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SUSTAINABLE WASTE MANAGEMENT

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

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Abstract

Peat-based growing media are not ecologically sustainable and peat extraction threatens sensitive peatland ecosystem. In this study, olive-stone waste (OSW) and paper waste (PW) were used in different ratios—as growing media—for ornamental crop production, as peat (P) substitutes. Marigold (*Calendula officinalis* L.), petunia (*Petunia x hybrita* 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:PW (60%:20%:20%). The physicochemical properties of these substrates and the effects on plant growth were determined. The addition of 10–30% OSW into the substrate increased marigold height compared to plants grown in 100% peat. No differences in plant size, plant biomass (leaves and flowers), and dry matter content were found. Adding PW, in combination with OSW, maintained marigold 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 size and fresh weight, but not plant height. No differences were observed when plants grew in lower OSW (i.e., 10%) content. Petunia's height, its total number of flowers and flower earliness (flower opening) were increased in the presence of OSW compared to the plants grown in 100% peat. The addition of OSW did not affect petunia's size and fresh weight among treatments. The addition of PW suppressed several plant growth-related parameters for both matthiola and petunia. The insertion of OSW did not change leaf chlorophyll content whereas the presence of PW decreased chlorophylls for marigold, petunia, and matthiola. Both OSW and PW altered the content of total phenolics and antioxidant capacity of 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) in leaves and flowers for marigold and petunia. Both 30% OSW and PW increased antioxidative enzyme metabolism due to the increased damage index and lipid peroxidation observed in plants. Leaf N and P content decreased in PW-based media, while matthiola displayed visual phytotoxicity symptoms when PW was added into the substrate. The present work indicates that up to 30% of OSW can replace peat for marigold and petunia growing and only up to 10% of OSW for matthiola, while the addition of PW on top of OSW is not recommended, so further research is needed.

Keywords Olive-mill waste · Paper waste · Peat · Growth · Earliness · Ornamentals · Antioxidants

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Introduction

Peat extraction for horticulture represents 14–20% of peatlands (IPS 2008). Peat is organic media most extensively used as growing substrate for seedlings and ornamental potted plant production in nurseries (Schmilewski 2009). This is associated with the wide use of non-renewable natural resources and the disruption of very important peatland ecosystems. Peat removal results into reduction and loss of carbon sinks and increases greenhouse gas emission (Kern et al.

2017).Various constraints have been imposed on peat use due to its negative environmental impacts [disturbing susceptible ecosystems, possible source of carbon production and emission (Bullock et al. 2012)]. Therefore, peat has to some extent become a deficient and costly growing medium for commercial potting applications (Lazcano et al. 2009). Moreover, peatlands are under a protective scheme of the Directive 92/43/EC which is related to natural habitats and wildlife (Ceglie et al. 2015). To that direction, several authorities are attempting to limit the use of peat as a growing medium and soil quality enhancer while at the same time both local and national authorities encourage organic waste reuse as a substrate component (Moral et al. 2013). Lately, there has been great interest in exploring and using alternative high-quality and low-cost components as growing media for horticultural crops due to increasing needs and the rising cost of peat (Morales-Corts et al. 2014).

The extended waste disposal into agricultural land resulted in ecosystem imbalances and therefore there is a growing public concern about environmental and human health consequences (Xu et al. 2006). In recent years, a growing interest has been shown in the reutilization of organic by-products and waste composts for agricultural use and this is the primary issue for sustainable waste management (Baziramakenga et al. 2001). Wastes can be turned in a different direction from landfills and can be used as substrate materials for plant production in nurseries, field, and greenhouse crops, replacing or reducing dependency on peat moss (Bugbee 2002).

The olive tree, globally cultivated as a perennial crop, is widely present across the Mediterranean region contributing to 98% of the world production. Olive oil is produced in traditional and/or industrial olive mills and the harvesting period is only a few months long, usually from October to December (Paredes et al. 2005). Most of the traditional enterprises have been replaced by industrial olive mills. This has actually resulted into large quantities of solid and liquid wastes and by-products in areas of extensive olive cultivation. In olive oil-producing areas, ornamental crops normally thrive too, because of the Mediterranean climate type. The dominant organic solid waste derived from olive oil production is the extracted olive press cake (EOPC) which has been widely used as a fuel source (Manios 2004). The nature of olive oil cakes and by-products depends on the processing method. However, EOPC solid fuel can emit smoke during burning, thus polluting the air, and affecting environmental sustainability. Therefore, due to environmental constraints, the use of EOPC as a fuel source is limited by lowering the material price. Alburquerque et al. (2007) revealed that adding composted material of olive-mill (OW) by-product into soil enhanced its physicochemical properties. Ghosheh et al. (1999) indicated herbicidal activity of olive husks, Pardo et al. (2014) stated that olive-mill waste compost improved vegetative cover crops in a trace-element-contaminated soil while D'Addabbo et al. (1997) reported *Meloidogyne incognita* decrease in soil amended with residues of olive crops. Olive stones are rich materials in several compounds such as oil, proteins, sugars, fibers, and phenolic glucosides (Fernfindez-Bolafios et al. 1999).

Another type of solid waste that recently has been of concern is the paper waste. Noticeable amount of solid waste generated by the paper and pulp industry are deposited directly in landfills (Mendez et al. 2009). Composting paper mill sludges and paper waste could provide products of considerable value to the horticultural and the container-grown industries. In such markets, compost quality and consistency is a high demand, and often unaffordable due to high cost for horticulture enterprises. However, the financial returns (sales revenue) can also be much higher. Normally, every compost producer or soil improvement business initially aims at the high-end market. However, that horticultural market is very hard to penetrate and sustain (Tucker and Douglas 2006).

The wide use of peat as the main component into growing media for the production of seedlings and potting plants in nurseries is linked with the high cost of peat extraction from peatlands and its processing until it reaches the end users. Therefore, it is substantial to search for local available alternatives of peat which will be both of good quality and low cost. Several materials, particularly waste components, have been examined as alternatives for peat replacement, including compost derived from green wastes, mushrooms, and animal manures as well as coir, vermicompost, paper mill residuals, paper waste, pine bark, rock wool, sawdust, straw, wood chips, cocoa shell, loam, perlite and vermiculite, and reclaimed fiber board (Tucker and Douglas 2006; Marinou et al. 2013; Christoulaki et al. 2014). However, there is no single verified material to match all the desirable properties of peat for successive peat replacement. Interestingly, several of the above materials performed well or even displayed better performance than peat for specific applications (Morrish and Hofstede 2000).

Various possible alternatives have been determined and demonstrated to be very promising (Garcia-Gomez et al. 2002), such as waste from the olive oil industry (olive leaves and olive stone waste) (Papafotiou et al. 2004; Kelepesi and Tzortzakis 2009; Sofiadou and Tzortzakis 2012). However, it is quite often that farmers have applied olive mill solid wastes on horticultural crops, without using composting processes or having knowledge of the possible negative effects and/or limitations. This fact attracts the interest for experimental studies on raw olive-stone waste (OSW) material and their suitability in ornamental potting production. Studies on paper waste (PW) have revealed that the combination of composting and vermicomposting processes is a good approach for chicken manure/paper waste mixture management and it is suggested that paper waste could be a good feed for earthworms due to its relatively non-toxic and biodegradable nature (Ravindran and Mnkeni 2016). Indeed, a large amount of raw PW material still remains to be managed and the application in agriculture is a putative use. The present study sought to evaluate the effect of varying the proportion of olive-stone waste and/or paper waste mixed with conventional peat substrates, as growing media in the nursery production of ornamentals. Therefore, two issues to be examined are (a) the possible use of raw organic material (OSW, PW) as a growing medium and (b) the alleviation of any mineral deficiencies/toxicities through the decomposition process of raw organic material if the fertigation is done by a basic hydroponic nutrient solution for growing 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 the 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 was obtained from a local olive press factory in Cyprus, by "Koroneiki" olive cultivar. Common A4 paper sheets with ink were used and this paper waste was shredded into pieces of 0.5 × 3–4 cm (wide × length). Both OSW and PW were analyzed in relation to several physicochemical parameters (in dry weight: dwt) as described below, which were pH (pr *EN*13037) 5.67 and 6.31; EC (pr *EN* 13038) 0.283 and 1.175 mS cm^{-1} (1:5 *v*/*v*); organic matter (pr *EN* 13039) 99.29 and 75.52% dwt; organic carbon 57.59 and 43.81% dwt; N 0.18 and 0.37% dwt; ratio C/N 431.2 and 153.9; and extraction of calcium chloride/DTPA (CAT) soluble nutrients (pr *EN* 13651) for K 647.6 and 2428.5 μg/g; P 96.1 and 389.2 μg/g; Ca 62,283.4 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 limits, as described previously (Chong 2005). Additionally, several heavy metals were under remarkable concentration (Cd, Cr, Pb) or under lower limits (for example Ni < 3.4 μg/g) (Wuana and Okieimen 2011).

Experimental design

The experiment was carried out at the Cyprus University of Technology experimental farm, in an unheated plastic greenhouse with a North-South orientation, in Cyprus. Seedlings were produced in expanded clay cubes (3 × 3 × 2 cm) and were acquired from a local agriculture nursery, at the growth stage of two true leaves. Three growing media, peat (P), OSW and PW, and mixtures of these, were used to create four substrates which were as follows: (1) P (100%- control), (2) P:OSW (90%:10%), (3) P:OSW (70%:30%), and (4) P:OSW:PW (60%:20%:20%). Following mixtures, in each substrate medium 10% (*v*/*v*) of perlite was added for adequate substrate aeration and drainage. The OSW and PW ratios were chosen based on preliminary trials and/or previous reports, as higher OSW proportion (i.e., 50%) caused plant growth reduction and/or phytotoxicity (Kelepesi and Tzortzakis 2009; Sofiadou and Tzortzakis 2012). Plants grown in the above substrates were fertigated every second day (or according to plant needs), manually, with hydroponic nutrient solution (as described in Christoulaki et al. 2014) of EC 1.9 mS cm^{-1} and pH 6.0. The nutrient solution used (1:100 *v*/*v* in water) contained the following concentrations of nutrients: NO_3-*N* = 14.29, K = 10.23, PO_4-*P* = 0.97, Ca = 3.74, Mg = 2.88, SO_4-S = 1.56, and Na = 1.30 mmol l^{-1}, respectively; and B = 18.52, Fe = 71.56, Mn = 18.21, Cu = 4.72, Zn = 1.53, and Mo = 0.52 μmol l^{-1}, respectively. The experiment consisted of eight individual (replications) plants per treatment, for each of the examined species.

The 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. The pots were placed at a distance of 0.10 m from each other. The plants were grown under common cultivation practices, without any pruning or pesticide application.

Measurements

The physicochemical properties of substrate mixtures were analyzed. 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 by ashing in oven at 550 °C, and the organic C was calculated. The electrical conductivity (EC) and pH determined according to 1:5 (*v*/*v*) dilution method, employing a portable pH/EC-meter (HI 98130 HR, Hanna Instruments, USA). After acid digestion of the sample ash, analysis for macronutrients and micronutrients was employed using inductively coupled plasma atomic emission spectrometry (ICP-OES; PSFO 2.0 Leeman Labs INC., USA) while total N was determined according to Kjeldahl method.

Plant growth and tissue analysis

Marigold, petunia, and matthiola were grown for 1.5 month. Starting from the second week after transplanting, the impact of growing media on the number of total and open flowers produced in each pot was recorded weekly. Plant marketability based on appearance and possible purchase at a commercial level was observed by employing a 1–4 scale [1: non marketable quality (i.e., decolorization, toxicity, necrotic tissue); 2: medium quality (i.e., small size, decolorization); 3: good quality; 4: high quality]. Only data at the end of the experiment presented.

At the end of the experiment, plant height (cm), plant diameter-size (cm), total plant, and flower biomass (fresh weight in g and the % of dry matter content) were determined. Leaf elemental analysis took place on four replications/ treatment (each replication was a pool of two individual plants' leaf samples). Samples were dried at 65 °C for 4 days, 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. The ash was then digested with 10 mL hydrochloric acid (2 N HCl). The solution was placed on a heater at 80 °C in order to achieve faster and better dilution. The solution was filtered through Whatman No. 4 filter paper and diluted to 50 mL with deionized water. Mineral determination of K and Na (flame photometer; Lasany Model 1832, Lasany International, India), P (yellow method, spectrophotometric; Multiskan GO, Thermo Fischer Scientific, USA), and N (in leaf grinded sample) by the Kjeldahl method (BUCHI, Digest automat K-439 and Distillation Kjelflex K-360, Switzerland) was performed. Data was expressed in grams per kilogram 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 (Chrysargyris et al. 2016).

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 the 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) method. Total phenolic content was determined with the Folin-Ciocalteu method at 755 nm according to Tzortzakis et al. (2011) and results were expressed as milligrams of gallic acid equivalents (Scharlau, Spain) per gram of fresh weight (mg GAE/g Fwt). Radical scavenging activity was determined as described previously (Chrysargyris et al. 2016). In 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 milligrams trolox/gram of fresh weight. The antioxidant capacity using the ABTS method was carried out according to Wojdylo et al. (2007). The results were expressed as milligrams of trolox equivalents per gram of fresh weight (mg of trolox/g Fwt).

Damage index: determination of content of H_2O_2 and lipid peroxidation

The H_2O_2 content was determined from four samples (two individual plants were pooled/sample) for each treatment as described previously (Chrysargyris et al. 2017). Leaf tissue (0.2 g) was ground in ice cold 0.1% trichloroacetic acid (TCA) and centrifuged at 15,000*g* for 15 min. Reaction mixture consisted of 0.5 mL of the supernatant extract, 0.5 mL of 10 mM potassium-phosphate buffer (pH = 7.0), and 1 mL of 1 M potassium iodide. The H_2O_2 concentration was determined using standards of 5 to 1000 μM of H_2O_2 and calibration curve plotted accordingly. The absorbance of standards and samples was measured at 390 nm and results were expressed as micromoles H_2O_2/gram fresh weight.

Lipid peroxidation was assessed according to Azevedo-Neto et al. (2006) and measured in terms of malondialdeyde content (MDA). Leaf tissue (0.2 g) was homogenized in 0.1% TCA and the extract was centrifuged at 15,000*g* for 10 min. Aliquot of 0.5 mL extract was mixed with 1.5 mL of 0.5% thioarbituric acid (TBA) in 20% TCA, and the reaction mixture was incubated at 95 °C for 25 min and then cooled on ice bath. The absorbance was determined at 532 nm and corrected for non-specific absorbance at 600 nm. MDA amount was measured using the extinction coefficient of 155 mM/cm. Results were expressed as nanomoles of MDA/gram fresh weight.

Activities of antioxidant enzymes

Fresh leaf tissue was homogenized in a chilled mortar using ice cold extraction buffer containing 1 mM ethylenediaminetetraacetic acid (EDTA), 1% (*w*/*v*) polyvinylpyrrolidone (PVPP), 1 mM phenylmethylsulfonyl fluoride (PMSF), and 0.05% Triton X-100 in 50 mM potassium-phosphate buffer (pH = 7.0) (Chrysargyris et al. 2017). The content of protein in the enzyme extracts was measured according to Bradford assay (1976) using bovine serum albumin (BSA) as a standard.

Catalase activity (CAT) (EC 1.11.1.6) and superoxide dismutase activity (SOD) (EC 1.15.1.1) were assayed

(Chrysargyris et al. 2017). Catalase activity was assayed in a reaction mixture (1.5 mL) containing 50 mM K-phosphate buffer (pH = 7.0), 10 mM H_2O_2 and an enzyme aliquot. The decomposition of H_2O_2 was followed at 240 nm. The results were expressed as CAT units/mg of protein (1 unit = 1 mM of H_2O_2 reduction/min). SOD was assayed using the photochemical method. Reaction mixture (1.5 mL) containing 50 mM K-phosphate buffer (pH = 7.5), 13 mM methionine, 75 μM nitro blue tetrazolium (NBT), 0.1 mM EDTA, 2 μM riboflavin, and an enzyme aliquot. Reaction started with the addition of riboflavin and placing tubes with the reaction mixture below a light source of two 15-W fluorescent lamps for 15 min. Reaction stopped by placing the tubes in the dark. Mixtures without enzyme extract developed maximal color (control) and non-irradiated mixture used as blank. The absorbance was determined at 560 nm and activity was expressed as units/mg of protein. One unit of SOD activity was defined as the amount of enzyme required to cause 50% inhibition of the NBT photoreduction rate. Peroxidase activity (POD) (EC 1.11.1.6) was determined as described by Tarchoune et al. (2012) following the increase in absorbance at 430 nm. Calculations were performed using the coefficient of extinction of 2.47 mM/cm. One POD unit was defined as the amount of enzyme to decompose 1 μmol of H_2O_2 per minute. Results were expressed as units of peroxidase/mg of protein.

Statistical analysis

Data was tested for normality and was subsequently 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. Statistical analyses were performed using SPSS (SPSS Inc., Chicago, Ill.).

Results and discussion

The effects of OSW and/or PW on substrate physicochemical properties are presented in Table 1. The replacement of peat (up to 30%) with OSW had increased the bulk density of the substrate. It also decreased the total porosity and the air-filled porosity of the media (Table 1) being in agreement with previous reports when OSW compost was used for foliage potted plants (Papafotiou et al. 2005). Additionally, Papafotiou et al. (2005) reported that adding OSW in high levels (i.e., 50–75%) not only reduced the substrate's total porosity but also reduced the pores size (empty spaces were filled up with OSW particles) and as a consequence, it decreased the substrate available water holding capacity (held less water) compared to the control (i.e., 100% peat). In our study, the low ratio of OSW (i.e., 10–30%) increased the pore size, due to the increased particle

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

	P:OSW:PW (100:0:0)	P:OSW (90:10)	P:OSW (70:30)	P:OSW:PW (60:20:20)
Organic matter (%)	99.41	90.35	92.95	92.13
Organic C (%)	57.58	52.41	53.92	53.44
pH	4.63	4.75	5.06	5.11
EC (mS/cm)	0.169	0.155	0.179	0.237
C/N	105.8	123.9	235.7	231.4
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)	0.17 (0.19)	0.38 (0.43)	0.21 (0.40)
K (g/L)	0.32 (1.58)	1.58 (1.51)	2.43 (2.66)	1.56 (1.84)
Ca (g/L)	3.22 (10.91)	1.48 (5.12)	3.19 (7.15)	11.48 (11.38)
Na (mg/L)	0.31 (2.42)	0.42 (1.39)	0.98 (2.07)	0.58 (1.69)
Mg (mg/L)	0.99 (2.73)	0.55 (1.49)	2.80 (4.02)	0.49 (2.11)
Fe (mg/L)	612.13 (701.89)	189.06 (328.41)	809.28 (2014.09)	280.14 (462.22)
Mn (mg/L)	16.11 (20.16)	10.59 (12.60)	18.72 (32.40)	12.51 (21.23)
Zn (mg/L)	16.61 (18.21)	24.72 (14.71)	27.95 (46.44)	10.48 (13.47)
Cu (mg/L)	141.84 (141.42)	111.28 (430.74)	442.82 (722.17)	44.33 (48.13)
B (mg/L)	n.d. (112.94)	n.d. (111.01)	n.d. (51.48)	n.d. (118.35)
TPS (%)	89.43	65.74	67.81	67.41
AFP (%)	28.51	11.90	8.57	9.52
AWHC (%)	59.84	53.84	59.24	57.89
BD (g/cm^3)	0.205	0.266	0.388	0.344

TPS total pore space, *AFP* air-filled porosity, *AWHC* available water holding capacity, *BD* bulk density by volume, *n.d.* not detectable

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

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

^{Y} Values ($n = 8$) in columns followed by the same small letter are not significantly different, $P \leq 0.05$

size (data not presented). Organic matter content was high in all the examined growing media, ranging from 92.13% in P:OSW:PW (60:20:20) to 99.41% in P (100%), indicating fertile growing media. The improved organic material into the growing media is of considerable nutrient value, as mixtures (20–50%) of compost derived from municipal solid wastes with perlite may be used as substrate without the need for additional mineral fertilizer (Castillo et al. 2004).

Olive waste has also high concentration of Ca, Fe, K, Mg, N, P, and Fe that can affect the fertility of the growing media (Ouzounidou et al. 2008). Several studies reported that the increase in the pH of the medium, as it has been found in lightweight potting mix (consisted of sphagnum peat, vermiculite, perlite, composted bark, and sand), is related to the decrease in the availability of P, Fe, Mn, and B (Papafotiou et al. 2001, 2004). All the examined growing media had slightly acidified pH. This supports the availability of minerals as P, Fe, Mn, and B into the substrate. However, any possible deficiency in leaves and flowers could not be stated in the present study, as mineral content of Fe, Mn, and B were not analyzed. At the end of the experiment, mineral concentration in growing media was of sufficient content, without any deficiencies or accumulation/toxicities on the plant tissue as observed macroscopically (Table 1). This could be related to the well-balanced fertigation through the complete nutrient solution application (both macronutrients and micronutrients were applied) during the experiment. Therefore, any putative nutrient imbalances derived by the mineral release through the substrate medium could be weakened by the appropriate (balanced) hydroponic solution. There are, however, certain limitations concerning the use of some organic material and composts due to their fertile status: increased salt content to

Table 3 Impact of olive-stone waste (OSW) and paper waste (PW) 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
Marigold	P:OSW:PW (100:0:0)	1.01^{a,Y}	0.29^{a}	1.31^{a}	3.98^{a}
	P:OSW (90:10)	0.94^{ab}	0.29^{a}	1.24^{ab}	4.00^{a}
	P:OSW (70:30)	0.92^{ab}	0.25^{a}	1.17^{ab}	3.81^{a}
	P:OSW:PW (60:20:20)	0.77^{b}	0.21^{a}	0.98^{b}	2.92^{b}
Petunia	P:OSW:PW (100:0:0)	0.72^{a,Y}	0.28^{a}	1.01^{a}	3.52^{b}
	P:OSW (90:10)	0.73^{a}	0.29^{a}	1.02^{a}	4.00^{a}
	P:OSW (70:30)	0.61^{a}	0.25^{a}	0.87^{a}	3.90^{a}
	P:OSW:PW (60:20:20)	0.52^{a}	0.23^{a}	0.75^{a}	2.78^{c}
Matthiola	P:OSW:PW (100:0:0)	0.54^{a,Y}	0.16^{a}	0.71^{a}	3.45^{a}
	P:OSW (90:10)	0.49^{a}	0.14^{a}	0.63^{a}	3.03^{ab}
	P:OSW (70:30)	0.65^{a}	0.19^{a}	0.84^{a}	1.85^{b}
	P:OSW:PW (60:20:20)	0.21^{b}	0.06^{b}	0.28^{b}	1.09^{c}

^{Y} Values ($n = 4$) in columns followed by the same small letter are not significantly different, $P \leq 0.05$

levels which might affect the growth of sensitive crops, heavy metal toxicity, low overall porosity, and a marked variation in physical/chemical properties (Spiers and Fietje 2000).

In marigold, low content of OSW (10%) increased plant height compared to the control plants (grown in 100% peat) and plants grown in P:OSW:PW (60:20:20) (Table 2). Moreover, marigold grown in 10% OSW produced the highest number of flowers. The presence of PW into the substrate reduced marigold biomass as a consequence of reduced leaf and flower fresh weight. However, marigold's total plant diameter (indicating plant size), flower's opening, and plant dry matter content were unaffected by the addition of OSW and/or PW into the substrate. In petunia, the PW addition affected plant growth parameters by causing reduction in plant height and diameter as well as in plant biomass (as a result of leaf weight reduction rather than of the flower's weight) (Table 2). Interestingly, the 10% OSW either improved (plant height, flower opening—indicating flower earliness) or maintained plant growth/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 petunias grown in 10–30% OSW compared to the control substrate. Both marigold and matthiola grown in 10–30% OSW maintained their foliage biomass at similar levels with the control, being in agreement with studies in *Codiaeum variegatum* and *Syngonium podophyllum* foliage potted plants

Fig. 1 Impact of olive-stone waste (OSW) and paper waste (PW) into commercial peat (P) on marigold, petunia, and matthiola during plant growth in greenhouse/nursery

(Papafotiou et al. 2005). Similarly, in matthiola, the PW addition 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 (Papafotiou et al. 2004). 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 that the effect of growing media on flowering time is highly depended on compost type and its concentration in the growing medium, and on the plant species (Papafotiou et al. 2001, 2004). In the present study, the observed reduction after the PW addition is not related to different plant nutrition, as Na content remained at non phytotoxic levels, similar to the levels of 100% peat. As a consequence, the observed plant growth reduction was due to the unsuitable substrate's physicochemical properties, such as total pore space and air-filled porosity. To this sense, efforts for the improvement of the substrate properties should be considered by either increasing inert material percentage (i.e., 15–20% of perlite) or adding a mixture of inert materials (perlite, sand, vermiculate, etc.). It is well known that plant can grow successfully in pure inorganic material (perlite, sand, pumice, etc.) when a complete in mineral nutrient solution is used. Therefore, since in our study the fertigation takes place through a hydroponic nutrient solution, the inert material could be as high as needed for growing medium property improvement.

Plant marketability was reduced in the presence of PW content (20% PW) for all the examined ornamentals (marigold, petunia, and matthiola). The same effect was noticed for matthiola after the application of 30% of OSW (Table 3). The decrease in marigold's and petunia's marketability was noticed at the end of the 4th week of plant growth (Fig. 1) whereas, in the case of matthiola, the first symptoms of reduced marketability/quality were evident even from the 2nd week (Fig. 1), indicating matthiola as a more sensitive species to the examined growing media compared to marigold or petunia. This is probably related to the growing media readily available water and air for different plants as well as to 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 (Greive et al. 2008) to cause a delay in flowering. Ornamental marketability is related to the appearance (considering plant vigorous, any discoloration, any marks/symptoms, any mineral deficiencies) as this is the main criterion that consumers take into consideration when buying ornamentals—neither the plant size nor the biomass produced. For instance, considering the relevant high scores (2.78; close to 3 which means good quality, according to the scale used) for marketability for petunia plants in P:OSW:PW (60:20:20), this is not reflected in the petunia's 68% biomass reduction.

The addition of OSW did not change leaf chlorophylls content whereas the presence of PW decreased chlorophylls for marigold, petunia, and matthiola (Table 3). The chlorophyll level is an index of the photosynthesis and any decrease in chlorophyll level leads to reduction in growth parameters (Kiarostami et al. 2010). This is more or less the case in growth parameters for the examined species, as there is a decrease in plant growth in the presence of PW. Total phenolics and antioxidant capacity (DPPH, ABTS) altered differently in leaves and flowers for the examined ornamental species (Figs. 2, 3, and 4). More specifically in marigold flowers, the

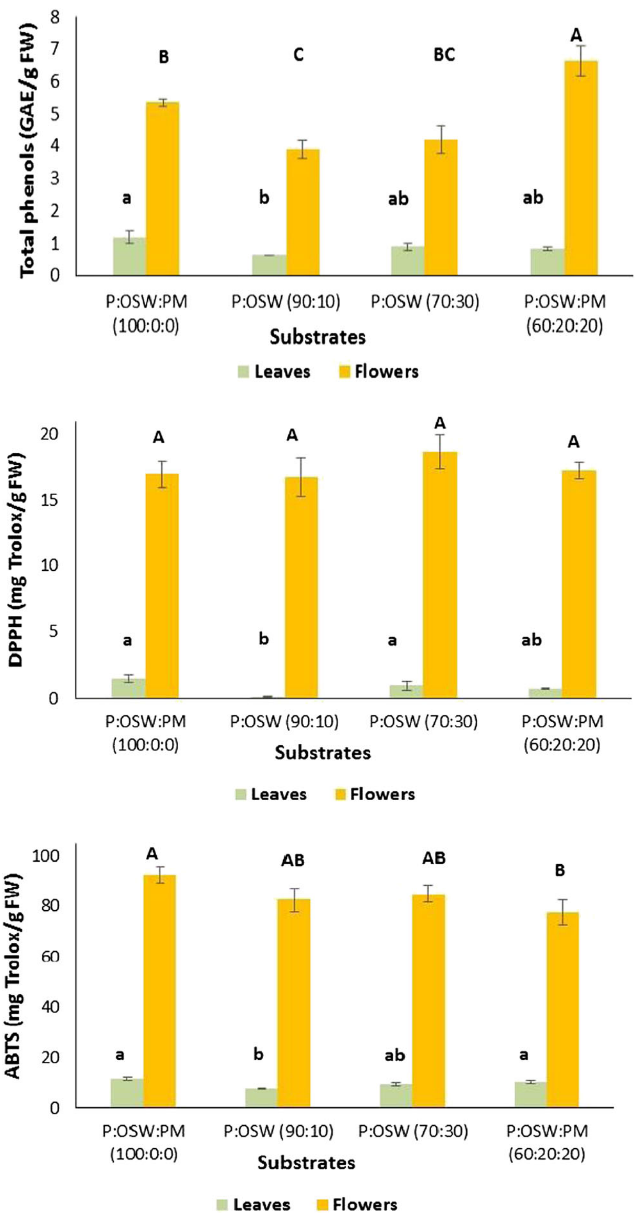

Fig. 2 Impact of olive-stone waste (OSW) and paper waste (PW) into commercial peat (P) on marigold content of total phenols and antioxidant activity (DPPH, ABTS) on ornamental plants grown in greenhouse/nursery. Mean ($n = 4$) values (± SE) followed by the same small or capital letter are not significantly different, $P \leq 0.05$

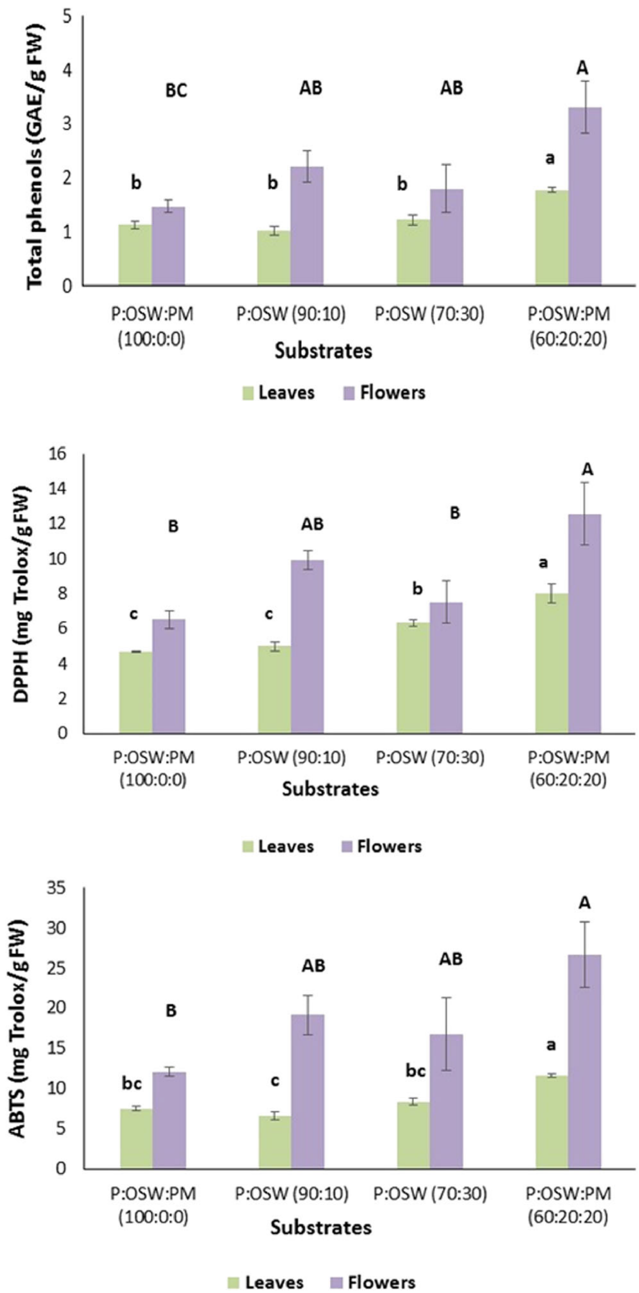

Fig. 3 Impact of olive-stone waste (OSW) and paper waste (PW) into commercial peat (P) on petunia content of total phenols and antioxidant activity (DPPH, ABTS) on ornamental plants grown in greenhouse/nursery. Mean ($n = 4$) values (± SE) followed by the same small or capital letter are not significantly different, $P \leq 0.05$

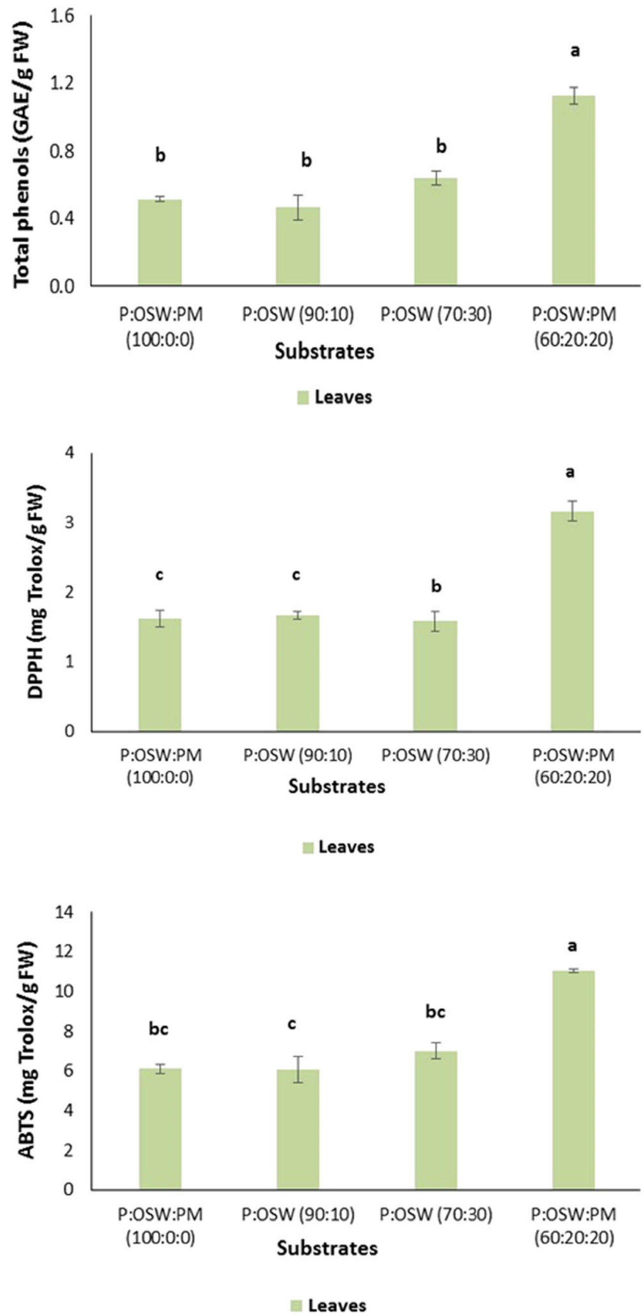

Fig. 4 Impact of olive-stone waste (OSW) and paper waste (PW) into commercial peat (P) on matthiola content of total phenols and antioxidant activity (DPPH, ABTS) on ornamental plants grown in greenhouse/nursery. Mean ($n = 4$) values (± SE) followed by the same small letter are not significantly different,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

phenol content was increased and the ABTS antioxidant activity was decreased in plants grown in the presence of paper waste (P:OSW:PW 60:20:20). However, the addition of 10% OSW into the substrate kept the total phenolic content and antioxidant activity of marigold leaves at lower levels, indicating lower or unmarkable stress conditions. The same effects were evident in petunia's flower antioxidant capacity, as paper waste increased the phenol content and antioxidant activities in both leaves and flowers. The phenolic content and antioxidant activity in matthiola leaves were also increased due to the increase of PW content into the substrate (Fig. 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, including marigold and petunia. Plant material of high phenolic content have developed active oxygen scavenging systems and can protect tissue oxidation by scavenging free radicals

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

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

[Y] Each value is means ± SE ($n = 4$). Values in rows followed by the same letter are not significantly different, $P \leq 0.05$

and inhibiting lipid peroxidation (Scherer et al. 2013). This can attract consumers' interest in edible flower consumption. However, in the present study, the edibility of flowers was not studied and further research to that direction is necessary.

As it has been widely reported, both biotic and abiotic stresses lead to the overproduction of reactive oxygen species (ROS) that cause oxidative damage. MDA is regarded as a marker for evaluation of lipid peroxidation that increases with environmental (abiotic) and biotic stresses. In the presence of oxidative stress, more lipid peroxidation products are formed due to cell damage. The increase in lipid peroxidation may be due to the incapability of antioxidants to scavenge ROS and this is related to the stress derived by the growing media and their physicochemical properties. This was noticed in the present study as well, where in all three plant species, the addition of both OSW and PW in the same substrate caused increment in MDA levels, indicating a stress situation, as cellular damage (Fig. 5). This fact is supported not only by the overproduction of H_2O_2 in plants grown at the same substrate, but also from the high activity of the antioxidant enzymes. Plants scavenging ROS capacity is directly related with the activity/content of the antioxidant enzymes (SOD, ascorbate peroxidase-APX, glutathione reductase-GR and CAT) which increase under stress conditions (Foyer and Noctor 2011; Chrysargyris et al. 2017), meaning that plants protect themselves against oxidative stress by activating such enzymes. Therefore, SOD provides the first line of defense against the toxic effects of elevated levels of ROS. The SODs convert $O_2^{\cdot-}$ to H_2O_2 while H_2O_2 is a strong nucleophilic oxidizing agent and the oxidation of SH-group is one major mode of its toxicity. The H_2O_2 produced is then scavenged by catalase and a variety of peroxidases (Tarchoune et al. 2010). Catalase dismutates H_2O_2 into water and molecular oxygen, whereas peroxidase (POX) decomposes H_2O_2 by oxidation of co-substrates such as phenolic compounds and/or antioxidants. In plant cells, the ascorbate/glutathione (ASH-GSH) cycle represents an alternative and more effective detoxification mechanism against H_2O_2 operating both in the chloroplasts and the cytosol (Sgherri and Navari-Izzo 1995). It may remove H_2O_2 in a series of enzymatic reactions involving APX and GR (Gill and Tuteja 2010; Ahmad et al. 2010). The net result is that two potentially harmful species, superoxide and hydrogen peroxide, are converted to water. For the three species tested in this study, using up to 30% of OSW in the potting medium, plants' enzyme activity levels did not change compared to the control (Fig. 5). The use of OSW up to 30% caused no additional physiological stress to plants tested. However, induced stress is noticed only when the PW was added in the growing media.

Mineral content fluctuated among plant species, while matthiola had visual phytotoxicity symptoms as they were observed macroscopically (Fig. 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 addition of PW into the growing media 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, adding PW 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 PW 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 the experiment, the plant mineral content and the physicochemical properties, as they are described in Table 1, it is

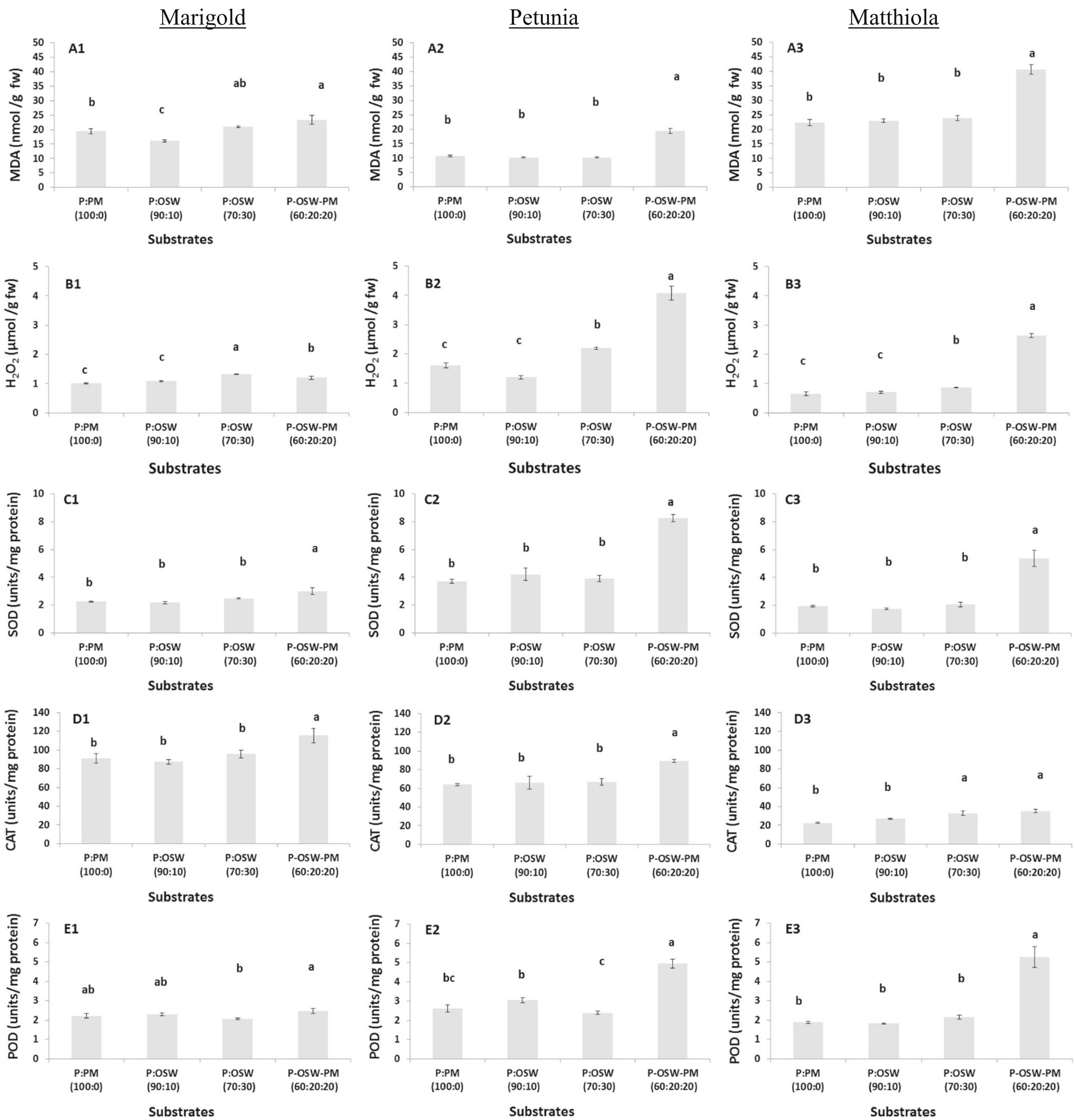

Fig. 5 Impact of olive-stone waste (OSW) and paper-mill waste (PMW) into commercial peat (P) on the plant damage index measured by hydrogen peroxide (H_2O_2) production, lipid peroxidation (MDA), and antioxidant enzyme activities measured by superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD) on marigold (A1, B1, C1, D1, E1), petunia (A2, B2, C2, D2, E2), and matthiola (A3, B3, C3, D3, E3), respectively, during plant growth in greenhouse/nursery. Mean ($n = 4$) values (± SE) followed by the same small letter are not significantly different, $P \leq 0.05$

obvious that the reduction in plant growth and marketability is mainly related to the altered growing medium properties and not to the growing medium nutrient content.

Growing media used for potting plants are often a mixture of two or more materials and rather rarely consist of a single component. Common potting media consist of soil, peat, and sand (while sand can be replaced by perlite or vermiculite) in different ratio for container plant production (Landis et al. 1990). However, the abovementioned materials might be fully or partly replaced with several organic waste products, such as rice hulls, kenaf, and pine bark. This replacement will compensate for certain environmental issues since ecosystem damage caused by soil, peat, perlite, and vermiculite extraction is prevented and the impact of waste accumulation is minimized. Furthermore, such a replacement will be profitable since waste usage means lower cost and more effective waste management (as reviewed by Tsakaldimi 2006). Various studies have reported the use and benefits of compost application in nursery plant production and have analyzed the chemical, physical, and biological properties (as reviewed by Herrera et al. 2008), the increased plant growth, and supplied soluble salts, i.e., K (Walker and Bernal 2008). However, raw waste material might have applications under specific cases and to that direction the present study took place.

The production of container-grown vegetables and ornamentals is a highly competitive business; fast, consistent seedling emergence and rapid growth are vital for profitable production. The use of good crop growing media is therefore critical. The selection of alternative materials to peat for more sustainable growing media is therefore of crucial importance. Alternative materials ought to have appropriate physical structure and create a suitable biological and chemical environment for the plant. In conclusion, the current work indicates that up to 30% of OSW can be used as a substitute for peat for marigold and petunia and only up to 10% of OSW for matthiola, while the addition of PW on top of OSW is not recommended, so further research for PW is needed.

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