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INFLUENCE OF POLYPHENOLIC COMPOUNDS ON *OCIMUM BASILICUM L.* DEVELOPMENT

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Abstract. The activities and role of phenolic compounds in the plant kingdom are well known. They are especially recognized for their function as plant growth regulators, but also for the important role in the biosynthesis process. Based on that, the aim of this work is to establish the influence of polyphenolic compounds, on the main physiological processes involved in basil cultivation under controlled conditions. Studies were carried out on sweet basil seeds (*Ocimum basilicum L.*) treated with different spruce bark polyphenolic extracts (aqueous extract and ultrasound assisted aqueous extract) on several concentrations. The germination energy and germination capacity, plants vegetative organelles development and photoassimilatory pigments content were investigated. The results show that the *Picea abies* extracts, rich in phenolic compounds, have an influence on the global development of plantlets. An increased value for the growth parameters and pigments concentration was observed, compare with a control sample. Also it was shown that the effect of phenolic compounds on plants development significantly depends on their concentration.

INTRODUCTION

Basil (*Ocimum basilicum* L.) is a popular aromatic culinary herb, belonging to the Laminaceae family, native to Iran, Afghanistan and India as described previously (Asghari *et al.*, 2012). Commonly it is used in food industry as a flavoring, being one of the major essential oils producing species from the *Ocimum* genus as described previously (Koca and Karaman, 2014). It's also used in cosmetics, perfumery and medicine, as cosmetic and pharmaceutical products, presenting great antioxidant, antimicrobial and antitumor activities due to their aromatic compounds content as described previously (Taie *et al.,* 2010). Basil can be as well used as a stomachic, anthelmintic, antipyretic, diaphoretic or as a carminative.

Based on these properties, there is an increase of interest on conservation and development of basil plants, many studies being carried out recently with the aim of establishing new and simple methodologies both to increase polyphenols content in plant, to further enhance of their overall nutritional value, and to ensure a high reproducibility and viability.

Polyphenols are one of the major groups of plant secondary metabolites being responsible for pigmentation, astringency, flavor, attraction of pollinators and offer protection against UV light and pests and well recognized for their antioxidant activity. Beside human health treating polyphenols are use in commercial applications as food and soft drinks colorants and additives, in cosmetics and pharmaceuticals products, as bioaccumulation agents, allelochemicals, chelating agents or as plants grown up regulators as described previously (Ignat *et. al.*, 2009; Popa *et al.*, 2008; Tanase *et al.*, 2011; Volf *et al.*, 2012).

In the present study spruce bark was chosen as raw material. The choice was based on resource availability, spruce bark coming as a waste resulted in large amounts from the wood processing. In our research group many studies have been done on this raw material. Taking in to account the obtained results regarding the reported total polyphenols content, the tannins content, anthocyanins, flavones, flavonols and other, it could be concluded that the spruce ritidom represent an important source of phenolic compounds as gallic acid, vanillic acid or catechine with a polyphenolic content up to 5.2 mg GAE/ g spruce bark, as reported previously (Tanase *et al*., 2014).

Application of polyphenols as a biostimulator on plant growth is an innovative concern in the plant science world and shows a real success as described previously (Popa *et al*., 2002). Their proprieties have been gratefully tested on a large group of plants as soybean and sunflower, bean, oat, rape, maize and tomatoes as described previously (Bălaş and Popa, 2007; Ignat *et al*., 2009; Ignat *et al*. 2011; Tanase *et al*., 2011; Tanase *et al*., 2014).

The main goal of this study was to compare the action of different aqueous polyphenolic extracts obtained using hot water extraction and ultrasound assisted extraction techniques, and to establish the influence of these compounds on *Ocimum basilicum* L. seeds. Thus, using different concentration and reported to a control, germination test were carried out and also radicles elongation, fresh biomass accumulation and photoassimilatory pigments content was determined.

MATERIALS AND METHODS

Extraction method

Extractions were performed by conventional extraction method (hot water extraction) and by ultrasound assisted extraction in order to establish the influence of the extraction method on the recovered polyphenolic content and the reactions induced by this on the main biosynthesis processes during seeds germination and plants development.

The hot water extraction was performed using two different solid/solvent ratios (5g/L (SB1) and 10 g/L (SB2)) of ground and dried spruce bark placed in a Erlenmeyer flask over which 125 mL distilled water were added. The mixture was incubated for 45 min on a water bath at 85-90 ^{0}C, shaking from time to time. Collected extracts were filtered and the extracted material was subjected to a second extraction with fresh distilled water. This operation was repeated 2-3 times until the spruce bark was fully exhausted (colorless extract). All extracts were pooled in a 1000 mL volumetric flask and mark up to volume mark with distilled water.

Ultrasound assisted extraction was performed using 5 g of spruce bark, immersed in 125 mL distilled water in a Erlenmeyer flask using a ultrasonic bath (Bandelin Sonorex), at 35 kHz frequency, 70 ^{0}C for 15 min, the extraction time being selected based on the literature data and previous preliminary studies. The operation was four times repeated, extracts being filtered and collected in a 500 mL volumetric flask and volume was completed with distilled water. In case of the SB3 solution the obtained extract was diluted 1:1 (v/v) with distilled water.

Aqueous extracts were used in four working solutions: SB1 - aqueous extract obtained by hot extraction with 5 g/L spruce bark concentration and 0.06 mg GAE/mL phenolic content, SB2 - aqueous extract obtained by hot extraction with 10 g/L spruce bark concentration and 0.13 mg GAE/mL phenolic content, SB3 - aqueous ultrasonic extract with 5 g/L spruce bark concentration and 0.32 mg GAE/ml phenolic content and SB4 - aqueous ultrasonic extract with 10 g/L spruce bark concentration and 0.65 mg GAE/mL phenolic content.

Total polyphenols content determination

Total polyphenolic content was determined by Folin-Ciocalteu method, using a UV-VIS GBS AVANTA spectrophotometer. The total phenols content was given by reading the absorbance at 765 nm wavelength and taking into account the calibration curve of gallic acid standard solution, results being expressed in milligrams gallic acid equivalents per mL spruce bark extract (mg GAE/mL).

Plant characterization

The germination tests were performed on *Ocimum basilicum* L. seeds obtained from SC. Agrosel SRL, Campia Turzii, Romania. 15 basil seeds were placed in Petri dishes covered with filter paper at approximately equal distances over which 10 mL of different extracts concentrations were added. The reference samples were prepared in same conditions using distilled water. Germination tests were performed in controlled conditions at 30^{0}C in a thermostatic room for 7 days, and wetted with 10 mL fresh solutions of polyphenolic extracts and distillated water, maintaining the specific working concentrations at every three days. On the third day after starting the experiments the germination energy (speed of the seeds germination) was determinate as a report between the germinated seeds number and total seeds number taken for experimental tests. 7 days after germination the germination capacity was determined as a numeric percentage of the total germinated seeds. For each experiment four repetitions were performed, the results being reported as percentages differences from control (Tanase *et al.,* 2014).

After germination the Petri dishes were kept for 3 days in daylight to allow the seedlings to synthesize assimilatory pigments as described previously (Tanase *et al.*, 2011). The young plantlets were analyzed by biometric and quantitative measurements in term of elongation of vegetative organs, biomass accumulation and photoassimilatory concentration determination.

For pigments content quantification 0.05 g of fresh vegetal material was milled in a mortar with quart sand and extracted in acetone (80%). The carotenoids and chlorophyll content (chlorophyll a and chlorophyll b) were than spectrophotometrically determined by reading absorbance at specific wavelengths (470, 646 and 663 nm) using mathematical formulas as described previously (Lichtenthaler and Wellburn, 1983).

Statistical analysis

The results are expressed as mean ± SD, where n = 3. Comparison of the means was performed by the Fisher least significant difference (LSD) test ($p \leq 0.05$) after ANOVA analysis using program PAST 2.14. Sampling and chemical analyses were examined in triplicate in order to decrease the experimental errors and to increase the experimental reproducibility.

RESULTS AND DISCUSSIONS

Analyzing the experimental data, it can be seen that the presence of aqueous polyphenolic extract has an stimulating effect both on energy and germination capacity (Fig.1, Fig. 2) influencing the germination process of basil seeds. Remarking is the incentive effect of polyphenolic extract obtained by ultrasonic extraction with 0.65 mg GAE/mL phenolic content (SB4), where the stimulation percentage of germination energy is 26.5% (±2.6) and 18.5% (±2.2) for germination capacity, compared to control.

Elongation and biomass accumulation in plant tissue were another parameters included in the study. As it can be seen from Figure 3, no significant differences were reported between experimental variants in terms of vegetative organs elongation.

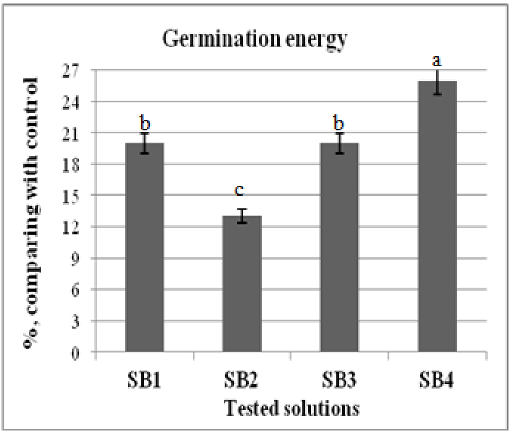

Fig. 1. The influence of spruce bark polyphenolic aqueous extracts on seed germination energy of *Ocimum basilicum* L. Bars show that the same letter is not significantly different at $p \leq 0.05$. Error bars represent the standard deviation of means (n = 3).

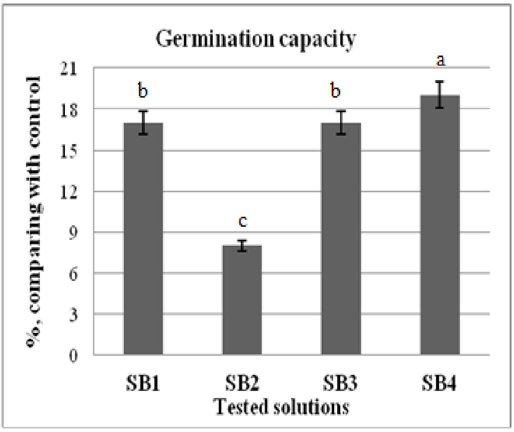

Fig. 2. The influence of spruce bark polyphenolic aqueous extracts on seed germination capacity of *Ocimum basilicum* L. Bars show that the same letter is not significantly different at $p \leq 0.05$. Error bars represent the standard deviation of means (n = 3).

It can be noted the fact that in case of using SB2 and SB3 extracts there is a decrease in growthness of the roots. This can be attributed to the increasing of extract concentration wich was applied. Regarding the biomass accumulation (Fig. 4) it was recorded an increase for all experiments. The highest value was recorded for SB1 version, where the applied extract concentration was 0.06 mg GAE/mL of phenolic content.

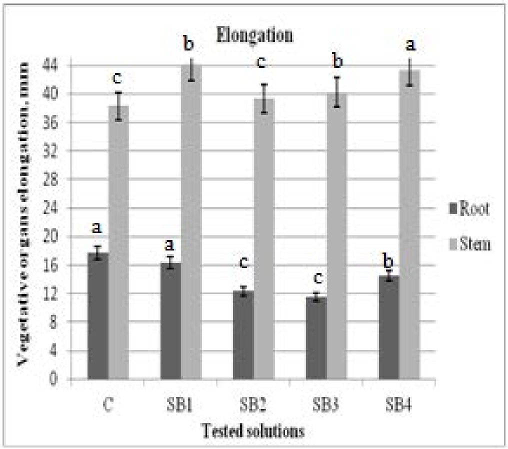

Fig. 3. The influence of spruce bark polyphenolic aqueous extracts on vegetative organs elongation of *Ocimum basilicum* L. Bars show that the same letter is not significantly different at p ≤ 0.05. Error bars represent the standard deviation of means(n = 3).

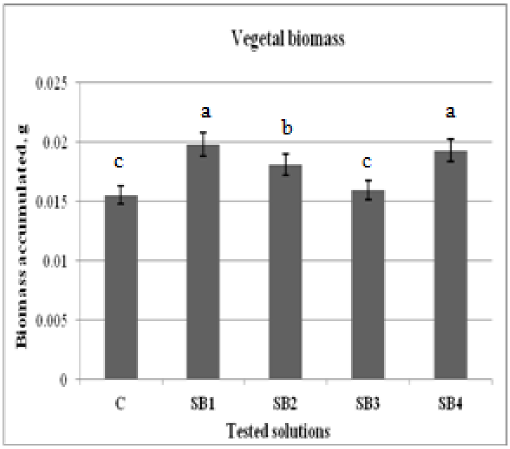

Fig. 4. The influence of spruce bark polyphenolic aqueous extracts on vegetal biomass accumulation of *Ocimum bas* Bars show that the same letter is not significantly different at p ≤ 0.05. Error bars represent the standard deviation of means(n = 3).

In Table 1 are presented the values obtained for photo-assimilatory pigments content which were synthesized in basil primary leaves. Analyzing these recorded values it can be observed an increase of chlorophyll and carotenoids pigments biosynthesis for SB1 and SB3 variations.

In case of SB2 and SB4 variants we can notice a decrease in chlorophyll pigments accumulation. Thus, compared with the control, on SB1 there was a decrease by 10% (±1.1) and 12% (±1.6) for chlorophyll *a* chlorophyll *b*. Also SB4 show a decrease by 12% (±1.4) for chlorophyll *a* and 16% (±1.6) for chlorophyll *b*. A particular situation was reported in SB2 where the amount of carotenoids is higher compared to control (41%, ±3.2). This situation can be attributed as being a defense reaction of plant to high concentration of the extract applied in the growth medium.

Table 1. The amount of photo-assimilatory pigments synthesized in primary leaves

	Chl a µg/g	***Chl b µg/g***	***Carotens µg/g***	***Chl a+b***	***Chl a/b***
C	123.68±2.23	29.96±0.51	62.22±0.73	153.64	4.128906
SB1	133.98±2.47	38.72±0.63	80.42±0.86	172.69	3.460537
SB2	96.63±1.45	26.08±0.22	88.46±0.81	122.71	3.704961
SB3	127.94±2.65	34.84±0.55	83.41±0.79	162.78	3.672757
SB4	108.76±2.01	25.11±0.21	61.83±0.53	133.87	4.331877

CONCLUSIONS

Results showed that aqueous polyphenolic extract obtained by spruce bark heating extraction has influence on *Ocimum basilicum* L. plant acting as a stimulating on seed germination energy and capacity, root and stem growth, biomass accumulation and photoassimiling pigments synthesis. A higher extract concentration was found to inhibit the synthesis of chlorophyll *a* and *b*.

Polyphenolic aqueous extract obtained by spruce bark ultrasonic extraction increase seeds germination capacity and energy, stimulates biomass accumulation and encourages photoassimiling pigments synthesis. At higher concentrations synthesis of chlorophyll and carotenoids pigments is inhibited.

Our data show that applying aqueous ultrasonic extracts have an overall accentuated positive influence on most of the investigated parameters (as germination capacity, seeds activation energy, elongation and biomass accumulation) comparing with the hot aqueous extracts, given the fact that the ultrasounds lead to a more complete and selective extraction, extracts being more rich in a large group of polyphenols. This fact is sustained by the literature data, as described (Santos *et al.*, 2013). However we do not exclude the possibility that other constituents present in the extracts, recovered during the extraction processes could also have an influence the main studied physiological processes.

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