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Valorization of *Eucalyptus globulus* bark as a growing-media component for potted plants

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Abstract *Eucalyptus globulus* bark is a waste from pulp and paper industries. This work aims to use *E. globulus* bark as raw-material for substrates formulation. Three types of bark were used: fresh bark (FB) milled to 6 mm particle size, and two hydrothermally treated barks (HTB1: 20’ 60°C; HTB2: 40’ 100°C). Barks were mixed at 25 and 50% (v v^{-1}) (S25; S50) with peat. FB was phytotoxic, causing low germination (91%) and root growth inhibition (0.1 cm length) of *Lepidum sativum* seeds. HTB1 and HTB2 reduced significantly toxicity with germination rates of 98 and 100%, and root lengths of 5.1 and 5.2 cm, respectively. Potting test, using Chinese cabbage, revealed lower germination (95%) in FB mixtures than in HTB1, HTB2 and commercial substrate (CS) (98-100%), reinforcing the FB phytotoxic. S50 decreased plant growth, probably related with lower water retention, as well as nitrogen immobilization inherent to woody substrates. S25 showed shoot weight, and roots growth statistically equal or higher than CS, encouraging use of this proportion of hydrothermally treated bark in substrate formulation.

Keywords: *Eucalyptus globulus*, bark, industrial waste, hydrothermal treatment, substrate.

1. Introduction

In Southern European countries, *Eucalyptus globulus* is the predominant species for pulp and paper production (Domingues *et al*., 2010). Currently, one fifth of the total bleached pulp, apart from other biomass residues, remains bark excess (Domingues *et al*., 2010; Neiva *et al*., 2016). In 2015, Portugal produced around 500 000 tons of *E. globulus* bark (CELPA, 2015). As industrial by-product, it is mostly burned for energy production (Gruda *et al*., 2013; Neiva *et al*., 2016), which remains low added value product application. The increased environmental awareness of peatlands conservation has established intensive research aiming new peat alternative materials. Organic materials derived from agricultural and municipal waste streams, as well industrial by-products have become common (Cunha-Queda *et al*., 2006; Gruda *et al*., 2013; Barrett *et al*., 2016). Wood-based fibers from dedicated forest plantations (Gruda *et al*., 2013; Barrett *et al*., 2016) have been studied as growing-media component to optimize their physical properties (Caron *et al*., 2010; Barrett *et al*., 2016). With very high air-filled porosity and low bulk density, wood fiber addition is used to improve aeration and reduce shrinkage of peat-based substrates (Buamscha *et al*., 2008; Gruda *et al*., 2009). Despite the good performances, phytotoxicity is a common issue of woody biomass caused by natural chemical barriers (presence of phenolic compounds, terpenes, acetic acid, etc.) (Domingues *et al*., 2013; Neiva *et al*., 2016). The role of these chemical compounds has a protection effect against diseases or infections of native wood, although they also act as toxins for other cultivations in growing media applications (Gruda *et al*., 2013). Secondary process treatments are required to eliminate toxic compounds and promote stability before wood material effective use. They are broadly depending on raw-material. Cunha-Queda *et al*. (2006) and Jackson *et al*. (2010) proposed composting bark to remove phytotoxycity and lower media electrical conductivity. Gruda *et al*. (2009) reported improvements in germination rate and radicle growth after washing/leaching pine tree substrate. Buamscha *et al*. (2008) studied the differences in growth and nitrogen availability between fresh and aged *Pseudotsuga menziesii* bark and found out that plants were smaller in fresh bark with a greater N immobilization rate than in aged. From techno-economical point of view, the more material transformations required the higher associated cost (Barrett *et al*., 2016). Hydrothermal treatment is attractive due to its simplicity, rapid implementation without causing significant material corrosion, using water as main reagent, and low construction material cost requirement (Neiva *et al*., 2016). The purpose of this work was to study the possibility of using *E. globulus* bark as an organic component in growing-media application. Therefore, hydrothermal treatments were performed aiming to remove phytotoxins from raw-material that are known to have an inhibitory effect on plants performance.

2. Materials and Methods

Fresh *E. globulus* bark (FB) was collected from the Navigator Company pulp mill (Setúbal, Portugal) in November 2015, air dried and grinded in a hammer mill (Ø = 6mm).

Based on a preliminary test (data not shown) two hydrothermal treatments of barks were used (HTB1: 60 °C for 20’; HTB2: 100 °C for 40’). The HTB1 and HTB2 were performed in autoclave reactor/conditions placing 5 individual hermetic vessels (1 L) containing 90 g (10% moister content) of bark and 900 ml water each. Excess water of treated material was removed by centrifugation. All experiments were performed in randomized order to minimize uncontrolled factors.

Barks (FB, HTB1 and HTB2) were mixed with peat moss slightly decomposed (H2-H5 on Von post scale) amended with 8 g L^{-1} of limestone, in volumetric proportion of 25 and 50% (bark/peat) (S520; S50). All substrates mixes were fertilized (15 mmol NO_3^- L^{-1}, 8 mmol K L^{-1}, 4 mmol Ca L^{-1}, 1,5 mmol Mg L^{-1}, 1,25 mmol SO_4^{2-} L^{-1}, 1,5 mmol $H_2PO_4^-$ L^{-1}, 15 µmol Fe L^{-1}, 8 µmol Mn L^{-1}, 4 µmol Zn L^{-1}, 25 µmol B L^{-1}, 0,75 µmol Cu L^{-1}, 0,5 µmol Mo L^{-1}), and tested in a pot trial, using a peat-based commercial substrate (CS) as control.

Electrical conductivity (EC), pH, and water-soluble N, P, K, Ca, Mg and Na of barks and substrates were measured in the water extract 1:5 by volume, according to the European Norms EN 13037, EN 13038 and EN 13652. The incubation experiment (14 days) for N immobilization and respiration rates (NIR and RR) measurements was adapted from Buamscha *et al*. (2008). The following physical properties were determined according to Verdonck and Gabriëls (1992): total porosity (TP), bulk density (BD), easily available water between 10 and 50 cm of water column (EAW), water-buffering capacity between 50 and 100 cm of water column (WBC), air-filled porosity at 10 cm of water column (AFP_{10}) and shrinkage.

Barks and substrates germination rate (GR), root length per plant (RLP), and Munoo-Liisa vitality index (MLVI) were evaluated in a petri dish test, according to the European Norm EN 18086-2, using cress (*Lepidum sativum*) as test plant.

Chinese cabbage (*Brassica napa* ssp. *pekinensis*) seeds (10 seeds/pot) were sown in 290 mL pots (diameter: 8.5 cm, height: 8.2 cm) filled with the substrates, and grown for 5 weeks (May and June) in an unheated glass greenhouse (ISA-ULisboa Campus, Lisbon, Portugal, 38°42'29.1"N 9°11'06.6"W). Pots were daily irrigated, by weight, to the container capacity. Germination rate (GR) after 5 days, and fresh weigh (FW), dry weight (DW) after dried samples at 65 ° C for 48h, and root visual rating (1=worst; 5=best) at the end of the growing period, were evaluated.

Data were subjected to ANOVA analysis, followed by the LSD test (p = 0.05) using RStudio software.

3. Results and discussion

3.1. Eucalyptus globulus bark properties

Cress seeds test revealed that FB is phytotoxic (table 1), causing low germination rate (GR) and root growth inhibition with only 0.1 mm of root length per plant (RLP). The hydrothermal treatment of barks reduced significantly bark toxicity, with GR of 98.3 and 100%, and RLP of 5.1 and 5.2 cm, in HTB1 and HTB2, respectively, showing that toxic compounds were removed and both treatments were effective. Gruda *et al*. (2009) also recorded reduction of toxins levels, associated with decline of resin acids, fatty acids and phenols content, after pine tree substrates aqueous washing. Domingues *et al*. (2010) and Neiva *et al*. (2016) demonstrated that bark tissues of *E. globulus* trees are rich in extractable phenolic, triterpenic and other inhibitory compounds. Thus, like strongly recommended for other wood-based fibers (Buamscha *et al*., 2008; Gruda *et al*., 2009; Caron *et al*., 2010; Jackson *et al*., 2010; Gruda *et al*., 2013), *E. globulus* barks must be treated before use as growing-media.

All barks were biologically unstable in contrast with peat that showed a very low microbial activity (table1). After 14 days incubation, higher N immobilization (NIR) and respiration rates (RR) were measured in FB and HTB2, followed by HTB1. Although treatments removed part of organic material, maximum NIR in HTB2 (0.9 mmol N L^{-1} d^{-1}) might be explained by fiber structural fragmentation, associated to higher treatment temperatures, that increased cellulose digestibility (Neiva *et al*., 2016) and sturdily promoted microorganism activity (Depardieu *et al*., 2016).

Gruda *et al*. (2013) stated that extremely high amount of nitrogen consumption by microorganism in wood fiber materials can be adjusted by additional N fertilization. According to Buamscha *et al*. (2008), biological activity promotes simultaneous release of CO_2 and proportional capacity to immobilize N, and if there is a microbial need for N, it may occur soon after potting and therefore N fertilization should be applied before seeding/planting.

Low cress root growth, RLP of 0.4 cm, in peat may be a consequence of the very low pH of this material (table 1). Indeed, in a later test using the same peat amended with limestone reveled a pH of 5.75, a GR of 100% and a RPL of 6.1 cm.

Acidic pH of 4.0 was verified in peat (before liming), followed by FB with 4.9 and treatments significantly increased barks pH (table 1). No mineral nitrogen was detected in barks neither in peat (levels below the quantification limit of 5 mg L^{-1}) and FB had higher levels of water-soluble P, K, Ca, Mg and Na. Indeed, the use of demineralized water in the hydrothermal treatments leached the soluble elements, decreasing their concentration in HTB1 and HTB2 and, consequently, reducing the EC. However, all materials had low EC values and low levels of available nutrients, with the exception of K and Mg. It is noticeable that peat, HTB1 and HTB2 showed similar chemical composition regarding water-soluble nutrients.

Air-water relationships are present in table 2. The hydrothermal treatments did not affect the bark physical properties, with all barks presenting low bulk density (BD) and water availability and very high total porosity (TP) and aeration (AFP_{10}) greater than 80% (v v^{-1}). On the contrary, peat had low AFP_{10} (8.3%) and high water availability. Gruda *et al*. (2013) pointed wood fibers relative lightweight and very high air capacity as advantages for good drainability and aeration improvement in peat-based substrate. Similar shrinkage was observed in all barks and due to low value, it may reduce the shrinkage of peat mix in the pot.

Table 1. Eucalyptus barks and peat germination rate (GR), root length per plant (RLP), nitrogen immobilization rate (NIR), respiration rate (RR), pH, electrical conductivity (EC), and water-soluble nutrients (water extract 1:5 by volume): mineral nitrogen (N_{min}), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg) and sodium (Na).

Raw-material	GR	RPL	NIR	RR	pH	CE	N_{min}	P	K	Ca	Mg	Na
	%	cm	mmol N L^{-1} d^{-1}	mmol CO_2 L^{-1} d^{-1}		mS m^{-1}	mg L^{-1}					
FB	91 *b*	0.1 *b*	0.80 *a*	15.4 *a*	4.9 *c*	22 *a*	*nd**	8	167 *a*	9 *a*	22 *a*	38 *a*
HTB1	98 *a*	5.1 *a*	0.63 *b*	14.1 *a*	5.8 *a*	6 *b*	*nd**	*nd**	32 *b*	3 *a*	5 *b*	3 *b*
HTB2	100 *a*	5.2 *a*	0.90 *a*	15.7 *a*	5.5 *b*	6 *b*	*nd**	*nd**	27 *b*	3 *a*	4 *b*	3 *b*
Peat	98 *a*	0.4 *b*	0.00 *c*	1.3 *b*	4.0 *d*	5 *b*	*nd**	*nd**	6 *b*	3 *a*	1 *c*	3 *b*

Means followed by the same letter within the same column do not differ significantly at p = 0.05 by LSD-test

* *nd* = not detected, below the quantification limit (5 mg L^{-1})

Table 2. Eucalyptus barks and peat physical properties: bulk density (BD), total porosity (TP), air-filled porosity at 10 cm of water column (AFP_{10}), easy available water (EAW), water buffering capacity (WBC), available water (AW) and shrinkage.

Raw-material	BD	TP	AFP_{10}	EAW	WBC	AW	Shrinkage
	g L^{-1}	% (v v^{-1})					
FB	58.0 *c*	96.3 *a*	80.1 *a*	3.0 *b*	0.0 *b*	3.0 *b*	8.2 *b*
HTB1	64.7 *b*	95.9 *a*	80.7 *a*	2.1 *b*	0.1 *b*	2.2 *b*	11.2 *b*
HTB2	52.3 *d*	96.7 *a*	83.4 *a*	1.8 *b*	0.1 *b*	1.9 *b*	8.1 *b*
Peat	119.8 *a*	92.4 *b*	8.3 *b*	32.3 *a*	8.0 *a*	40.3 *a*	34.9 *a*

Means followed by the same letter within the same column do not differ significantly at p = 0.05 by LSD-test

3.2. Eucalyptus bark-based substrates properties

Table 3 shows the results of the Munoo-Liisa vitality index (MLVI) including its components (GR and RLP), using the commercial substrate (CS) as control. Equal GR (100%) was recorded for all substrates, but there were significant differences concerning MLVI. Increasing the percentage of bark in the mixture led to MLVI reduction, with lower values in S50 substrates. In S50 substrates, FB presented the lowest MLVI (73.8%), against 80.3% in HTB1 and 88.5% in HTB2, showing, again, the positive effect of the hydrothermal treatment on phytotixicity reduction. S25 substrates showed high MLVI values (>90%), suggesting that mixing 25% of bark with peat-based growing media may present favorable results for further consideration in substrates formulation. Regarding literature, the percentage of bark in a mixture could influence the extent of phytotoxicity (Gruda *et al*., 2009) and generally recommended peat substitution by wood fiber materials is up to 30% (v v^{-1}) (Gruda *et al*., 2013; Barret *et al*., 2016).

All bark-based substrates were fertilized with a complete nutrient solution (see materials and methods), with an extra amount of 100 mg N L^{-1} (total 200 mg N L^{-1}) compared to CS (100 mg L^{-1}), to compensate potential bark mineral N competition. All substrates pH values were within recommended range (5.3-6.5) and EC values lower than the threshold value of 60 mS m^{-1} (table 3). Considering the other nutrients, the values are within or slightly higher than the recommended range for a growing-media (Ansorena-Miner 1994; Weber *et al*., 2005) and, consequently, no limitations to plant growth are expected.

Bark addition to peat had a significant effect on substrates physical properties (table 4). Bark increased total porosity and improved peat aeration. AFP_{10} raised from 8.3 % in peat (table 4) to an average of 26.9% in S25, and 46.1% in S50 substrates. Following an inverse trend, with gradual bark addition water availability decreased. Shrinkage was also reduced by bark increment and tended to meet previous results from table 2. Shrinkage is often related to hydrophobic effects caused by drying and it is a problem mainly for outside plant production. In these cases, due to channeling, irrigation water drains very fast through the cracks or the void between container wall and the substrate (Blok *et al*., 2008). Concerning standard range for substrate physical properties (Noguera *et al*., 2003) all S25 mixtures fitted in recommended values. Generally, is assumed that high AFP_{10} promote air supply to the roots but can compromise water availability (Jackson *et al*., 2010) but with addition of 25% of bark, aeration is improved while water availability remains adequate (table 4). Gruda *et al*. (2013) pointed out the relevance of higher irrigation frequency when wood fibers are used as a component of growing media to maintain container water content.

Table 3. Substrates root length per plant (RLP), germination rate (GR), Munoo-Liisa vitality index (MLVI), pH, electrical conductivity (EC), and water-soluble nutrients (water extract 1:5 by volume): mineral nitrogen (N_{min}), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg) and sodium (Na).

Substrate	Bark type	RPL	GR	MLVI	pH	EC	N_{min}	P	K	Ca	Mg	Na
		cm	%			mS m^{-1}	mg L^{-1}					
S25	FB	5.5 *b*	100 *a*	90.2 *b*	5.7 *a*	59 *a*	193 *a*	50 *a*	313 *ab*	154 *c*	61 *a*	22 *c*
	HTB1	5.7 *b*	100 *a*	93.4 *b*	5.6 *a*	56 *ab*	203 *a*	45 *a*	257 *c*	164 *bc*	58 *a*	12 *d*
	HTB2	5.8 *ab*	100 *a*	95.1 *ab*	5.7 *a*	58 *ab*	197 *a*	39 *a*	268 *c*	155 *c*	65 *a*	12 *d*
S50	FB	4.5 *d*	100 *a*	73.8 *d*	5.7 *a*	58 *a*	196 *a*	34 *a*	336 *a*	167 *bc*	69 *a*	32 *b*
	HTB1	4.9 *c*	100 *a*	80.3 *c*	5.7 *a*	53 *b*	209 *a*	35 *a*	279 *bc*	172 *bc*	62 *a*	12 *d*
	HTB2	5.4 *b*	100 *a*	88.5 *b*	5.7 *a*	53 *b*	203 *a*	36 *a*	282 *bc*	192 *b*	64*a*	11 *d*
CS	-	6.1 *a*	100 *a*	100 *a*	5.9 *a*	37 *c*	94 *b*	26 *a*	181 *d*	264 *a*	22 *b*	40 *a*
Acceptable range*			-	-	-	<60	50-250	19-75	51-400	50-110	16-80	<100

Means followed by the same letter within the same column do not differ significantly at p = 0.05 by LSD-test
[1] Acceptable range, adapted from Ansorena-Miner (1994) and Weber at al. (2005).

Table 4. Substrates physical properties: bulk density (BD), total porosity (TP), air-filled porosity at 10 cm of water column (AFP_{10}), easy available water (EAW), water buffering capacity (WBC), available water (AW) and shrinkage.

Substrate	Bark type	BD	TP	AFP_{10}	EAW	WBC	AW	Shrinkage
		g L^{-1}	% (v v^{-1})					
S25	FB	109.3 b	93.3 b	28.0 bc	23.1 b	5.1 bc	28.2 b	24.1 bc
	HTB1	110.2 b	93.2 b	29.1 b	23.4 b	5.1 bc	28.5 b	26.5 b
	HTB2	113.6 b	93.0 b	23.7 c	26.0 b	5.2 b	31.2 b	25.6 b
S50	FB	96.8 c	94.0 a	45.1 a	16.4 c	4.3 bc	20.7 c	19.4 cd
	HTB1	94.8 c	94.1 a	46.1 a	16.3 c	3.6 c	20.0 c	15.5 d
	HTB2	92.9 c	94.3 a	47.1 a	15.8 c	4.2 bc	20.0 c	17.9 d
CS	-	139.1 a	91.5 c	10.5 d	30.9 a	8.3 a	39.2 a	39.7 a
Acceptable. range[1]		< 400	> 85	10 - 30	20 - 30	4 - 10	24 - 40	< 30

Means followed by the same letter within the same column do not differ significantly at p = 0.05 by LSD-test
[1] Acceptable range, adapted from Ansorena-Miner (1994) and Noguera *et al*. (2003).

3.3. Plant growth

Potting test, using Chinese cabbage (table 5), revealed lower germination rate (95%) in FB mixtures than in HTB1, HTB2 and CS (98-100%), reinforcing the FB phytotoxicity. S25 substrates showed shoot weight and roots rating statistically equal or higher than CS. S25 substrates increased Chinese cabbage growth by creating favorable conditions for plant and root development as bark incorporation improved substrate aeration while maintained adequate water availability (table 4). In addition, it seems that the N surplus applied (100 mg N L^{-1}) was enough to counteract bark N immobilization in S25 substrates. Plant growth was lower in substrates with 50% bark (S50), probably related with unsuitable water retention properties (table 4), as well as N immobilization (Gruda *et al*., 2013; Depardieu *et al*., 2016) due to a big percentage of bark in the substrate (50% bark).

4. Conclusions

The study shows that hydrothermal treatments were effective regarding phytotoxicity removal from *E. globulus* fresh bark. Due to less energy and time consumption, and less N immobilization, the HTB1 treatment (20’ and 60°C) seems to be adequate. Mixing 25% (in volume) of treated bark with peat shows simultaneously improvements in substrates aeration properties, while adequate water content is maintained, compared to peat. An additional N-fertilization (100 mg N L^{-1}) in 25% bark-based substrates (to counteract N-immobilization), allowed a Chinese cabbage growth (shoot weight and roots rate) statistically equal or higher than in commercial substrate, encouraging use of this proportion of hydrothermally treated bark in substrates formulation.

Table 5. Germination rate (GR), fresh and dry shoot weight (FW; DW) and root rating of chinese cabbage plants grown in bark-based and commercial substrates

Substrate	Bark type	GR	FW	DW	Root
		%	g pot^{-1}		rating
S25	FB	95 b	17.4 ab	2.9 ab	4.7 a
	HTB1	100 a	15.7 ab	2.8 ab	4.0 a
	HTB2	100 a	18.5 b	3.3 a	3.7 ab
S50	FB	95 b	9.0 c	1.5 c	2.7 cd
	HTB1	100 a	8.3 c	1.5 c	2.7 cd
	HTB2	98 ab	6.9 c	1.2 c	2.4 d
CS		100 a	14.3 b	2.6 b	3.2 bc

Means followed by the same letter within the same column do not differ significantly at p = 0.05 by LSD-test

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