fw).

2.5. Lipid Peroxidation and Hydrogen Peroxide Content

The content of hydrogen peroxide (H_2O_2) was determined according to Loreto and Velikova [42] from six samples (two individual plants were pooled/sample) for each treatment. The H_2O_2 concentration was calculated using standards from 5 to 1000 μM of H_2O_2. The absorbance of samples and standards was measured at 390 nm, and results are expressed as μmol H_2O_2/g fresh weight.

Lipid peroxidation was assessed according to De Azevedo Neto et al. [43] and measured in terms of malondialdeyde content (MDA). The absorbance was determined at 532 nm and corrected for non-specific absorbance at 600 nm. The MDA amount was determined using the extinction coefficient of 155 mM/cm. Results are expressed as nmol of MDA/g fresh weight.

2.6. Statistical Analysis

Measurements were performed in four to six biological replicates per treatment (each replicate included two individual measures/samples). Statistical analysis was performed using the SPSS statistical software (SPSS v.22; IBM, Armonk, NY, USA). Data means were also subjected to one-way analysis of variance (ANOVA), and Duncan's multiple range test was used for the mean comparison of the treatments at $p < 0.05$.

3. Results and Discussion

Various plant residues make excellent materials for the growth of seedlings or cuttings in nurseries, as well as the growth of plants in pots. The pH and saline levels, along with other phytochemical components (i.e., phenols, toxic elements) found in these residues, could restrict their utilization and application since they generate toxic effects in plants [44]. In this paradigm, investigating the impacts of the growing medium composition on plant growth and production is a very complex issue that requires the evaluation of various physical chemical, and biological factors. The use of residues derived from the oliviculture and/or grapevines sector in different ratios (0–5–10–20%) as well as their mixtures in the current experiment revealed they were mineral-rich and significantly enhanced the growth media properties when utilized in various ratios with peat (Tables 1–3). In particular, OW had neutral pH and high organic content, as well as high levels of N, K and Na, which resulted in a greater EC in contrast to peat (Table 1). In contrast, OW had low contents of Ca, P and Mg, as well as a low bulk density, in comparison to peat. Considering the GW material, it was found to have almost neutral pH, enriched organic matter content and bulk density, as well as raised levels of N, K, P and Na (Table 2). However, GW revealed lower Ca and Mg, a decreased C/N ratio and decreased available water-holding capacity, as well as decreased total pores' space and air-filled pores' space when compared to peat. Therefore, both materials (OW and GW) had properties within limitations [45]. The low EC values indicate that the wastes can be used safely without endangering young plants [46].

Table 1. Growing medias' (peat and olive-mill waste (OW)) physicochemical properties before plant transplantation.

	Peat 100%	**OW 5%**	**OW 10%**	**OW 20%**	**OW 100%**
pH	6.43 ± 0.18	6.88 ± 0.06	7.17 ± 0.05	7.31 ± 0.08	7.21 ± 0.04
EC (mS/cm)	0.85 ± 0.03 b	0.83 ± 0.08 b	0.89 ± 0.06 ab	1.03 ± 0.03 a	1.02 ± 0.01 a
Organic matter (%)	72.73 ± 0.97 e	77.49 ± 0.86 d	81.33 ± 1.25 c	87.92 ± 0.68 b	94.67 ± 0.89 a
Organic C (%)	42.19 ± 0.56 e	44.95 ± 0.50 d	47.17 ± 0.72 c	50.99 ± 0.39 b	55.86 ± 0.52 a
C/N ratio	50.29 ± 1.51 ab	57.03 ± 2.02 a	42.63 ± 2.65 c	47.51 ± 1.85 bc	50.90 ± 2.72 ab
N (g/kg)	8.48 ± 0.18 b	7.94 ± 0.26 b	11.21 ± 0.52 a	10.84 ± 0.48 a	11.11 ± 0.57 a
K (g/kg)	2.03 ± 0.02 d	2.09 ± 0.15 d	3.84 ± 0.09 c	4.72 ± 0.12 b	10.34 ± 0.27 a
P (g/kg)	1.13 ± 0.03 a	1.20 ± 0.05 a	0.91 ± 0.09 a	0.89 ± 0.04 a	0.43 ± 0.17 b
Ca (g/kg)	14.95 ± 0.31 a	10.12 ± 0.51 b	9.69 ± 0.19 b	10.31 ± 0.32 b	3.78 ± 0.07 c
Mg (g/kg)	0.78 ± 0.03 a	0.61 ± 0.01 b	0.55 ± 0.01 bc	0.50 ± 0.04 c	0.12 ± 0.00 d
Na (g/kg)	0.96 ± 0.02 b	0.77 ± 0.08 c	0.84 ± 0.00 bc	0.92 ± 0.02 bc	3.97 ± 0.06 a
Total porosity (% v/v)	85.54 ± 0.74 ab	77.78 ± 2.26 c	89.68 ± 2.72 a	77.03 ± 1.87 c	81.64 ± 2.08 bc
Air filled porosity (% v/v)	18.94 ± 0.77 a	16.70 ± 1.22 ab	14.89 ± 1.46 bc	12.74 ± 0.74 c	16.54 ± 0.14 ab
Bulk density (g/cm^3)	0.14 ± 0.01 d	0.23 ± 0.00 c	0.25 ± 0.01 c	0.29 ± 0.01 b	0.54 ± 0.01 a
Container capacity (% v/v)	66.61 ± 1.02 b	61.08 ± 1.10 c	74.78 ± 1.36 a	64.29 ± 1.23 bc	65.10 ± 2.05 bc

Total porosity (TP), available water-holding capacity (AWHC—container capacity), air-filled porosity (AFP) and bulk density (BD) by volume. Values are mean ± SE (n = 6). In each row, values followed by the same letter do not differ significantly at $p < 0.05$.

Table 2. Growing medias' (peat and grape-mill waste (GW)), physicochemical properties before plant transplantation.

	Peat 100%	**GW 5%**	**GW 10%**	**GW 20%**	**GW 100%**
pH	6.43 ± 0.18	6.53 ± 0.04	6.77 ± 0.05	6.82 ± 0.05	7.11 ± 0.12
EC (mS/cm)	0.85 ± 0.03 c	1.10 ± 0.08 a	0.86 ± 0.04 bc	0.99 ± 0.02 bc	0.93 ± 0.03 ab
Organic matter (%)	72.73 ± 0.97 c	72.24 ± 1.89 c	82.96 ± 1.04 b	83.25 ± 0.94 b	91.93 ± 0.78 a
Organic C (%)	42.19 ± 0.56 c	41.90 ± 1.10 c	48.12 ± 0.61 b	48.29 ± 0.54 b	53.32 ± 0.45 a
C/N ratio	50.28 ± 1.51 a	34.20 ± 0.76 c	42.41 ± 2.08 b	27.29 ± 1.38 d	27.30 ± 0.57 d
N (g/kg)	8.48 ± 0.18 d	12.37 ± 0.50 c	11.49 ± 0.45 c	17.69 ± 0.89 b	19.67 ± 0.27 a
K (g/kg)	2.03 ± 0.02 d	5.37 ± 0.10 c	5.41 ± 0.18 c	9.69 ± 0.16 b	18.43 ± 0.50 a
P (g/kg)	1.13 ± 0.03 d	1.94 ± 0.06 b	1.48 ± 0.05 c	1.92 ± 0.07 b	3.00 ± 0.14 a
Ca (g/kg)	14.95 ± 0.31 a	15.09 ± 0.59 a	11.77 ± 0.38 b	15.19 ± 1.11 a	8.89 ± 0.32 c
Mg (g/kg)	0.76 ± 0.03 b	0.97 ± 0.02 a	0.76 ± 0.04 b	0.95 ± 0.02 a	0.31 ± 0.04 c
Na (g/kg)	0.96 ± 0.02 c	1.09 ± 0.02 b	0.90 ± 0.02 c	0.92 ± 0.01 c	3.42 ± 0.07 a
Total porosity (% v/v)	85.54 ± 0.74 a	61.59 ± 4.41 c	59.71 ± 4.16 c	52.78 ± 2.29 c	76.86 ± 1.11 b
Air filled porosity (% v/v)	18.94 ± 0.77 a	9.94 ± 1.89 c	9.76 ± 2.27 c	10.28 ± 0.86 c	12.75 ± 0.81 b
Bulk density (g/cm^3)	0.14 ± 0.01 d	0.17 ± 0.00 c	0.20 ± 0.00 b	0.23 ± 0.00 b	0.35 ± 0.01 a
Container capacity (% v/v)	66.61 ± 1.02 a	51.64 ± 2.56 c	49.94 ± 2.37 bc	42.49 ± 1.94 c	61.81 ± 0.84 b

Total porosity (TP), available water-holding capacity (AWHC—container capacity), air-filled porosity (AFP) and bulk density (BD) by volume. Values are the mean ± SE (n = 6). In each row, values followed by the same letter do not differ significantly at $p < 0.05$.

Table 3. Growing medias' (peat, olive-mill waste (OW) and grape-mill waste (GW)), physicochemical properties before plant transplantation.

	Peat 100%	OW + GW 5%	OW + GW 10%	OW + GW 20%
pH	6.43 ± 0.18	6.34 ± 0.04	6.41 ± 0.04	6.72 ± 0.05
EC (mS/cm)	0.85 ± 0.03 b	1.20 ± 0.05 a	1.30 ± 0.15 a	1.09 ± 0.01 a
Organic matter (%)	72.73 ± 0.97 c	79.24 ± 2.05 b	81.43 ± 1.30 b	86.25 ± 1.26 a
Organic C (%)	42.19 ± 0.56 c	45.96 ± 1.19 b	47.23 ± 0.75 b	50.03 ± 0.73 a
C/N ratio	50.28 ± 1.51 a	40.81 ± 0.77 b	41.65 ± 0.67 b	36.11 ± 1.67 a
N (g/kg)	8.48 ± 0.18 c	11.38 ± 0.52 b	11.43 ± 0.30 b	14.04 ± 0.86 a
K (g/kg)	2.03 ± 0.02 c	4.98 ± 0.15 b	5.24 ± 0.11 b	7.29 ± 0.69 a
P (g/kg)	1.13 ± 0.03 b	1.88 ± 0.09 a	1.94 ± 0.07 a	1.74 ± 0.08 a
Ca (g/kg)	14.95 ± 0.31 bc	22.11 ± 0.99 a	17.41 ± 0.99 b	13.70 ± 1.01 c
Mg (g/kg)	0.76 ± 0.03 b	1.25 ± 0.07 a	1.11 ± 0.05 a	0.87 ± 0.07 b
Na (g/kg)	0.96 ± 0.02 a	1.03 ± 0.01 a	0.96 ± 0.02 a	0.86 ± 0.04 b
Total porosity (% v/v)	85.54 ± 0.74	80.93 ± 2.58	82.09 ± 1.68	78.06 ± 3.74
Air filled porosity (% v/v)	18.94 ± 0.77 a	14.09 ± 1.69 b	12.81 ± 0.79 b	9.99 ± 1.68 b
Bulk density (g/cm^3)	0.14 ± 0.01 c	0.15 ± 0.00 c	0.17 ± 0.00 b	0.23 ± 0.01 a
Container capacity (% v/v)	66.61 ± 1.02	66.84 ± 1.41	69.25 ± 1.09	68.06 ± 2.57

Total porosity (TP), available water-holding capacity (AWHC—container capacity), air-filled porosity (AFP) and bulk density (BD) by volume. Values are the mean ± SE (n = 6). In each row, values followed by the same letter do not differ significantly at $p < 0.05$.

The partially substitution of peat with OW and GW impacted the physicochemical characteristics of the tested mixtures (Tables 1–3). The presence of ≥10% OW or GW increased the pH of the mixture compared to peat's and/or lower OW levels in the growing media, whereas the pH levels were found to be higher above the suggested range of 5.3–6.5 [45]. When OW or GW were added to the growth media instead of peat, the amount of organic carbon and organic matter rose. This might have fewer impacts in organic growing media in general, but it might be of great importance in soil. Taking into consideration that the soil organic matter has low levels (i.e., up to 3–5%) in several Mediterranean countries, indicating a poor soil fertility, enriching the soil with organic materials is of major importance in the Mediterranean region and in other areas of the world. Indeed, mixtures that contained GW and OW + GW media were more stable, having lower C/N ratios, which is of preference during plant growth, and therefore the N will be more available to the roots for plants growth rather than to the opportunistic microorganisms for progressing the decomposition process of organic material. Therefore, the tested OW, GW and OW + GW-enriched growing media in the present study revealed higher N and K, while P was increased only in cases of GW and OW + GW media. The residues-based media (OW, GW and OW + GW) exhibited N of 7.94–17.69 g/kg, K of 2.09–9.69 g/kg, P of 0.89–1.94 g/kg, Mg of 0.50–0.97 g/kg, Ca of 9.69–15.19 g/kg and Na of 1.77–1.09 g/kg. The mineral quantities found in the combination of OW + GW were typically intermediate between those of the OW and GW that were being studied. The suggested EC levels for the growing medium, according to Abad et al. [45], are lower than 0.5 mS/cm; nevertheless, the EC of the tested growing media were higher (ranging from 0.85 to 1.30 mS/cm). Likewise, when composted residues derived from the wineries were employed as a partial peat substitute in growing media, the EC values ranged from 0.61 to 2.41 mS/m [10]. In fact, proper irrigation and fertilization management can address the high EC values, which indicate the availability of minerals to the plant's roots. In case the mineral levels are raised, inert materials such as sand, pumice or perlite can also be used [47].

The chemical characteristics of a growing medium, such as pH, EC and ion translocation, can be adjusted after the media has been prepared and/or throughout the development of plants; nevertheless, the physical characteristics (like the total porosity, water- and air-filled porosity, and bulk density) of the medium cannot be changed after the plants are transplanted into the growing media. Therefore, the physical properties of a growing medium need to be amended before plant transplanting in the media, as the physical properties are critical for crop performance in soilless cultures. Both OW and GW residues reduced the total pores' space, AWHC and AFP of the growing media in most cases, while between the two, GW caused greater reductions than the OW, and their mixture obtained in-between values. A growing medium is recommended to have 20–30% AFP, while AFP ≥ 10% is compulsory for growing media [45]. In that sense, GW 5–10% were slightly in lower levels than the recommended threshold's AFP (≤10%); however, this was recovered when GW was mixed with OW, resulting in AFP ≥ 10%. Similar findings were observed when *Sonchus oleracea* was growing in mixtures of peat that included residues of medicinal and aromatic plants after distillation process [37]. When mint wastes were added after distillation, they boosted the mustard (*Brassica juncea*) yield and improved soil physicochemical features, and they may be utilized as an interim replacement for fertilizers [48]. The low porosity in the case of GW was related to the low particle sizes that decreased the free pores' space, and this can be altered by adding larger particles or incorporate inorganic material, i.e., perlite, sand, etc., to increase the size of particles and as a consequence the total pores' space and AFP.

At the end of the cultivation period, several growing media properties were altered (Table 4, Figure S2). For example, OW-based media enhanced with minerals, as P, Mg, Ca and Na were increased in all ratios of OW, and the N and K levels were increased at OW 5% only. However, the decreased EC at the end of the cultivation period, found at OW ≥ 10%, reflected the shortage of N and K at that OW level. In the case of the GW-based media, the levels of N, K and P were found to be lower at the end of the cultivation period compared to the initial material, while the Ca, Mg and Na levels were increased. An intermediate situation was observed when OW and GW were mixed in the growing media (Table 4, Figure S2).

Table 4. Growing medias' (peat, olive-mill waste (OW) and grape-mill waste (GW)) physicochemical properties after plant growth (end of experiment).

	Peat 100%	**OW 5%**	**OW 10%**	**OW 20%**
pH	6.50 ± 0.09	6.85 ± 0.02	7.01 ± 0.07	7.10 ± 0.04
EC (mS/cm)	1.37 ± 0.07 a	0.94 ± 0.03 b	0.85 ± 0.01 bc	0.71 ± 0.08 c
Organic matter (%)	72.78 ± 1.32 c	78.31 ± 1.76 b	86.67 ± 1.59 a	89.27 ± 0.58 a
Organic C (%)	42.21 ± 0.76 c	45.42 ± 1.02 b	50.27 ± 0.92 a	51.78 ± 0.34 a
C/N ratio	48.77 ± 1.68 b	49.02 ± 2.56 b	61.08 ± 1.10 a	57.78 ± 0.55 a
N (g/kg)	8.67 ± 0.25 ab	9.30 ± 0.42 a	8.23 ± 0.17 b	8.96 ± 0.13 ab
K (g/kg)	3.07 ± 0.22 b	3.71 ± 0.45 ab	2.94 ± 0.33 b	4.30 ± 0.06 a
P (g/kg)	1.56 ± 0.12	1.55 ± 0.29	1.29 ± 0.21	1.36 ± 0.10
Ca (g/kg)	24.16 ± 0.69 a	22.58 ± 2.10 a	14.00 ± 1.59 b	13.22 ± 0.28 b
Mg (g/kg)	1.85 ± 0.05 a	1.74 ± 0.16 a	1.03 ± 0.12 b	0.95 ± 0.02 b
Na (g/kg)	3.09 ± 0.12 a	2.45 ± 0.27 b	1.98 ± 0.19 bc	1.79 ± 0.02 c
	Peat 100%	**GW 5%**	**GW 10%**	**GW 20%**
pH	6.50 ± 0.09	6.75 ± 0.05	6.82 ± 0.03	6.65 ± 0.05
EC (mS/cm)	1.37 ± 0.07 a	1.08 ± 0.08 b	1.04 ± 0.00 b	1.10 ± 0.07 b
Organic matter (%)	72.78 ± 1.32 b	80.56 ± 0.40 a	83.63 ± 1.13 a	83.92 ± 3.18 a
Organic C (%)	42.21 ± 0.76 b	46.73 ± 0.23 a	48.51 ± 0.66 a	48.68 ± 1.84 a
C/N ratio	48.77 ± 1.68 a	39.41 ± 2.49 c	42.94 ± 1.21 bc	47.74 ± 1.10 ab
N (g/kg)	8.67 ± 0.25 c	11.94 ± 0.71 a	11.30 ± 0.25 ab	10.19 ± 0.20 b
K (g/kg)	3.07 ± 0.22 b	4.71 ± 0.05 a	4.79 ± 0.58 a	5.34 ± 0.19 a
P (g/kg)	1.56 ± 0.12 a	1.61 ± 0.15 a	0.68 ± 0.13 b	0.91 ± 0.18 b
Ca (g/kg)	24.16 ± 0.69 a	19.46 ± 1.10 b	17.82 ± 0.07 bc	15.45 ± 0.64 c
Mg (g/kg)	1.85 ± 0.05 a	1.62 ± 0.08 b	1.46 ± 0.02 b	1.18 ± 0.03 c
Na (g/kg)	3.09 ± 0.12 a	2.55 ± 0.05 b	2.19 ± 0.13 c	1.81 ± 0.02 d
	Peat 100%	**OW + GW 5%**	**OW + GW 10%**	**OW + GW 20%**
pH	6.50 ± 0.09	6.70 ± 0.01	6.73 ± 0.05	6.86 ± 0.01
EC (mS/cm)	1.37 ± 0.07	1.15 ± 0.05	1.28 ± 0.09	1.14 ± 0.03
Organic matter (%)	72.78 ± 1.32 b	80.07 ± 1.18 a	78.50 ± 1.30 ab	79.88 ± 2.90 a
Organic C (%)	42.21 ± 0.76 b	46.43 ± 0.68 a	45.53 ± 0.76 ab	46.33 ± 1.68 a
C/N ratio	48.77 ± 1.68 a	39.88 ± 2.60 b	35.77 ± 0.80 b	34.87 ± 3.18 b
N (g/kg)	8.67 ± 0.25 b	11.71 ± 0.56 a	12.74 ± 0.46 a	13.50 ± 1.34 a
K (g/kg)	3.07 ± 0.22 c	4.01 ± 0.37 bc	4.93 ± 0.55 b	5.81 ± 0.21 a
P (g/kg)	1.56 ± 0.12 b	1.72 ± 0.03 ab	1.99 ± 0.11 a	1.77 ± 0.03 ab
Ca (g/kg)	24.16 ± 0.69 a	19.22 ± 0.71 b	19.60 ± 0.05 b	17.50 ± 1.08 b
Mg (g/kg)	1.85 ± 0.05 a	1.49 ± 0.06 b	1.41 ± 0.07 b	1.21 ± 0.03 c
Na (g/kg)	3.09 ± 0.12 a	2.20 ± 0.16 b	2.28 ± 0.16 b	1.95 ± 0.06 b

Values are the mean ± SE ($n = 6$). In each row, values followed by the same letter do not differ significantly at $p < 0.05$.

The addition of the OW and GW into the peat-based growing media affected the carnation growth parameters (Table 5). The presence of 5–10% OW in the growing media increased the number of leaves produced compared to the plants grown in the control treatment (peat), but when higher OW levels were used (i.e., OW 20%), plants had fewer leaves than the control ones. The above-ground plant biomass was decreased, but the dry matter content was increased at OW 20% compared to the OW 5% and control treatments. Previous research found that 10% OW in the growing media was the greatest permissible level for the profitable and commercial production of marigold, petunia and matthiola in pots [32]. However, in leafy vegetables as purslane (*Portulaca oleracea*), the use of ≥10% OW in the growing media suppressed the plant height and leaf number [16]. In fact, Papafotiou et al. [49] found that 30% OW reduced the poinsettia's leaf number and fresh biomass.

Table 5. The effect of growing media (peat, olive-mill waste—OW, grape-mill waste—GW and their mixtures) on the carnation seedlings' height (cm), leaf number, above ground fresh weight (Fw; g/plant), dry weight (Dw; g/plant) and dry matter content (%) on plants grown in the greenhouse/nursery.

	Height	Leaf No	Fresh Weight	Dry Weight	Dry Matter Content (%)
Peat 100%	17.75 ± 1.36	22.75 ± 0.85 b	7.90 ± 0.74 a	1.15 ± 0.03	12.29 ± 0.24 b
OW 5%	19.50 ± 2.17	26.00 ± 0.00 a	7.20 ± 0.69 a	1.03 ± 0.10	12.69 ± 0.47 b
OW 10%	17.62 ± 1.21	26.25 ± 1.03 a	6.32 ± 0.57 ab	0.91 ± 0.03	13.99 ± 0.73 ab
OW 20%	16.62 ± 1.08	20.25 ± 0.47 c	4.51 ± 0.78 b	0.90 ± 0.21	15.66 ± 0.43 a
Peat 100%	17.75 ± 1.36	22.75 ± 0.85 ab	7.90 ± 0.74 a	1.15 ± 0.03	12.29 ± 0.24
GW 5%	17.12 ± 2.51	23.25 ± 1.10 a	7.43 ± 0.80 ab	1.08 ± 0.06	11.79 ± 0.57
GW 10%	14.75 ± 1.60	25.50 ± 0.95 a	6.56 ± 0.17 ab	0.91 ± 0.13	13.95 ± 1.69
GW 20%	17.62 ± 0.89	20.00 ± 0.81 b	5.50 ± 0.53 b	0.89 ± 0.07	13.08 ± 0.62
Peat 100%	17.75 ± 1.36 ab	22.75 ± 0.85 ab	7.90 ± 0.74	1.15 ± 0.03	12.29 ± 0.24
OW + GW 5%	21.37 ± 1.17 a	25.50 ± 1.19 a	7.96 ± 0.88	1.14 ± 0.21	12.33 ± 0.16
OW + GW 10%	15.87 ± 1.87 b	23.50 ± 0.86 ab	8.67 ± 0.84	1.27 ± 0.14	12.57 ± 0.30
OW + GW 20%	14.75 ± 0.25 b	21.00 ± 1.47 b	7.07 ± 0.36	0.96 ± 0.00	12.61 ± 0.59

Values are mean ± SE (n = 6). Values in the columns that are followed by the same letter for the different residues (OW, GW or the mixtures of OW + GW) are not significantly different ($p < 0.05$).

The GW addition into the media resulted in leaf number and fresh weight decline at 20% GW compared to the lower ratios of GW in the media and/or control treatment. Plant height and dry weight of the above-ground biomass did not differ among the examined OW- and GW-based media. The combination of OW + GW in the media increased the plant height and leaf number at OW + GW 5%, while carnations' fresh and dry weight as well as dry matter content did not differ by the combination of OW + GW in the media. This indicated that the combination of OW + GW alleviated the negative effects on plant growth derived mainly by the OW presence (Table 5). For ornamental plants, the maintenance of plant height and plant biomass (fresh weight) is preferable as short plants under nursery conditions can be stored and/or transported to the final market easier [32]. Previous studies on vegetables demonstrated that high residue levels in the growing media, i.e., 20–40% OW or GW, might have negatively affected the growth parameters for sowthistle and purslane plants [16]. The growth of plants was reduced due to features of the unsuitable substrate, such as total pores space and AFP. The available air in the growing media can be enhanced by adding up to 20–30% inert material, such as perlite, pumice or sand, resulting in greater plant growth and development.

Plant photosynthesis is directly related to leaf pigment level and to leaf stomatal closing. The chlorophylls (Chl a, Chl b and total Chls) and carotenoid content, as well as the SPAD values, were significantly decreased in carnation grown in OW 20%, and the OW + GW 10–20% mixtures, compared to plants grown in 100% peat (Table 6). In the case of GW, a high content of chlorophylls was found in plant growing in GW 5% and/or control treatments, while the total carotenoids content was remained unaffected. Additionally, plant grown at the high residue's levels of OW 20% and OW + GW 10% revealed decreased leaf chlorophyll fluorescence, while plants grown at GW 5% accelerated the SPAD values compared to peat 100% or ≥10% of GW. The decrease in the amount of chlorophylls may have been caused by the high breakdown of chlorophylls or by the decrease in chlorophylls' biosynthesis. Chl b is converted to Chl a via the breakdown of chlorophyll [50]; this could explain the decrease in chlorophyll content and increase in the ratio of Chl a to Chl b when leaves were stressed in plants grown in OW, GW and OW + GW-based growing media (Table 6). Moreover, leaf chlorophyll content and the Chl a:Chl b ratio have been shown to vary in connection with climatic and environmental factors such as air pollution [51] and salt stress [50]. Photoprotective pigments' content increases in stressful conditions, as seen by nitrogen deficiency [52] or drought [53]. The carotenoid:chlorophyll ratio increased in plants grown in GW 20% and OW + GW 20% growing media, indicating the reduction in leaf photosynthetic capacity and, to some extent, the reduction of plant growth as revealed in Table 5 [54]. Plants' response to different stress conditions with their leaf stomatal closure has been previously reported in tomato plants grown in sand and watered with olive-mill wastewater and reflected the water stress induced by the material's elevated EC [55]. However, in the present study, leaf stomatal conductance was not measured, but evidence of the leaf photosynthetic capacity decrease is supported, as described above. Despite the fact that the minerals were easily accessible to the plant, premature residues may have used some of the available N in the decomposition procedure, which reflected changes at the C/N ratio at the end of the experiment (Table 4; Figure S2). The reported decline in plant development in the present research was not only due to mineral levels in the growing medium but also to the unfavorable features of inadequate growing media, such as AFP. In this regard, efforts to improve the growing media's characteristics may explore either increasing the level of inert material (i.e., adding 20–30% perlite) or introducing other inert materials (perlite, sand, zeolite, vermiculite, etc.). Attempts are also needed to supply some N to equalize the N losses/utilization by microorganisms throughout the organic matter decomposition.

Table 6. The effect of growing media (peat, olive-mill waste—OW, grape-mill waste—GW and their mixtures) on the carnation chlorophyll fluorescence (Fv/Fm), SPAD values, chlorophylls (Chl a, Chl b, total Chls; mg/g Fw) and carotenoids (mg/g Fw) levels, as well as the their ratios of Chl a:Chl b and carotenoids: total Chls on plants grown in the greenhouse/nursery.

	Chlorophyll Fluorescence	SPAD	Chl a	Chl b	Total Chls	Carotenoids	Chla:Chlb	Carotenoids: Total Chls
Peat 100%	0.82 ± 0.01 ab	71.74 ± 5.56 ab	0.93 ± 0.02 a	0.38 ± 0.02 a	1.31 ± 0.03 a	0.20 ± 0.01 a	2.55 ± 0.14 b	0.16 ± 0.01
OW 5%	0.84 ± 0.01 a	86.24 ± 15.19 a	0.99 ± 0.09 a	0.34 ± 0.04 a	1.34 ± 0.14 a	0.20 ± 0.01 a	3.05 ± 0.11 a	0.16 ± 0.00
OW 10%	0.83 ± 0.01 ab	63.70 ± 8.24 ab	0.87 ± 0.02 a	0.29 ± 0.00 ab	1.17 ± 0.01 a	0.18 ± 0.00 ab	3.10 ± 0.14 a	0.16 ± 0.01
OW 20%	0.81 ± 0.00 b	55.55 ± 3.96 b	0.66 ± 0.02 b	0.21 ± 0.01 b	0.88 ± 0.03 b	0.13 ± 0.00 b	3.15 ± 0.05 a	0.16 ± 0.00
Peat 100%	0.82 ± 0.01	71.74 ± 5.56 b	0.93 ± 0.02 ab	0.38 ± 0.02 a	1.31 ± 0.03 ab	0.20 ± 0.01	2.55 ± 0.14 b	0.15 ± 0.01 bc
GW 5%	0.85 ± 0.02	93.78 ± 9.10 a	1.05 ± 0.03 a	0.38 ± 0.01 a	1.43 ± 0.04 a	0.21 ± 0.01	2.94 ± 0.08 a	0.16 ± 0.00 c
GW 10%	0.82 ± 0.00	69.18 ± 7.62 b	0.84 ± 0.12 b	0.27 ± 0.04 b	1.12 ± 0.16 b	0.18 ± 0.02	3.09 ± 0.07 a	0.17 ± 0.00 ab
GW 20%	0.83 ± 0.01	70.94 ± 5.54 b	0.80 ± 0.01 b	0.28 ± 0.00 b	1.10 ± 0.01 b	0.20 ± 0.02	2.91 ± 0.02 a	0.16 ± 0.00 a
Peat 100%	0.82 ± 0.01 b	71.74 ± 5.56	0.93 ± 0.02 a	0.38 ± 0.02 a	1.31 ± 0.03 ab	0.20 ± 0.01 ab	2.55 ± 0.14 b	0.16 ± 0.01 b
OW + GW 5%	0.83 ± 0.01 ab	91.16 ± 15.85	1.01 ± 0.06 a	0.36 ± 0.02 a	1.37 ± 0.08 a	0.21 ± 0.01 a	2.96 ± 0.03 a	0.16 ± 0.00 b
OW + GW 10%	0.85 ± 0.01 a	82.92 ± 7.10	0.81 ± 0.08 ab	0.27 ± 0.02 b	1.10 ± 0.10 bc	0.17 ± 0.02 ab	3.08 ± 0.11 a	0.16 ± 0.00 ab
OW + GW 20%	0.83 ± 0.00 ab	83.06 ± 7.70	0.73 ± 0.01 b	0.24 ± 0.01 b	0.97 ± 0.01 c	0.16 ± 0.00 b	3.05 ± 0.03 a	0.17 ± 0.01 a

Values are mean ± SE (n = 6). Values in the columns that are followed by the same letter for the different residues (OW, GW or the mixtures of OW + GW) are not significantly different (p < 0.05).

Table 7 indicates the accumulated minerals in carnations grown in OW- or GW- or OW + GW-based growing media after a 35-day cultivation period. The addition of OW into the peat decreased the carnations' N and P content at ≥10% OW. The Ca and Mg content was increased at OW 5% and decreased at OW 20% compared to the peat treatment. Potassium was higher in plants grown at OW 5%, while Na was accumulated more in plants grown at 5% and 10% of OW.

Table 7. Effects of growing media (peat, olive-mill waste—OW, grape-mill waste—GW and their mixtures) on mineral element contents (g/kg dry weight) in carnation plants grown in the greenhouse/nursery.

	N	K	P	Ca	Mg	Na
Peat 100%	29.91 ± 0.56 a	34.01 ± 2.40 c	6.79 ± 0.22 ab	13.29 ± 1.12 b	3.44 ± 0.33 b	11.34 ± 0.78 ab
OW 5%	28.49 ± 0.56 a	42.71 ± 0.47 a	6.84 ± 0.15 a	15.84 ± 0.29 a	4.12 ± 0.10 a	12.65 ± 0.16 a
OW 10%	18.17 ± 0.63 b	39.88 ± 0.53 ab	6.26 ± 0.13 b	14.00 ± 0.35 ab	3.64 ± 0.03 ab	12.22 ± 0.16 a
OW 20%	12.56 ± 0.76 c	37.66 ± 0.52 bc	4.61 ± 0.14 c	8.86 ± 0.15 c	2.01 ± 0.06 c	10.45 ± 0.11 b
Peat 100%	29.91 ± 0.56 b	34.01 ± 2.40 c	6.79 ± 0.22 b	13.29 ± 1.12	3.44 ± 0.33 b	11.34 ± 0.78
GW 5%	30.10 ± 0.39 b	39.02 ± 0.43 b	6.75 ± 0.21 b	16.80 ± 1.88	4.11 ± 0.14 a	11.62 ± 0.34
GW 10%	32.33 ± 0.18 a	40.99 ± 0.46 b	6.87 ± 0.11 b	14.64 ± 0.27	4.06 ± 0.04 ab	11.91 ± 0.13
GW 20%	28.78 ± 0.56 b	47.51 ± 0.26 a	7.50 ± 0.19 a	13.98 ± 0.41	3.99 ± 0.02 ab	12.24 ± 0.20
Peat 100%	29.91 ± 0.56 a	34.01 ± 2.40 c	6.79 ± 0.22 a	13.29 ± 1.12 ab	3.44 ± 0.33 ab	11.34 ± 0.78
OW + GW 5%	28.26 ± 0.14 b	45.94 ± 0.52 ab	6.56 ± 0.15 a	13.85 ± 0.13 a	3.66 ± 0.02 a	12.05 ± 0.05
OW + GW 10%	25.06 ± 0.24 c	44.67 ± 0.41 b	5.75 ± 0.25 b	11.64 ± 0.17 bc	3.01 ± 0.05 bc	10.99 ± 0.16
OW + GW 20%	23.85 ± 0.18 c	49.96 ± 0.24 a	6.61 ± 0.25 a	10.70 ± 0.19 c	2.81 ± 0.05 c	10.98 ± 0.12

Values are mean ± SE (n = 4). The values in the columns that are followed by the same letter for the different residues (OW, GW or the mixtures of OW + GW) are not significantly different ($p < 0.05$).

Carnation plants grown at GW 20% growing media had higher K and P contents compared with the plants grown in peat or ≤10% GW. Nitrogen was obtained more in plants grown at 10% GW, whereas plants grown at 5% GW gathered more Mg compared to plants grown in peat. The GW presence in the growing media did not affect the accumulation of Ca and Na in plants (Table 7).

An intermediate effect was observed with the mixture of the wastes (OW + GW). Therefore, N content was less accumulated as the ratio of the wastes was increased. Potassium content increased in plants grown at ≥10% OW + GW compared to the control treatment. Magnesium and Ca were accumulated more in plants grown at OW + GW 5%, while P was accumulated less in the OW + GW 10% growing media. Sodium content was similar in plants grown in all mixtures, independently from the ratio of the wastes (OW + GW) added in the mixture.

Adding organic residues in the growing media, the level of N might be increased, but since the N is mainly in organic form, it is less available to the plants. Moreover, a part of N was most probably consumed by microorganisms during organic matter decomposition, as indicated by the C/N values, the decreased N levels at the growing media at the end of the crop period (Table 4, Figure S2) and the decreased of N content in plant tissue (Table 7), especially for the residues' high levels in the growing media. In that sense, additional N fertigation could alleviate this N shortage during the crop duration. This could justify the slow plant growth, less chlorophyll and photosynthetic capacity for the plants grown in media with high residue levels [37,56]. The mineral profile and release into the growing media when raw or not fully composted material was used, and it was not easy to record and manage; however, a supplement of a low-strength fertigation scheme (nutrient solution) could harmonize the abovementioned differences [32].

The use of OW and GW residues for the partial peat substitution in growing media affected the content of total phenolics and total flavonoids, as well as the antioxidant activity of the carnation plants (Figure 1). In the case of OW, total phenols in carnation were increased up to 36.5% in plants grown in ≥10% OW compared to plants grown in peat (Figure 1(A1)). Carnation antioxidant capacity, as assayed by FRAP and ABTS, increased by up to 63.5% and 86.0%, respectively, with higher values found at the OW 20% when compared to the relevant plants grown in peat (Figure 1(A3,A4)). Total flavonoid contents in carnation plants fluctuated among the tested mixtures without a specific trend (Figure 1(A5)), while DPPH levels were remained unchanged (averaged in 0.191 mg trolox/g fresh weight) (Figure 1(A2)). Interestingly, the total phenols, total flavonoids and antioxidant capacity in carnation plants grown in GW-based media were at similar levels. They averaged a phenols content of 0.662 mg GAE/g fresh weight, a flavonoids content of 0.027 mg rutin/g fresh weight, and an antioxidant capacity of 0.163, 0.173 and 0.522 mg trolox/g fresh weight for DPPH, FRAP and ABTS, respectively (Figure 1(B1–B5)). In case of the OW + GW mixtures, it seems that the GW material harmonized the changes occurred by the OW material, and the total flavonoids and antioxidant capacity in carnation plants grown in OW + GW-based media were unchanged (Figure 1(C2–C5)). Indeed, only the total phenols content was increased at the 5–10% OW + GW treatment compared to the control (100% peat) (Figure 1(C1)). Comparable findings have been observed in *Brassica* plants cultivated in OW-based growing media [57]. The increased antioxidant capacity of the carnation plant when exposed to high OW levels is of great interest, as similar observations could possibly be found in carnation flowers, which are edible [58]. However, in the present study, since flowers were not produced due to the short crop cycle, the flowers' antioxidant capacity was not measured, and it needs to be addressed in future research. The increased interest of edible flowers, enriched in antioxidants, has been well explored, for example, in the case of marigold (*Calendula officinalis* L.), petunia (*Petunia* x *hybrita* L.) and matthiola (*Matthiola incana* L.) plants that were grown in OW and paper waste growing media under hydroponic conditions [32].

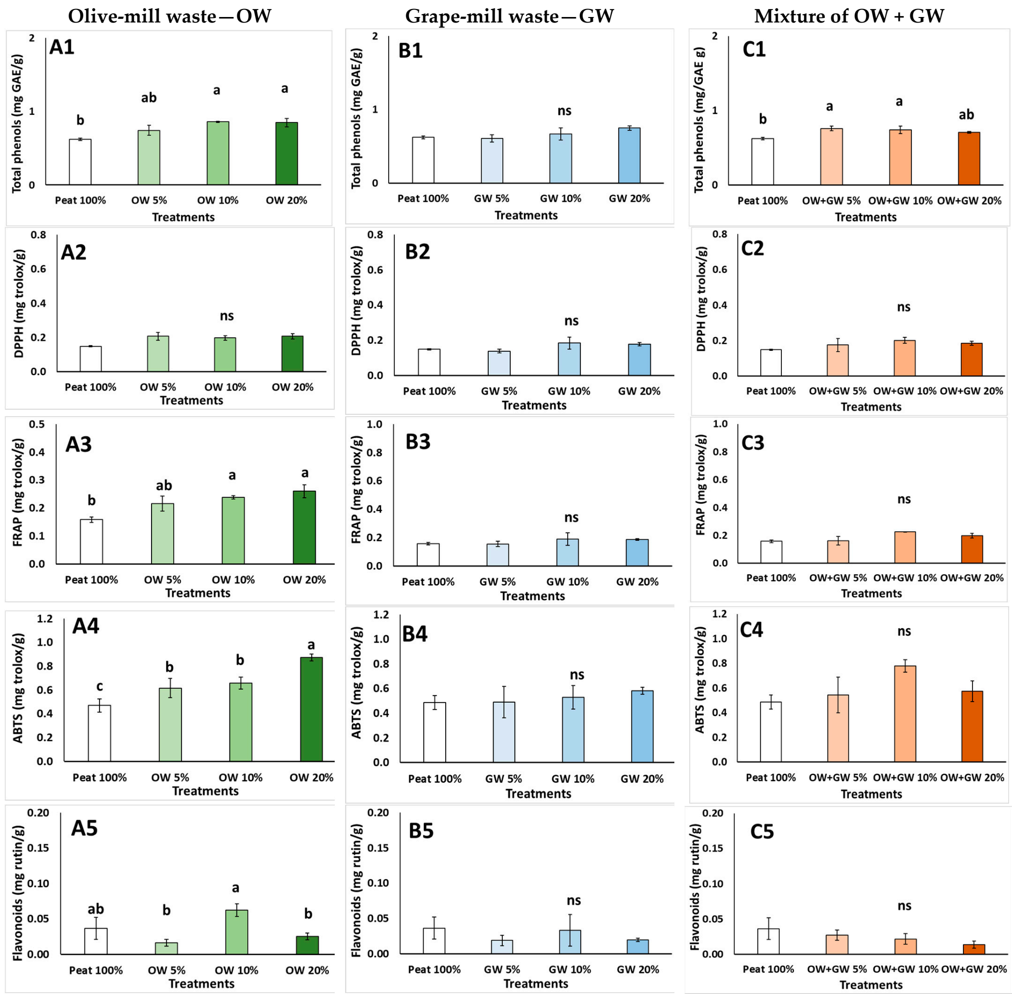

Figure 1. Effects of growing media (peat, olive-mill waste—OW, grape-mill waste—GW and their mixtures) on total phenols (mg GAE/g Fw), antioxidant activity (DPPH, FRAP, ABTS: mg trolox/g Fw), and flavonoids (mg rutin/g Fw) in carnation plants (OW: (**A1**–**A5**); GW: (**B1**–**B5**); OW + GW: (**C1**–**C5**)). Values are mean ± SE (n = 6). Mean values followed by the same letter do not differ significantly at $p \geq 0.05$, according to Duncan's Multiple Range Test. ns: no significance.

Plants are exposed to a plethora of adverse conditions throughout their life period. Often, these conditions involve biotic (which include diseases, pests, and weeds) together with abiotic (harsh temperatures, drought, very salty and osmotic soils, and elevated mineral/heavy metal levels) conditions. Plants have developed a range of detoxification processes that remove excessive amounts of reactive oxygen species (ROS) generated in cells during stressful conditions. MDA generation, which is linked to increased lipid peroxidation in stress conditions, constitutes among the most extensively utilized stress markers. MDA levels rise when plant antioxidants lose their capacity to scavenge ROS as a first stage in detoxifying what is triggering the stress conditions. In this case, MDA levels increased in carnation plants growing in media with ≥10% OW, where high levels of H_2O_2 production is a sign of a stressful situation that damages cells in plants (Figure 2(A1,A2)). In the case of the carnations growing in GW-based media, the MDA and H_2O_2 production were almost unchanged (Figure 2(B1,B2)), indicating no stressful condition, as this was also well proven by the unchanged levels of phenols and antioxidants (Figure 1). In the case of the OW + GW mixtures, MDA and H_2O_2 production were increased at 5% OW + GW mixture compared to peat (Figure 2(C1,C2)). Plants are able to initiate non-enzymatic (such as phenols, flavonoids, proline, ascorbic acid, etc.) and enzymatic antioxidants (superoxide dismutase, catalase, peroxidase, ascorbate peroxidase, etc.) mechanisms to detoxify the ROS accumulation in cells, which is raised under stress conditions [57]. Indeed, in the present study, several non-enzymatic molecules were studied, clearly indicating their activation (Figure 1(A1–A5)) toward the induced stress condition (Figure 2(A1)). Activation of non-enzymatic metabolism under stress conditions was evidenced in sowthistle grown in medicinal/aromatic plants residues [37], in ornamentals grown in OM and paper waste made growing media [32] and in *Myrtus communis* (L.) plants gown in saline conditions [59].

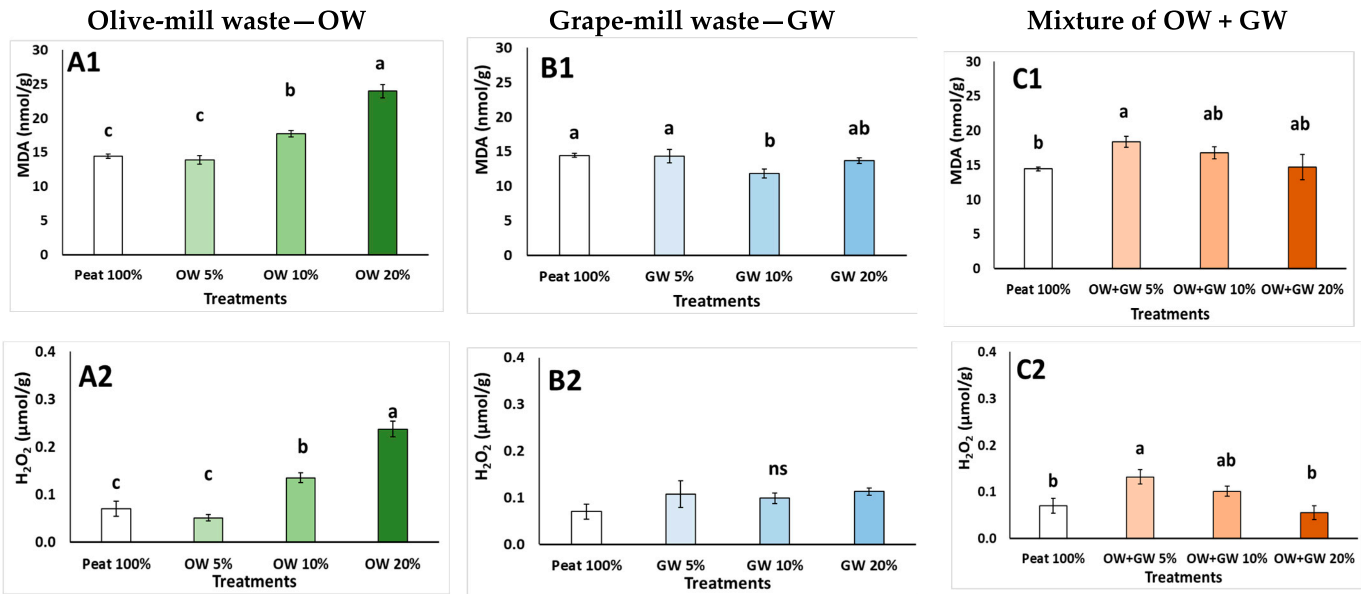

Figure 2. Effects of growing media (peat, olive-mill waste—OW, grape-mill waste—GW and their mixtures) on hydrogen peroxide—H_2O_2 (µmol/g) and lipid peroxidation—MDA (nmol/g) in carnation plants (OW: (**A1**,**A2**); GW: (**B1**,**B2**); OW + GW: (**C1**,**C2**)). Values are mean ± SE (n = 6). Mean values followed by the same letter do not differ significantly at $p \geq 0.05$, according to Duncan's Multiple Range Test.

It is critical to produce fruits, vegetables, ornamentals, seedlings and plants in pots economically by considering the fast plant growth and development, as well as by keeping plants in good health. Furthermore, when introducing peat substitutes to growing media mixtures, it is critical to choose environmentally friendly substitutes. Alternative materials need to be naturally produced, being available in large quantities and providing the appropriate physicochemical properties to the growing media [60,61]. Reduced peat extraction and utilization could lower the expenses of cultivating seedlings while also assisting in maintaining peatlands, which is an environmental limitation. Future research should not only focus on varying the ratios of the materials used for making growing media but also take into consideration the fertilizers and irrigation schedule applied. Plant wastes are high in nutrients and organic matter, which may enhance the soil as well as the characteristics of growing media. Although certain potting studies may result in improved soil properties when MAP residues are added, there are some variations between pot testing and actual agricultural practice in field conditions in regard to the context of MAP wastes disposal in soil and fertilizer substitution [62].

4. Conclusions

In the current study, the partial peat substitution was tested using OW and GW as well as their mixtures (OW + GW) for the growth of carnation potted plants under nursery conditions. Both residues' materials, when incorporated in the peat-based mixtures, impacted the physicochemical properties of the tested mixtures. Both OW and GW decreased the total pore space, which also diminished the growing media's ability to retain water and free air. In contrast, the addition of OW, GW and OW + GW increased the content of the organic matter and mineral levels available to the plants. The the high levels of OW and GW mainly negatively affected the plant growth-related parameters and photosynthetic performance of the carnation. As a result, by the decomposition of the organic material of the wastes, minerals accumulated in plant tissues when low OW levels were applied or when higher GW levels were applied, mainly for N, K and P accumulation. The non-enzymatic antioxidant metabolisms, including the total phenolics, total flavonoids and antioxidant activity of carnation, were triggered in plants grown in OW-based media to detoxify the oxidative stress occurred, as determined by the increased MDA levels. In contrast, the GW material did not cause any oxidative stress to plants. The increased antioxidants found in plants grown in OW-based media could be of interest, since carnation flowers are edible flowers, and the relevant decrease in plant growth was observed at high residues levels, i.e., 20% of OW. Therefore, both OW and GW can be used at ratios of 10%, while their combination of OW + GW is also recommended at 20% as it is alleviating the negative impacts that OW may occur, yet the additional improvement of growing media characteristics along with potential fertigation must be investigated. Therefore, the combination of different wastes might merge their properties and result in a mixture that has putative use in growing media preparation. In that sense, different parts of the world may paradigm to assess and combine various materials from local industrial sectors. Furthermore, additional studies might look into the impacts of composted wastes on plant growth, along with the impact of applying raw residues directly to the soil in field conditions.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy14030605/s1, Figure S1: Experimental lay out and representative images of plants grown in different growing media (Peat, Olive waste-OW, Grape waste-GW and their mixtures). Figure S2: Changes of growing media (Peat, Olive waste-OW, Grape waste-GW and their mixtures) chemical properties (% of changes from initial to final material) after plant harvesting (at the end of the growing period).

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