



## Effect of fresh and composted spent coffee grounds on lettuce growth, photosynthetic pigments and mineral composition

Teresa Gomes<sup>1</sup>, José Alberto Pereira<sup>1</sup>, Elsa Ramalhosa<sup>1</sup>, Susana Casal<sup>2</sup> & Paula Baptista<sup>1</sup>

<sup>1</sup>Mountain Research Centre (CIMO), School of Agriculture – Polytechnic Institute of Bragança, *Campus* Sta Apolónia, Apartado 1172, 5301-855 Bragança, Portugal. Email: pbaptista@ipb.pt

<sup>2</sup>REQUIMTE/Laboratory of Bromatology and Hydrology, Faculty of Pharmacy, Porto University, Rua Jorge Viterbo Ferreira, 228, 4050-313 Porto, Portugal

### Abstract

The coffee industry has experienced a constant growth and, as a consequence, large amounts of residues are generated worldwide. One of the main coffee residues are spent coffee grounds (SCG), which are the solid residues obtained after preparation of the coffee beverages. References to its use as organic fertilizer in domestic cultures, especially in gardens, are common. However, scientific evidence of its effectiveness or even safety remains largely unknown.

The main objective of this work was to assess the effect of SCG, both composted and uncomposted, on *Lactuca sativa* L. growth and mineral composition under greenhouse conditions. It is also expected to find the optimum dose of SCG that would give maximum lettuce growth. With this purpose, lettuce plants cv. "Four seasons" were grown on topsoil (control) or in topsoil mixed with different concentrations of fresh (2.5; 5; 10; 15; 20%, v/v) or composted (5; 10; 15; 20; 30%, v/v) SCG. After 39 days of transplantation, the plants were collected and used to evaluate several growth parameters, as well as photosynthetic pigments and mineral contents on leaves.

The application of both fresh and composted SCG, as well as their concentrations, had significant influence on all the parameters measured. Fresh SCG stimulated the plant growth at low concentrations (2.5-5%) but without significant differences when compared to control. A similar effect was observed for composted SCG, but only when applied at high concentrations ( $\geq 10\%$ ). By contrast, the foliar-N, -P and K- contents were, in general, reduced by fresh and composted SCG. When comparing to fresh SCG, the composted SCG resulted, in average, in higher carotenoids content, foliar-N and -K contents, and plant growth. Both treatments could be used to recycle coffee grounds with demonstrated horticultural benefits.

Keywords: *Lactuca sativa*, leaf-macronutrient content, chlorophyll a and b, carotenoids.

## Efeito da borra de café fresca e compostada no crescimento, teores em pigmentos fotossintéticos e composição mineral de plantas de alface

### Resumo

Nos últimos anos, tem sido observado um crescimento constante da indústria de café, e consequentemente, dos vários resíduos que são originados durante o seu processamento. Um desses resíduos é a borra de café resultante da extração de café bebida. Várias são as referências acerca da sua utilização como fertilizante orgânico em culturas domésticas, especialmente em jardins. No entanto, evidências científicas acerca da sua eficácia ou até mesmo segurança permanecem desconhecidas.

Neste trabalho pretendeu-se avaliar o efeito da borra de café fresca e compostada no crescimento e composição mineral de *Lactuca sativa* L., em condições de estufa. Pretendeu-se ainda determinar a dose de borra de café que permite obter o máximo de crescimento. Para tal, plantas de alface cv. "Quatro estações" foram plantadas em terra vegetal (controlo) ou em terra vegetal misturada com diferentes concentrações de borra de café fresca (2,5, 5, 10, 15, 20%, v/v) ou compostada (5, 10, 15, 20, 30%, v/v). Após 39 dias, as plantas foram colhidas e utilizadas para avaliar o seu crescimento, o teor em pigmentos fotossintéticos e a composição mineral das folhas.

A aplicação da borra de café fresca e compostada, bem como a sua concentração influenciaram significativamente todos os parâmetros analisados. A borra de café fresca estimulou o crescimento das plantas a baixas concentrações (2,5-5%), apesar de não diferir significativamente do controlo. Um efeito semelhante foi observado para a forma compostada, mas apenas quando aplicada em concentrações elevadas ( $\geq 10\%$ ). Pelo contrário, os teores foliares de N, P e K foram, em geral, reduzidos em plantas cultivadas em borra de café. As alfaves cultivadas em borra compostada obtiveram, em média, maiores teores em carotenóides, N e K, e maior crescimento, em comparação com alfaves cultivadas em borra de café fresca. Ambos os tratamentos podem ser utilizados para reciclar a borra de café uma vez que demonstraram benefícios hortícolas.

Palavras-chave: *Lactuca sativa*, teor foliar em macronutrientes, clorofila a e b, carotenóides.

## Introduction

In the last years, an increase of coffee beverages consumption has been observed all over the world. The preparation of this beverage generates high amounts of spent coffee grounds (SCG), which is usually mixed with common garbage (Tokimoto et al., 2005; Mussatto et al., 2011; Cruz et al., 2012), causing major environmental issues. Therefore, finding strategies to valorise the utilization of this organic residue is of much interest. The chemical composition of SCG indicates several applications to this residue. For instance, the presence of nitrogen (about 1.2 – 2.3%), phosphorus (0.02 – 0.5%) and potassium (0.35%) contents (Mussatto et al., 2011; Cruz et al., 2012) suggests its utilization on agriculture as fertilizer or as soil improver (Kondamudi et al., 2008). These nutrients increase soil fertility and are equally important for healthy plant growth. There are several references about the use of SCG as organic fertilizer on domestic agriculture; however the scientific evidence of its effectiveness remains largely unknown. In fact, the presence of caffeine (about 0.2%) in espresso SCG (Cruz et al., 2012), together with tannins and chlorogenic acids, might impose some toxicity to soil microorganisms and plants (Batish et al., 2008), and its direct application might not be totally beneficial to the plants. An alternative to reduce these potential damaging effects could be through SCG composting.

Therefore, the main objective of this study was to assess the effect of espresso SCG, fresh or after composting, on *Lactuca sativa* L. growth, as well as on photosynthetic pigments and mineral leaves contents, under greenhouse conditions.

## Material and methods

The fresh SCG were collected from various coffee establishments in the Porto city (Portugal), serving espresso coffee on a regular basis. The composted SCG were obtained from fresh SCG, after being subject to a composting process during 4 months, conducted on the premises of LIPOR – Intermunicipal Waste Management of Greater Porto. The mineral composition (N, P, K, Ca and Mg) of fresh and compost SCG, as well as topsoil (Siro® Germe, Leal and Soares, Lda.; Portugal) used to performed the mixtures of the experiment, are presented in Table 1. Total N content determination was carried out by Kjeldahl's method. The contents on P were determined following the molybdate-ascorbic acid blue method and K by flame photometric method.

Seeds of *Lactuca sativa* L. cv. “Four Seasons” were germinated on topsoil (Siro® Germe), under greenhouse conditions (day/night thermal regime of 23°/18° ± 2°C, and 70 ± 10% relative humidity). One month after sowing, uniform plantlets were selected and transplanted into 1L pots, with topsoil (Siro® Germe) mixed with different percentages of fresh (2.5%, 5%, 10%, 15% and 20%) or composted (5%, 10%, 15%, 20% and 30%) SCG, calculated on a volume basis taking into account their apparent densities. Plain topsoil was used as control (0%). For each treatment and control 42 pots (each one with one lettuce plantlet) were prepared and arranged at random in the same above-mentioned greenhouse conditions. Lettuce plants were harvested 39 days after transplantation, and were further used to evaluate several growth parameters, as well as photosynthetic pigments and mineral leaves contents. Dry weight (dw) and daily growth rate ( $\Delta x/\Delta t$ ) were determined for the aerial part of the lettuce plants. Chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*) and total carotenoids (Car) contents were quantified by the method of Ozerol and Titus (1965). Mineral leave contents were determined as described above.

Data are presented as the mean of three to fifteen independent experiments followed by the corresponding standard deviations (SD). The significance of differences among means was tested by analysis of variance (ANOVA), using SPSS v.20 software (SPSS Inc.), in which the averages were compared using Tukey test ( $p \leq 0.05$ ).

**Table 1.** Mineral composition of fresh and composted SCG, as well as topsoil used to perform the several mixtures of the experiment.

Minerals	Spent coffee grounds		Topsoil
	Fresh	Composted	
N (g kg <sup>-1</sup> fw)	12.07 ± 0.139	2.39 ± 0.011	2.98 ± 0.031
P (g kg <sup>-1</sup> fw)	0.27 ± 0.018	0.17 ± 0.001	0.23 ± 0.007
K (g kg <sup>-1</sup> fw)	9.14 ± 0.793	28.96 ± 5.403	5.12 ± 0.149

fw - fresh weight

## Results and discussion

The results showed that both fresh and composted SCG, as well as the concentration used had influence on the lettuce growth (Tables 2 and 3). Fresh SCG when applied at low concentrations (2.5%) increased slightly the dry weight (dw) and daily growth rate ( $\Delta x/\Delta t$ ) when compared to control, even though the differences were not statistically significant. This improvement in plant growth could be related to the richness of fresh SCG on mineral nutrients, especially on N (Table 1). However, for higher fresh SCG amounts ( $\geq 10\%$ ), the biomass was reduced to values inferior to those observed in control samples. Fresh SCG has known to contain high amounts on toxic compounds such as caffeine, tannins and chlorogenic acids (Batish et al., 2008). Therefore, an increased on fresh SCG amounts in the soil may consequently result in an increased of levels on these toxic compounds, which could ultimately reduce plant growth. The results from the composted SCG treatment indicated that the composting process probably reduced the amounts on these phytotoxic compounds. In fact, the incorporation of composted SCG into the soil has showed to enhance lettuces plant growth as compared to control, only when applied at concentrations equal or higher than 10% (Table 3).

The leaves contents on chl *a* and chl *b* of lettuces grown on both fresh and composted SCG did not differed significantly from control plants (Table 2 and 3). Leaf chlorophyll content is usually an indicator of nutritional stress (Chen et al., 2010), especially of nitrogen-deficiency (Wang et al., 2004; Chen et al., 2010). Therefore, the absence of differences between control plants and treatments on chlorophyll contents, suggested that lettuce plants grown on both fresh and composted SCG had sufficient mineral nutrients to meet their growing needs. The carotenoid concentration in lettuces was significantly affected only by the presence of composted SCG (Table 3) and was found to follow the same pattern as the lettuces plant growth. This suggested that the increase on carotenoids contents lead to an improved photosynthetic rate when compared to control plants, which ultimately result in an enhancement of plant growth.

The N, P and K contents in lettuce leaves were significantly affected by the presence of SCG. In fresh SCG treatment, both K and P leaves contents were significantly lower as compared to control, for all the concentrations tested (Table 2). By contrast, N leaves content have decreased significantly in relation to the control for fresh SCG concentrations  $\geq 15\%$ . Composted SCG showed a greater tendency to increase the mineral nutrients in lettuces (especially K) than fresh SCG (Table 3). When compared to control, composted SCG increased significantly K leaves contents at any of the concentrations tested; and decreased P leaves contents when applied at concentrations  $\geq 10\%$ . N leaves contents on plants grown on composted SCG were similar to the control. Thus, since in lettuces grown on composted SCG was observed higher leaf content on N, K and plant growth as compared to plants grown on fresh SCG, this may indicate greater soil mineralization and uptake of mineral nutrients under composted SCG.

**Table 2.** Effect of fresh SCG, at different concentrations (0-20%, v/v) on lettuce growth, photosynthetic pigments and mineral leaves composition.

Fresh SCG								
Concentration	Plant growth		Photosynthetic pigments			Mineral composition		
	Dw	$\Delta x/\Delta t$	Cla (mg/g)	Clb (mg/g)	Car (mg/g)	N (g/Kg)	K (mg/Kg)	P (mg/Kg)
0%	1.54±0.08 <sup>a</sup>	0.42±0.02 <sup>a,b</sup>	1.40±0.11 <sup>a</sup>	0.94±0.09 <sup>a</sup>	0.10±0.01 <sup>a</sup>	38.40±0.21 <sup>a</sup>	208.97±1.63 <sup>a</sup>	959.19±6.29 <sup>a</sup>
2.5%	1.64±0.17 <sup>a</sup>	0.44±0.02 <sup>a</sup>	1.79±0.16 <sup>a</sup>	1.24±0.11 <sup>a</sup>	0.19±0.04 <sup>a</sup>	35.97±0.07 <sup>ba</sup>	131.26±5.43 <sup>cd</sup>	853.87±13.93 <sup>b</sup>
5%	1.32±0.07 <sup>a</sup>	0.38±0.03 <sup>a,b</sup>	1.68±0.17 <sup>a</sup>	1.16±0.11 <sup>a</sup>	0.15±0.01 <sup>a</sup>	33.35±0.34 <sup>b</sup>	120.26±7.40 <sup>d</sup>	793.23±10.20 <sup>c</sup>
10%	0.99±0.14 <sup>b</sup>	0.34±0.02 <sup>b</sup>	1.36±0.11 <sup>a</sup>	0.95±0.07 <sup>a</sup>	0.17±0.08 <sup>a</sup>	36.08±0.64 <sup>ba</sup>	161.16±1.60 <sup>b</sup>	823.97±16.51 <sup>b</sup>
15%	0.68±0.07 <sup>b</sup>	0.20±0.01 <sup>c</sup>	1.54±0.11 <sup>a</sup>	1.01±0.08 <sup>a</sup>	0.19±0.08 <sup>a</sup>	28.74±0.25 <sup>c</sup>	118.55±3.31 <sup>d</sup>	784.60±17.73 <sup>c</sup>
20%	0.84±0.12 <sup>b</sup>	0.19±0.02 <sup>c</sup>	1.68±0.17 <sup>a</sup>	1.15±0.10 <sup>a</sup>	0.19±0.01 <sup>a</sup>	28.72±0.89 <sup>c</sup>	151.19±5.35 <sup>c</sup>	799.97±15.76 <sup>c</sup>

Dw - dry weight;  $\Delta x/\Delta t$ - daily growth rate; Different letters in a row show statistically significant differences ( $p < 0.05$ ) from the given mean.

**Table 3.** Effect of composted SCG, at different concentrations (0-30%, v/v) on lettuce growth, photosynthetic pigments and mineral composition.

Composted SCG								
Concentration	Plant growth		Photosynthetic pigments			Mineral composition		
	Dw	$\Delta x/\Delta t$	Cla (mg/g)	Clb (mg/g)	Car (mg/g)	N (g/Kg)	K (mg/Kg)	P (mg/Kg)
0%	0.53±0.13 <sup>b</sup>	0.29±0.02 <sup>c</sup>	2.01±0.53 <sup>a</sup>	1.34±0.43 <sup>a</sup>	0.10±0.07 <sup>b</sup>	38.40±0.20 <sup>a</sup>	208.9±1.63 <sup>d</sup>	959.19±6.29 <sup>a</sup>
5%	0.88±0.15 <sup>ab</sup>	0.29±0.02 <sup>c</sup>	1.78±0.39 <sup>a</sup>	1.23±0.28 <sup>a</sup>	0.13±0.05 <sup>b</sup>	38.36±1.27 <sup>a</sup>	271.45±3.47 <sup>a</sup>	924.88±13.41 <sup>a</sup>
10%	1.24±0.23 <sup>ab</sup>	0.43±0.03 <sup>ab</sup>	2.25±0.72 <sup>a</sup>	1.66±0.69 <sup>a</sup>	0.19±0.04 <sup>a</sup>	38.41±0.23 <sup>a</sup>	238.63±4.08 <sup>c</sup>	822.63±7.86 <sup>b</sup>
15%	1.28±0.16 <sup>ab</sup>	0.45±0.02 <sup>a</sup>	2.18±0.36 <sup>a</sup>	1.54±0.31 <sup>a</sup>	0.20±0.03 <sup>a</sup>	38.27±0.60 <sup>a</sup>	247.35±1.28 <sup>b</sup>	731.06±20.90 <sup>c</sup>
20%	1.09±0.20 <sup>ab</sup>	0.38±0.02 <sup>bc</sup>	1.62±0.16 <sup>a</sup>	1.14±0.11 <sup>a</sup>	0.14±0.03 <sup>ab</sup>	37.62±0.54 <sup>a</sup>	241.81±3.21 <sup>b</sup>	831.06±20.01 <sup>b</sup>
30%	1.80±0.30 <sup>a</sup>	0.34±0.02 <sup>bc</sup>	2.11±0.48 <sup>a</sup>	1.51±0.35 <sup>a</sup>	0.18±0.03 <sup>a</sup>	37.35±0.51 <sup>a</sup>	244.68±0.74 <sup>b</sup>	810.66±10.11 <sup>b</sup>

Dw - dry weight;  $\Delta x/\Delta t$ - daily growth rate; Different letters in a row show statistically significant differences ( $p < 0.05$ ) from the given mean.

## Conclusions

With this work was possible to conclude that fresh and composted SCG can be used as fertilizer in agriculture. Both forms of SCG (fresh and composted) have showed to improve significantly lettuces plant growth when compared to the control. This effect was also found to depend greatly of their application rate. Overall, the results indicate that fresh SCG should be applied at low concentrations (2.5 and 5%, v/v), whereas composted SCG should be applied at higher concentrations ( $\geq 10\%$ , v/v). Specifically, the lettuces plant growth was significantly increased at concentrations of 10% of fresh SCG and 30% of composted SCG, when compared to control. The application of fresh SCG at concentrations above 10%, showed to induce plant stress, probably due to the increase of phytotoxic compounds (such as caffeine), resulting in a reduction of macronutrients plant-uptake and plant growth. The composted form appears to be less toxic to the plants, even when applied at high concentrations. This form leads to a higher plant uptake of N, K and plant growth when compared to the fresh SCG.

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