

## Alternative soilless media using olive-mill and paper-mill wastes for growing ornamental plants

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### Abstract

The performance of ornamental crops in greenhouse using growing media with olive-stone waste (OSW) and paper-mill waste (PMW) in different ratio, as peat (P) replacement, was determined. Therefore, marigold (*Calendula officinalis* L.), petunia (*Petunia x hybrida* L.) and matthiola (*Matthiola incana* L.) plants were grown in 1) P (100%), 2) P:OSW (90%:10%), 3) P:OSW (70%:30%) and 4) P:OSW:PMW (60%:20%:20%). Substrate physicochemical properties were determined. Different ratio of wastes affected growth and nutritional related parameter. In marigold, the addition of 10-30% OSW into the substrate increased plant height compared to plants grown in 100% peat. No differences on plant diameter as well as on leaves and flowers plant fresh weight and dry matter content were found. Adding PMW in combination with OSW maintained plant height and total number of flowers produced to similar levels as in plants grown in 100% peat. In matthiola, adding 30% OSW into the substrate reduced plant diameter and fresh weight, but not plant height, while no differences were evidence in lower OSW (i.e. 10%) content. Petunia's height, the total number of flowers and flower earliness (flower opening) were increased with the presence of OSW comparing to the plants grown in 100% peat. The adding of OSW did not affect plant diameter and fresh weight among treatment. The adding of PMW suppressed several plant growth-related parameters for both matthiola and petunia. The addition of OMW did not cause any changes on the leaf chlorophyll content (Chl a, Chl b, total Chl) whereas the presence of PMW decreased chlorophyll content for marigold, petunia and matthiola. Both of OMW and PMW altered the content of total phenolics and antioxidant capacity (DPPH, FRAP) in leaves and flowers for marigold and petunia. Leaf N and P content decreased in PMW-based media, while matthiola had visual phytotoxicity symptoms when PMW was added into the substrate. The current work indicating that up to 30% of OSW can substitute to peat for marigold and petunia and only up to 10% of OSW for matthiola, while the addition of PMW on top of OSW is not recommended, and further research is needed.

**Keywords:** Olive-mill waste; paper-mill waste; peat; growth; earliness; ornamentals

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### Introduction

Peat is the amendment most widely used in commercial potting growing media for horticultural seedling production and ornamental plants [1], but this involves the exploitation of non-renewable resources and the degradation of highly valuable peatland ecosystems. In many countries several restrictions have been established for the use of this material owing to environmental concerns (destruction of ecosystems highly fragile [2], potential source of C emissions [3] and indeed peat has become a somewhat scarce and expensive potting substrates [4]). Furthermore, peatlands are under the safeguard of the Directive 92/43/EC for natural habitats and wild fauna and flora [5]. In this sense, several governments are trying to reduce the use of peat as a substrate and as a soil improver, as well as encouraging the re-use of organic wastes as substrate components instead of their disposal [6]. Nowadays, the search for alternative high-quality and low-cost materials as growing media in horticulture is a necessity due to the increasing demand and rising costs for peat [7].

The continuous inputs of wastes on agricultural land have caused imbalance in ecosystems as well as there is a growing public concern about the environmental impacts and human health [8]. Over recent years, an increasing interest has been shown in the reutilization of organic by-products and waste composts for agriculture use and is a major key for sustainable waste management [9]. Recycling diverts wastes from landfills and, when used as substrate materials for production of nursery and horticultural crops, replaces or reduces dependency on peat moss [10].

Olive culture is a major crop worldwide and extensively cultivated in Mediterranean basin consisting the 98% of world production, and is produced by traditional and industrial olive mills usually from October to December [11]. The industrial olive oil sector generates large quantities of solid and liquid wastes and by-products in areas of expanded olive cultivation. In olive oil producing areas usually ornamental horticulture is also developed because of the climate type. Extracted olive press cake (EOPC) is the major organic solid waste from the olive oil industry and it has been used extensively as a fuel source [12]. However, smoke emission from

burning the waste have restricted this practice due to environmental constraints, resulting in material price decline. Albuquerque et al. [13] detected an overall positive effect and improvement of soil conditions by adding olive-mill (OW) by-product compost to soil. Ghosheh et al. [14] assessed herbicidal activity of olive husks, while D'Addabbo et al. [15] reported suppression of *Meloidogyne incognita* (Kofoid and White) in soil amended with olive residues. Relevant information is available on whole olive seed chemistry including its oil, proteins, sugars, fibers and phenolic glucosides [16].

Considerable amount of solid wastes generated by the paper and pulp industry are deposited directly in landfill [17]. Composted paper mill sludges could provide products that are of value to the horticultural and the container-grown industries. In such markets, the demands on compost quality and consistency become very much higher, and often unaffordable for horticulture enterprises. However, the financial returns (sales revenue) can also be very much higher. Typically, every compost producer or soil amendment business initially aims for the high-end market. However, that horticultural market is very difficult to penetrate and sustain [18].

Many materials, especially waste materials, have been considered for use as potential peat alternatives, including green waste compost, mushroom compost, (composted) animal manures, coir, vermicompost, paper mill residuals, paper waste, pine bark, rock wool, sawdust, sands, straw, wood chips, loam, cocoa shell, perlite and vermiculite, and reclaimed fibre board [18]. To date, none of the materials have proved ideal in matching all the desirable properties of peat. However, many of the substances have been shown to match or even surpass peat for specific applications [19]. Therefore, the present study sought to evaluate the effect of varying the proportion of olive-stone waste and/or paper mill waste mixed with conventional peat substrates, as a growing media in the nursery production of ornamentals such as marigold, petunia and Matthiola.

## **Material and methods**

### ***Plant material, olive-stone and paper waste***

In the present study, ornamental plants of marigold (*Calendula officinalis* L.), petunia (*Petunia x hybrita* L.) and Matthiola (*Matthiola incana* L.) were grown under natural light during winter period of 2017. Commercial peat (Professional peat, Gebr. Brill Substrate GmbH & Co.KG, Georgsdorf, Germany) and perlite (Perloflor, Protectivo EPE, Athens, Greece) were used. Olive-stone waste (OSW) was obtained from a local olive press factory in Cyprus, by "Koroneiki" olive cultivar. Common paper sheets with ink were used and paper-mill waste (PMW) shredded at particles of 0.5 x 3-4 cm (wide x length). Both OSW and PMW were analysed for several physicochemical parameters (in dry weight: dwt) which were pH: 5.67 and 6.31; EC: 0.283 and 1.175 dS/m (1:5 v/v); organic matter: 13.78% and 43.39% dwt; organic carbon: 8.01% and 25.17% dwt; N: 0.18% and 0.37% dwt; ratio C/N: 44.4 and 68.1; K: 647.6 and 2428.5 µg/g; P: 96.1 and 389.2 µg/g; Ca: 62283.4 and 1804.8 µg/g; Mg: 700.9 and 332.7 µg/g; Na: 2036.7 and 489.3 µg/g; Al: 254.2 and 54.8 µg/g; Fe: 156.9 and 85.9 µg/g; Mn: 9.3 and 6.8 µg/g; Cu: 134.8 and 34.6 µg/g; Si: 27.4 and 43.6 µg/g; Zn: 29.8 and 12.9 µg/g; respectively. The physicochemical parameters are within levels as described previously [20]. Several heavy metals were undetectable (Cd, Cr, Pb) or under low limits (for example Ni < 3.4 µg/g) [21].

### ***Experimental design***

The experiment was carried out in an unheated plastic greenhouse with a North-South orientation at the experimental farm of Cyprus University of Technology, in Cyprus. Seedlings were produced in expanded clay cubes (3 x 3 x 2 cm) and were acquired from local agriculture nursery, at the growth stage of 2 true leaves. Three medium, peat (P), OSW and PMW, and mixtures of these, were used to create four substrates which were: 1) P (100%), 2) P:OSW (90%:10%), 3) P:OSW (70%:30%) and 4) P:OSW:PMW (60%:20%:20%). Plants grown in the above substrates were fertigated every second day (or according to plant needs), manually, with basic hydroponic nutrient solution (as described in Christoulaki et al. [22] of EC: 1.9 mS/cm and pH: 6.0 with 8 individual (replications) plants per treatment for each examined species.

Seedlings were transplanted in single pots (filled with substrate; 0.25 L capacity pot) and arranged randomly on plastic trays in order to maintain drainage solution. The drainage solution was collected in trays in each pot and was available for plant water needs through capillary suction. Pots were 0.10 m apart each other. Plants were grown under common cultivation practices, without any pruning or pesticide applications.

### ***Measurements***

The physicochemical properties of individual mixtures were analysed and total pore space (TPS), air filled porosity (AFP), available water holding capacity (AWHC) and bulk density (BD) by volume were measured. Organic matter content was determined based on the Walkley-Black chromic acid wet oxidation method, and the organic C was calculated. The electrical conductivity (EC) and pH determined according to 1:5 dilution method, employing a portable pH/EC-meter (HI 98130 HR, Hanna Instruments, USA). After acid digestion of the sample ash, nutrients analysis for macronutrients and micronutrients employ inductively coupled plasma atomic

emission spectrometry (ICP-AES; PSFO 2.0 Leeman Labs INC., USA) was determined while total N determined through Kjeldahl method.

#### *Plant growth and tissue analysis*

Marigold, petunia and matthiola were grown for 1.5 months. Beginning second week after transplanting, it was studied weekly the impact of growing media on number of total and open flowers produced in each pot. Plant marketability was observed by employing a 1-4 scale [1 not marketable quality (i.e. decolourization, toxicity, necrotic tissue); 2: medium quality (i.e. small size, decolourization); 3: good quality; 4: extra quality]. At the end of the experiment, plant height (cm), plant diameter-size (cm), total plant as well as flowers biomass (fresh weight and the % of dry matter) were determined. Leaf elemental analysis took place on four replications/treatment (each replication was a pool of two individual leaf samples). Samples were dried at 65 °C for 4 d, weighted, and grounded in a Wiley mill to pass through a 40 mesh screens. Sub samples (0.2-0.3 g) were placed in a furnace (450 °C for 6 h) for ashing (Carbolite, AAF 1100, GERO, Germany), in porcelain cups. After removal from the oven the ash was digested in 10 mL hydrochloric acid (2 N HCl). The solution was placed on a heater at 80 °C in order to achieve a faster and better dilution. The solution was filtered through Whatman No. 4 filter paper and diluted to a 50 mL volume with deionized water. Determination of K, P, and Na was done by flame photometer (Model 1382, Lasany International, India) and atomic absorption spectrometry (AA500FG, PG Instruments, UK) and N by the Kjeldahl (BUCHI, Digest automat K-439 and Distillation Kjeldahl K-360) method. Data were expressed in g per kg of dry weight.

Plant tissue (four replications/treatment; each replication consisted of a pool of two plants tissue; 0.1 g) was incubated in heat bath at 65 °C for 30 min, in the dark, with 10 mL dimethyl sulfoxide (DMSO) for chlorophyll extraction. Photosynthetic leaf pigments, chlorophyll a (Chl a), chlorophyll b (Chl b) and total chlorophyll (t-Chl) content were calculated [23].

#### *Polyphenol content and antioxidant activity of ornamental species*

Polyphenols were extracted from four samples (two individual plants were pooled/sample) for each treatment. Plant tissue (1 g) was milled (for 60 s) with 10 mL methanol (50% v/v) and extraction was assisted with ultrasound for 30 min. The samples were centrifuged for 15 min at 4000 g at 4 °C (Sigma 3-18K, Sigma Laboratory Centrifuge, Germany). The supernatant was stored at -20 °C until use for analysis of total phenolic content and total antioxidant activity by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) and ferric reducing antioxidant power (FRAP) method. The total phenolic content was determined with the Folin-Ciocalteu method at 755 nm according to Tzortzakis et al. [24] and results were expressed as mg of gallic acid equivalents (Scharlau, Spain) per g of fresh weight (GAE/g Fwt). Radical-scavenging activity was determined as described previously [25]. In more details, DPPH radical scavenging activity of the plant extracts was measured at 517 nm from the bleaching of the purple-colored 0.3 mM solution of DPPH. Standard curve was prepared using different concentrations of trolox [(±)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid], and results were expressed as mg trolox/g of fresh weight. The antioxidant capacity using the 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) method was carried out according to Wojdylo et al. [26]. The results were expressed as equivalents of trolox per g of fresh weight (mg of Trolox/g Fwt).

#### *Statistical analysis*

Data were tested for normality, and then subjected to analysis of variance (ANOVA). Significant differences between mean values were determined using Duncan's Multiple Range test ( $P < 0.05$ ) following one-way ANOVA. Significant differences on percentage values (% dry matter) were logarithmically transformed prior to analysis. Statistical analyses were performed using SPSS (SPSS Inc., Chicago, Ill.).

#### **Results and discussion**

The extensive use of peat as the main substrate component for the production of seedlings and potting plants in nurseries, with high cost of extraction from peatlands and transformation, as well as the increasing environmental pressures against peat extraction leads to an increasing interest on the feasibility of substituting peat by organic wastes and by-products. Therefore, it is important to look for good quality and local available low cost substituents of peat. A number of potential alternatives have been identified [Abad et al., 2001], and have proved to be very promising [27], such as olive leaves and olive stones waste [28-30]. However, several farmers applied olive mill solid wastes on crops, without composting process or awareness of the possible negative impacts and/or limitations. This fact increase the need for experimental studies on raw OSW material and their performance in ornamental potting production.

Replacing of peat by OSW increased the bulk density and decreased the total porosity as well as the air filled porosity of the media (Table 1), being in agreement with previous studies when OSW compost used for foliage potted plants [31-32]. Papafotiou et al. [32] reported that composted OSW had a higher number of small pores

than peat, because media with a high OSW (i.e. 50-75%) level held less water initially than the control (i.e. 100% peat). In our study, the OSW particle size improved the pores space, due to the increased particles size (data not presented). Organic matter was high in all the examined growing media, ranging from 16.10% in P:OSW:PMW (60:20:20) to 19.18% in P:OSW (90:10). The improved organic material into the growing media is of considerable nutrients value, as mixtures (20–50%) of compost derived by municipal solid wastes with perlite may be used as substrates without the need for additional mineral fertilizer [33].

Olive waste also has high concentration of Ca, Fe, K, Mg, N, P and Fe that can affect the growing media fertility [34]. Several studies reported that the high pH of the medium which has been found that in a lightweight potting mix (consisted of sphagnum peat, vermiculite, perlite, composted bark and sand) decreased the availabilities of P, Fe, Mn and B [28, 35]. All the examined growing media had light acidified pH, which prevent any P, Fe, Mn and B deficiencies. At the end of the experiment, mineral content in growing media were of sufficient content, without any deficiencies or accumulation/toxicities on the plant tissue, which is related to the well balance fertigation through the complete nutrient solution application. There are, however, certain limitations on the use of some composts: increase in salt content to levels which might affect the growth of sensitive crops; heavy metal toxicity; low overall porosity and a marked variation in physical/chemical properties [36].

**Table 1.** Physicochemical properties of substrate medium consisting of olive-stone waste (OSW) and paper-mill waste (PMW) into commercial peat (P) resulting in four media. Mineral values in parenthesis, are referred to the mineral content at the end of experiment.

	<b>P:OSW:PMW (100:0:0)</b>	<b>P:OSW (90:10)</b>	<b>P:OSW (70:30)</b>	<b>P:OSW:PMW (60:20:20)</b>
Organic matter (%)	18.52	19.18	18.37	16.10
Organic C (%)	10.74	12.46	10.66	9.34
pH	4.63	4.75	5.06	5.11
EC (mS/cm)	0.169	0.155	0.179	0.237
C/N	19.2	27.7	39.5	37.4
Total N (%)	0.55 (0.87)	0.42 (0.43)	0.22 (0.16)	0.23 (0.56)
P (g/L)	0.34 (0.44)	0.38 (0.43)	0.17 (0.19)	0.21 (0.40)
K (g/L)	0.32 (1.58)	2.43 (2.66)	1.58 (1.51)	1.56 (1.84)
Ca (g/L)	3.22 (10.91)	3.19 (7.15)	1.48 (5.12)	11.48 (11.38)
Na (mg/L)	0.31 (2.42)	0.98 (2.07)	0.42 (1.39)	0.58 (1.69)
Mg (mg/L)	0.99 (2.73)	2.80 (4.02)	0.55 (1.49)	0.49 (2.11)
Fe (mg/L)	612.13 (701.89)	809.28 (2014.09)	189.06 (328.41)	280.14 (462.22)
Mn (mg/L)	16.11 (20.16)	18.72 (32.40)	10.59 (12.60)	12.51 (21.23)
Zn (mg/L)	16.61 (18.21)	27.95 (46.44)	24.72 (14.71)	10.48 (13.47)
Cu (mg/L)	141.84 (141.42)	442.82 (722.17)	111.28 (430.74)	44.33 (48.13)
B (mg/L)	n.d. <sup>Z</sup> (112.94)	n.d. <sup>Z</sup> (111.01)	n.d. <sup>Z</sup> (51.48)	n.d. <sup>Z</sup> (118.35)
TPS (%) <sup>Z</sup>	89.43	65.74	67.81	67.41
AFP (%) <sup>Z</sup>	28.51	11.90	8.57	9.52
AWHC (%) <sup>Z</sup>	59.84	53.84	59.24	57.89
BD (g/cm <sup>3</sup> ) <sup>Z</sup>	20.51	26.67	38.88	34.40

<sup>Z</sup> Total pore space (TPS), air filled porosity (AFP), available water holding capacity (AWHC), bulk density (BD) by volume. n.d. not detectable.

In marigold, low content of OSW (10%) increased plant height compared with control plants (grown in 100% peat) and plants grown in P:OSW:PMW (60:20:20) (Table 2). Moreover, plants grown in 10% OSW produced the highest number of flowers. The presence of PMW into the substrate reduced plant biomass as a consequence of reduced leaf and flowers fresh weight. However, plant diameter, flower's opening and plant dry matter content were unaffected by the adding of OSW and/or PMW into the peat. In petunia, the PMW add into the substrate reduced plant growth parameters by causing reduction in plant height and diameter as well as plant biomass (as a result of leaf weight reductions rather than of the flower's weight) (Table 2). Interestingly, the 10% OSW either improved (plant height, flower opening - indicating flower earliness) or maintained the plant development which was observed in control treatment (100% peat). No differences were found in flower weight among treatments, despite the increased number of flowers (more than 2-fold) in plants grown in 10-30% OSW compared to the control substrate. Both marigold and matthiola grown in 10-30% OSW maintained their foliage biomass to similar levels with the control, being in agreement with studies in *Codiaeum variegatum* and *Syngonium podophyllum* foliage potted plants [32]. Similarly, in matthiola, the PMW add into the substrate reduced plant height, diameter and plant biomass but increased dry matter content compared to the control

treatment (Table 2). Additionally, the high OSW content (30% OSW) reduced plant biomass and diameter, being in accordance with previous studies in poinsettia [28]. Indeed, in poinsettia, the decreased leaf number and foliage fresh weight was due to the earlier senescence of the basal leaves, as a result of the lower readily available water. Previous studies reported the effect of growing media on flowering time is highly depended on compost type and its concentration in the growing medium, as well, as on the plant species [28, 35]. In the present study, the observed reduction with the PMW add is not related to different plant nutrition, as Na content was remained at non phytotoxic levels similar to the levels on 100% of peat. As a consequence, the observed plant growth reduction was due to unsuitable substrate physicochemical properties, such as available water holding capacity.

**Table 2.** Impact of olive-stone waste (OSW) and paper-mill waste (PMW) into commercial peat (P) on marigold, petunia and matthiola seedling height (cm/plant), diameter-size (cm), leaf and flower fresh weight (fwt; g/plant), dry matter content (dm; %), total biomass as well as flower number produced and opened on ornamental plants grown in greenhouse/nursery.

	Mixtures	Height	Diameter	Leaf fwt	Flower fwt	Leaf dm	Flower dm	Total biomass	No flowers	Open flowers
<b>Marigold</b>	<b>P:OSW:PMW (100:0:0)</b>	14.32 <sup>c,Y</sup>	13.92 <sup>a</sup>	6.92 <sup>a</sup>	10.16 <sup>a</sup>	12.20 <sup>a</sup>	13.45 <sup>a</sup>	17.19 <sup>a</sup>	3.38 <sup>ab</sup>	1.25 <sup>a</sup>
	<b>P:OSW (90:10)</b>	15.81 <sup>a</sup>	14.87 <sup>a</sup>	7.42 <sup>a</sup>	9.78 <sup>a</sup>	12.45 <sup>a</sup>	13.50 <sup>a</sup>	17.21 <sup>a</sup>	4.25 <sup>a</sup>	1.50 <sup>a</sup>
	<b>P:OSW (70:30)</b>	15.43 <sup>ab</sup>	14.87 <sup>a</sup>	6.49 <sup>ab</sup>	8.55 <sup>a</sup>	12.34 <sup>a</sup>	13.58 <sup>a</sup>	15.05 <sup>a</sup>	3.12 <sup>b</sup>	1.12 <sup>a</sup>
	<b>P:OSW:PMW (60:20:20)</b>	14.56 <sup>bc</sup>	14.00 <sup>a</sup>	5.13 <sup>b</sup>	7.41 <sup>b</sup>	13.01 <sup>a</sup>	13.15 <sup>a</sup>	12.47 <sup>b</sup>	2.87 <sup>b</sup>	1.00 <sup>a</sup>
<b>Petunia</b>	<b>P:OSW:PMW (100:0:0)</b>	10.05 <sup>bc,Y</sup>	15.82 <sup>a</sup>	23.11 <sup>a</sup>	1.34 <sup>a</sup>	7.31 <sup>b</sup>	10.76 <sup>a</sup>	24.43 <sup>a</sup>	2.12 <sup>b</sup>	1.50 <sup>b</sup>
	<b>P:OSW (90:10)</b>	11.75 <sup>ab</sup>	17.68 <sup>a</sup>	23.80 <sup>a</sup>	2.52 <sup>a</sup>	8.31 <sup>b</sup>	12.46 <sup>a</sup>	26.33 <sup>a</sup>	4.62 <sup>a</sup>	4.12 <sup>a</sup>
	<b>P:OSW (70:30)</b>	12.06 <sup>a</sup>	17.81 <sup>a</sup>	22.02 <sup>a</sup>	3.03 <sup>a</sup>	7.75 <sup>b</sup>	10.59 <sup>a</sup>	25.03 <sup>a</sup>	4.37 <sup>a</sup>	3.25 <sup>ab</sup>
	<b>P:OSW:PMW (60:20:20)</b>	8.68 <sup>c</sup>	12.31 <sup>b</sup>	6.80 <sup>b</sup>	1.09 <sup>a</sup>	12.10 <sup>a</sup>	12.70 <sup>a</sup>	7.62 <sup>b</sup>	2.00 <sup>b</sup>	1.50 <sup>b</sup>
<b>Matthiola</b>	<b>P:OSW:PMW (100:0:0)</b>	13.30 <sup>a,Y</sup>	11.82 <sup>a</sup>	12.09 <sup>a</sup>	-	13.61 <sup>b</sup>	-	12.09 <sup>a</sup>	-	-
	<b>P:OSW (90:10)</b>	12.25 <sup>a</sup>	10.62 <sup>ab</sup>	8.23 <sup>ab</sup>	-	13.63 <sup>b</sup>	-	8.23 <sup>ab</sup>	-	-
	<b>P:OSW (70:30)</b>	12.06 <sup>a</sup>	9.18 <sup>b</sup>	5.18 <sup>bc</sup>	-	13.97 <sup>b</sup>	-	5.18 <sup>bc</sup>	-	-
	<b>P:OSW:PMW (60:20:20)</b>	9.06 <sup>b</sup>	6.31 <sup>c</sup>	1.49 <sup>c</sup>	-	19.67 <sup>a</sup>	-	1.49 <sup>c</sup>	-	-

<sup>Y</sup> values (n=8) in columns followed by the same small letter are not significantly different,  $P \leq 0.05$ .

Plant marketability was reduced in the presence of PMW content (20% PMW) for all the examined ornamentals (marigold, petunia and matthiola), including the high OSW content of 30% for matthiola (Table 3). The decrease in marigold's and petunia's marketability was marked at the end of the 4<sup>th</sup> week of plant growth (Figure 1) whereas in case of matthiola, the first symptoms of reduced marketability/quality were found even at the 2<sup>nd</sup> week of plant growth (Figure 1). This is probably related to the growing media readily available water for different plants as well as the irrigation/drainage needs for each plant species. Matthiola water needs are not well characterized, however plant sensitivity to salinity and/or osmotic stress (for example the increased N) was reported [37], which caused delays in plant flowering.

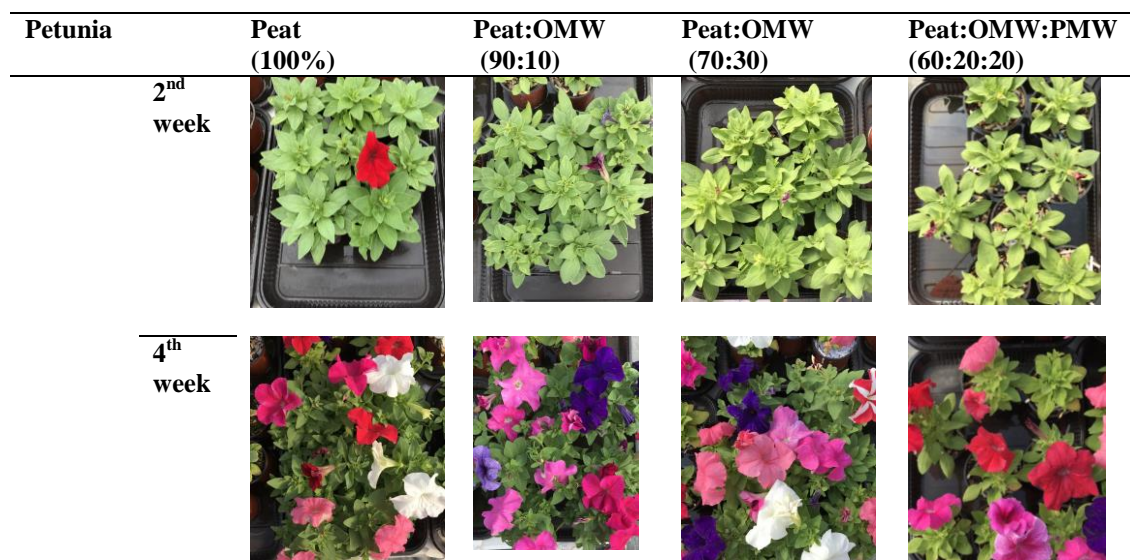
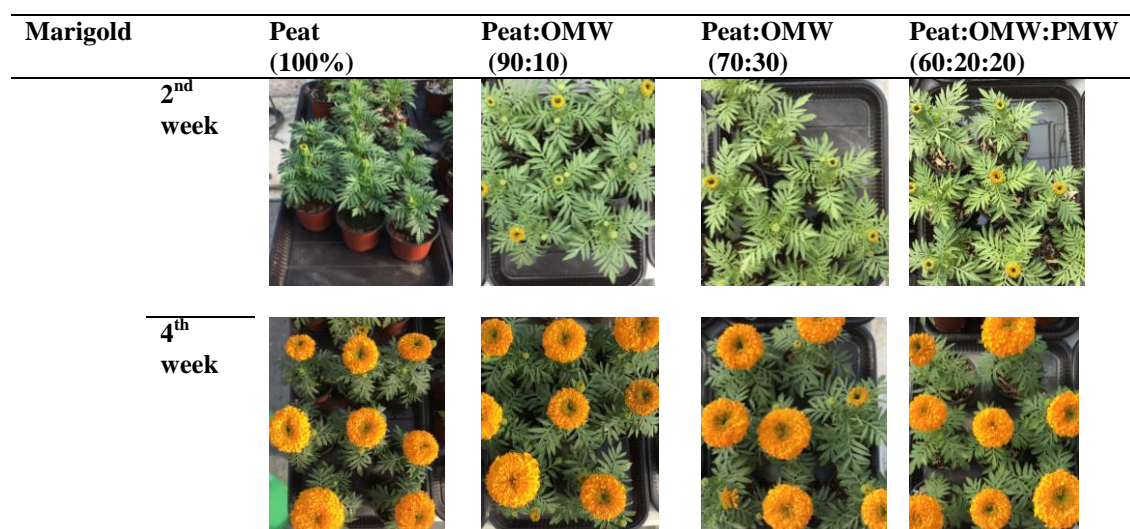
The total leaf chlorophyll content decreased in marigold and matthiola grown in P:OSW:PMW (60:20:20) and this was mainly due to the reduction of Chl a content (Table 3). The fluorescence yield of chlorophyll a, is dependent on the micro-environment in the thylakoid, therefore indicating structural or organizational changes of the chloroplast membranes and elucidating the physiological and biochemical bases for changes in the ability of leaves to assimilate CO<sub>2</sub> [38-39].

**Table 3.** Impact of olive-stone waste (OSW) and paper-mill waste (PMW) into commercial peat (P) on marigold, petunia and matthiola chlorophyll (Chl a, Chl b and total Chl) content and marketability (scale 1-4 from lower to higher quality) on ornamental plants grown in greenhouse/nursery.

	Mixtures	Chl a	Chl b	Total Chl	Marketability
<b>Marigold</b>	<b>P:OSW:PMW (100:0:0)</b>	1.01 <sup>a,Y</sup>	0.29 <sup>a</sup>	1.31 <sup>a</sup>	3.98 <sup>a</sup>
	<b>P:OSW (90:10)</b>	0.94 <sup>ab</sup>	0.29 <sup>a</sup>	1.24 <sup>ab</sup>	4.00 <sup>a</sup>

	<b>P:OSW (70:30)</b>	0.92 <sup>ab</sup>	0.25 <sup>a</sup>	1.17 <sup>ab</sup>	3.81 <sup>a</sup>
	<b>P:OSW:PMW (60:20:20)</b>	0.77 <sup>b</sup>	0.21 <sup>a</sup>	0.98 <sup>b</sup>	2.92 <sup>b</sup>
<b>Petunia</b>	<b>P:OSW:PMW (100:0:0)</b>	0.72 <sup>a,Y</sup>	0.28 <sup>a</sup>	1.01 <sup>a</sup>	3.52 <sup>b</sup>
	<b>P:OSW (90:10)</b>	0.73 <sup>a</sup>	0.29 <sup>a</sup>	1.02 <sup>a</sup>	4.00 <sup>a</sup>
	<b>P:OSW (70:30)</b>	0.61 <sup>a</sup>	0.25 <sup>a</sup>	0.87 <sup>a</sup>	3.90 <sup>a</sup>
	<b>P:OSW:PMW (60:20:20)</b>	0.52 <sup>a</sup>	0.23 <sup>a</sup>	0.75 <sup>a</sup>	2.78 <sup>c</sup>
<b>Matthiola</b>	<b>P:OSW:PMW (100:0:0)</b>	0.54 <sup>a,Y</sup>	0.16 <sup>a</sup>	0.71 <sup>a</sup>	3.45 <sup>a</sup>
	<b>P:OSW (90:10)</b>	0.49 <sup>a</sup>	0.14 <sup>a</sup>	0.63 <sup>a</sup>	3.03 <sup>ab</sup>
	<b>P:OSW (70:30)</b>	0.65 <sup>a</sup>	0.19 <sup>a</sup>	0.84 <sup>a</sup>	1.85 <sup>b</sup>
	<b>P:OSW:PMW (60:20:20)</b>	0.21 <sup>b</sup>	0.06 <sup>b</sup>	0.28 <sup>b</sup>	1.09 <sup>c</sup>

<sup>Y</sup> values (n=4) in columns followed by the same small letter are not significantly different,  $P \leq 0.05$ .



<b>Matthiola</b>	<b>Peat (100%)</b>	<b>Peat:OMW (90:10)</b>	<b>Peat:OMW (70:30)</b>	<b>Peat:OMW:PMW (60:20:20)</b>
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Figure 1. Impact of olive-stone waste (OSW) and paper-mill waste (PMW) into commercial peat (P) on marigold, petunia and matthiola during plant growth in greenhouse/nursery.

Total phenolics and antioxidant capacity (DPPH, ABTS) altered differently in leaves and flowers for the examined ornamental species (Figures 2-4). Therefore, in marigold flowers, the phenols content increased and the ABTS antioxidant activity was decreased in plants grown with the presence of paper-mill waste (P:OSW:PMW 60:20:20). However, the 10% OSW adding into the substrate kept lower the content of total phenolics and antioxidant activity of marigold leaves. The same effects were evidence in petunia's flower antioxidant capacity as paper-mill waste increased the content of phenols and antioxidant activities in both leaves and flowers. The content of phenolics and antioxidant activity in matthiola leaves were also increased due to the increase PMW content into the substrate media (Figure 4). In both marigold and petunia, the content of polyphenols is greater in flowers compared to the leaves. This is of great importance as several potted plants have edible flowers, and this include marigold and petunia. Plant material of high phenolic content can protect tissue oxidation by scavenging free radicals and inhibiting lipid peroxidation [40]. However, in the present study, the edibility of flowers was not study, and further research to that direction is necessary.

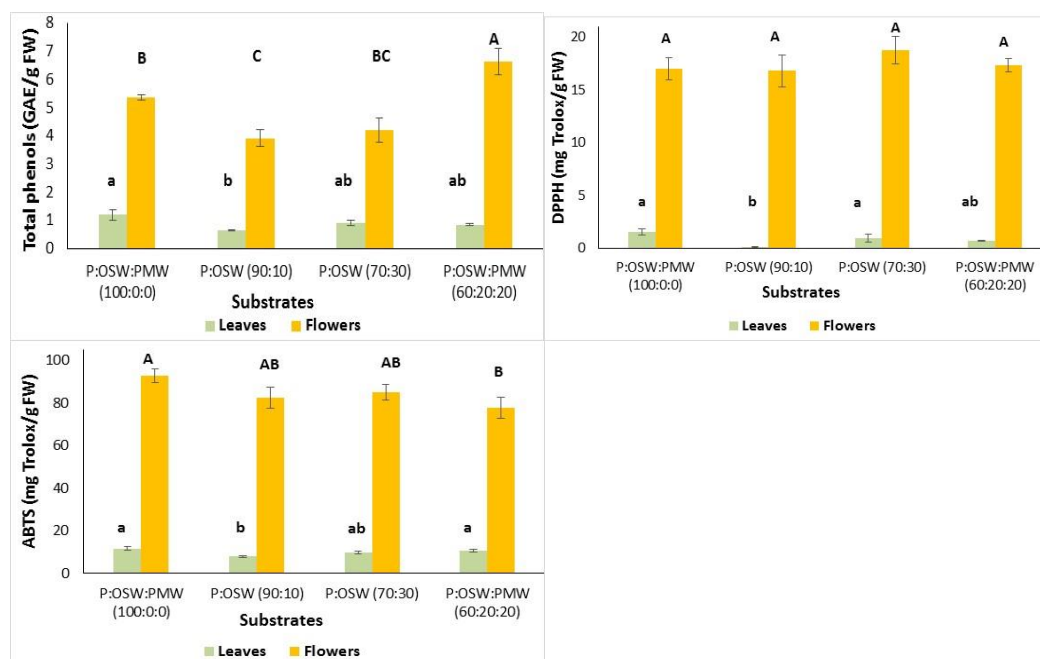
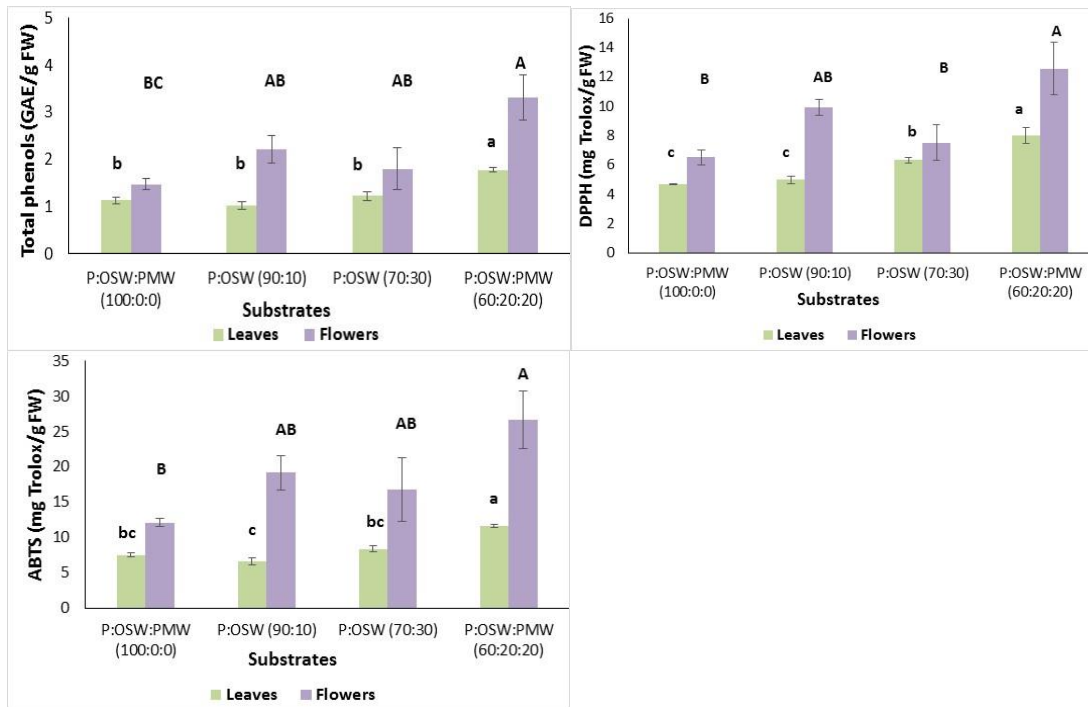
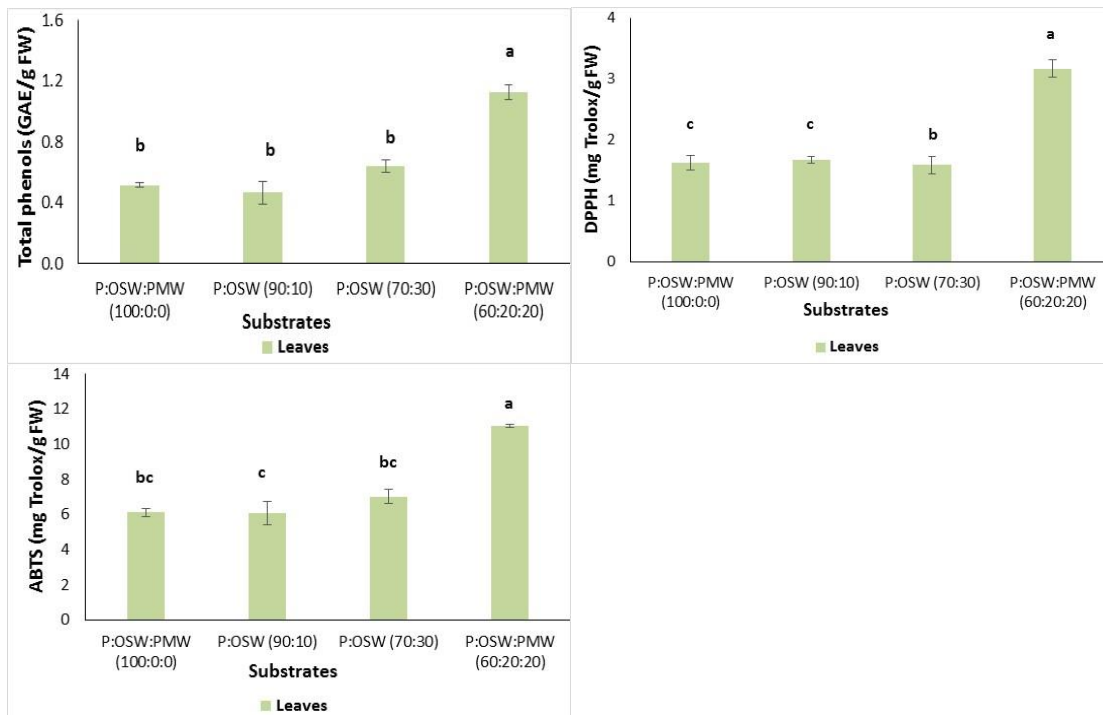


Figure 2. Impact of olive-stone waste (OSW) and paper-mill waste (PMW) into commercial peat (P) on marigold content of total phenols and antioxidant activity (DPPH, ABTS) on ornamental plants grown in greenhouse/nursery. Means values followed by the same small or capital letter are not significantly different,  $P < 0.05$ .



**Figure 3.** Impact of olive-stone waste (OSW) and paper-mill waste (PMW) into commercial peat (P) on petunia content of total phenols and antioxidant activity (DPPH, ABTS) on ornamental plants grown in greenhouse/nursery. Means values followed by the same small or capital letter are not significantly different,  $P \leq 0.05$ .



**Figure 4.** Impact of olive-stone waste (OSW) and paper-mill waste (PMW) into commercial peat (P) on matthiola content of total phenols and antioxidant activity (DPPH, ABTS) on ornamental plants grown in greenhouse/nursery. Means values followed by the same small letter are not significantly different,  $P \leq 0.05$ .

Mineral content was fluctuated among plant species, while matthiola had visual phytotoxicity symptoms as they were obtained macroscopically (Figure 1). In marigold, N level was reduced in plants grown in OSW-based media, while K content was greater in plants grown in 30% OSW (Table 4). The add of PMW into growing media, resulted in decreased marigold leaf N, P and Na content but increased K content compared to the control



treatment (plants grown in 100% peat). In petunia, N content decreased in 30% OSW, K content increased in 30% OSW and Na content decreased in 10-30% OSW compared to plants grown in peat. Similarly to marigold, the add of PMW in growing media reduced petunia's N and Na content and increased K leaf content. Matthiola plants grown in 30% OSW had lower Na content but higher K content compared to control media. The effects of PMW in matthiola's leaf elemental content were similar to that of marigold and petunia. Considering the mineral content into growing media at the end of experiment, the plant mineral content and the physicochemical properties as described in Table 1, it is obvious that the reduction in plant growth and marketability is mainly related to the altered growing media properties and not to the growing media nutrient content.

**Table 4.** Leaf elemental analysis of marigold, petunia and matthiola plants grown in substrate medium consisting of olive-stone waste (OSW) and paper-mill waste (PMW) into commercial peat (P).

Mixtures	N (g/kg)	K (g/kg)	P (g/kg)	Na(g/kg)
<b>Marigold</b>				
P:OSW:PMW (100:0:0)	40.48 <sup>a,Y</sup>	28.42 <sup>b</sup>	5.35 <sup>a</sup>	0.98 <sup>a</sup>
P:OSW (90:10)	32.03 <sup>b</sup>	27.79 <sup>b</sup>	4.24 <sup>ab</sup>	0.86 <sup>ab</sup>
P:OSW (70:30)	31.33 <sup>b</sup>	36.36 <sup>a</sup>	4.95 <sup>a</sup>	0.84 <sup>ab</sup>
P:OSW:PMW (60:20:20)	25.96 <sup>c</sup>	33.85 <sup>a</sup>	3.74 <sup>b</sup>	0.76 <sup>b</sup>
<b>Petunia</b>				
P:OSW:PMW (100:0:0)	29.44 <sup>a,Y</sup>	29.45 <sup>b</sup>	4.01 <sup>a</sup>	8.65 <sup>a</sup>
P:OSW (90:10)	28.63 <sup>a</sup>	35.40 <sup>ab</sup>	4.35 <sup>a</sup>	6.70 <sup>b</sup>
P:OSW (70:30)	22.37 <sup>b</sup>	41.40 <sup>a</sup>	4.14 <sup>a</sup>	5.84 <sup>bc</sup>
P:OSW:PMW (60:20:20)	11.23 <sup>c</sup>	29.89 <sup>b</sup>	3.26 <sup>b</sup>	4.18 <sup>c</sup>
<b>Matthiola</b>				
P:OSW:PMW (100:0:0)	29.86 <sup>a,Y</sup>	26.53 <sup>b</sup>	2.51 <sup>a</sup>	7.37 <sup>a</sup>
P:OSW (90:10)	35.84 <sup>a</sup>	28.64 <sup>b</sup>	2.36 <sup>a</sup>	7.02 <sup>ab</sup>
P:OSW (70:30)	28.82 <sup>a</sup>	32.05 <sup>a</sup>	2.23 <sup>a</sup>	6.84 <sup>b</sup>
P:OSW:PMW (60:20:20)	12.16 <sup>b</sup>	32.02 <sup>a</sup>	1.46 <sup>b</sup>	6.07 <sup>c</sup>

<sup>Y</sup>Each value is means  $\pm$  SE (n=4). Values in rows followed by the same letter are not significantly different,  $P \leq 0.05$ .

A potting medium rarely contains a single ingredient, often being composed of two or more materials. Highly valuable materials such as soil, peat, sand, perlite and vermiculite are commonly used as substrates for container plant production [41]. Nevertheless, these materials might be fully or partially replaced with various organic waste products such as rice hulls, kenaf, pine bark, etc., thus achieving environmental benefits since ecosystem damage caused by soil, peat, perlite and vermiculite extraction is avoided and the impact of residue accumulations is minimized. There are also economic benefits, because the use of residues means lower costs (as reviewed by Tsakalimi [42]). Numerous studies have addressed the use of compost in nursery plant production, and have analyzed the chemical, physical and biological properties (as reviewed by Herrera et al. [43]), the increased plant growth and supplied soluble salts i.e. K [44]. However, raw waste material might have applications under specific cases.

The production of container-grown vegetables and ornamentals is a highly-competitive business; fast, uniform seedling emergence and rapid growth are essential for efficient production. Use of good crop substrates is therefore critical [45]. The current work indicating that up to 30% of OSW can substitute to peat for marigold and petunia and only up to 10% of OSW for matthiola, while the addition of PMW on top of OSW is not recommended, and further research is needed.

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