

„*IN VITRO*” EFFECT OF SOME INDUSTRIAL BY-PRODUCTS ON *LAVANDULA ANGUSTIFOLIA* MILL. EXPLANT GROWTH

CORNELIU TANASE^{1,2*}, SMARANDA VANTU², VALENTIN I. POPA¹

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Abstract: After many studies, it was observed that lavender has many therapeutic effects, such as sedation, activities spasmolytic, antiviral, antibacterial. Thus, given the importance of lavender in different areas of human life, in the present study, we studied the influence of natural products bioregulators separated from industrial by-products on some lavender stems explants. These explants were inoculated *in vitro* on MS nutrient media. In these culture media were added polyphenolic extracts obtained from spruce bark and hemp shives, and evaluated their influence on lavender stems explants. The results obtained were compared with those obtained for the control variant, where MS culture medium was used as standard. It was found that the addition of aqueous extract from spruce bark of concentration of 130 mg GAE / L, in the growth of explants of *Lavandula angustifolia* Mill, an increase in the elongation of the main stem, number of leaves formed, the amount of photoassimilating pigments synthesized and causes the phenomenon of shoots formation. At a higher concentration of the extract (26 mgGAE/100g) values are lower.

INTRODUCTION

In the last years, most studies have been directed towards the establishment extraction methods of active principles from fruits and vegetables (Pinelo et al., 2005). Extraction of polyphenolic compounds is a very important step in the isolation, identification and use. The most common techniques for the extraction and isolation of polyphenolic compounds are solvent extraction (Baydar, 2004; Bucic-Kojic, 2007) and supercritical fluid extraction (Bleve et al., 2008; Nahar and Sarker, 2005). A large number of articles in the literature is focused on the extraction and analysis of polyphenols from plant materials (fruits, vegetables, bark, leaves etc.) (Tanase et al. 2011; Ignat et al. 2011; Luthria and Pastor-Corrales, 2006; Balasundram et al. 2006; Nacz and Shahidi, 2006).

The spruce bark aqueous extract was characterized in terms of total polyphenol content, color intensity, pH and was observed to contain a considerable amount of bioactive aromatic compounds, especially catechins and vanillic acid (Tanase et al. 2013). This was revealed by the identification of polyphenolic compounds by HPLC analysis. Regarding, aqueous extract from hemp shives was found that the amount of total polyphenol content expressed in mg GAE/100g is 164 ± 15.23 . The chromatographic analysis were identified and quantified phenolic acids and catechins in hemp shives aqueous extract (catechins - 52.17 mg plant material GAE/100g, vanillic acid - 96.8 mg GAE/100g acid, p-coumaric - 8.06 mg GAE/100g, ferulic acid - 3.2 mg GAE/100g) (Tanase et al. 2013). Regarding the characteristics, DDW (25 ppm isotopic concentration) is a microbiological pure distilled water, obtained by isotopic distillation in vacuum of a natural water with an isotopic concentration of 145 ppm D / (D + H) (Tanase et al., 2013).

Lavender is one the most useful herbs. From commercially point lavender, is an important source of essential volatile oil, which is widely used in the industries of flavors, including soaps, colognes, perfumes, skin lotions and other cosmetics (Paul et al. 2004). In food processing industry, lavender oil is used in flavored drinks, ice cream, candy, baked goods, and chewing gum (Kim and Lee, 2002). Recently, aromatherapy is used for a wide range of growing and lavender is used in aromatherapy as a relaxant (Lis-Balchin and Hart, 1999; Ghelardini et al., 1999). After extensive research, it was found that lavender has many therapeutic effects, such as sedation, activities spasmolytic, antiviral, antibacterial (Gamez et al. 1990; Buchbauer et al. 1991).

Thus, given the importance of lavender in different areas of human life, in the present study we followed the influence of natural products on some lavender stems explants. These explants were inoculated *in vitro* on MS nutrient media. In these culture medium were added polyphenolic extracts (DDW, DDW + M1, M1, M2, P1, P2) and evaluated their influence on lavender stems explants. The results obtained were compared with control, where MS culture medium was used as standard.

MATERIALS AND METHODS

Meristematic explants taken from *Lavandula angustifolia* Mill. obtained under aseptic conditions have been tested "*in vitro*" for regenerative capacity.

Explants were treated with 70% ethanol - 1 min and then with calcium hypochlorite (10%) - 15 minutes. After repeated washing with sterile distilled water, the explants were inoculated on agar nutrient medium (MS medium) solubilized in the polyphenolic extract used for each experimental variant (Table 1). Samples were maintained in growth

chamber at 23 ± 1 C to 16 hours photoperiod for 30 days. Observations were made at predetermined intervals (1, 5, 10, 20, 30 days), the number of leaves formed, the length of explants, shoot regeneration and photoassimilating pigments content were investigated.

Table 1 - Experimental variants

Experimental variant	Abbreviation	Vegetable material (g)	Total polyphenolic content (mg GAE/L)
Control	Control	0	0
Deuterium depleted water	DDW	0	0
Deuterium depleted water and spruce bark polyphenolic extract (1:1)	DDW+M1	5	96
Spruce bark polyphenolic extract	M1	10	191
Spruce bark polyphenolic extract	M2	5	130
Hemp shives polyphenolic extract	P1	10	164
Hemp shives polyphenolic extract	P2	5	78

Quantification of photoassimilating pigments. 0.05 g fresh vegetal material was extracted in 80% acetone by grinding with a spatula tip of quartz sand. Chlorophyll extract was analyzed spectrophotometrically by reading absorbance at various specific wavelengths: 470, 646, 663 nm. In order to determine the concentration of chlorophyll pigments (chlorophyll a and b) and carotenoid pigments were used formula proposed by Lichtenthaler and Welburn (1983):

$$\text{Chlorophyll a } (\mu\text{g} / \text{mL}) = 12.21 (A_{663}) - 2.81 (A_{646})$$

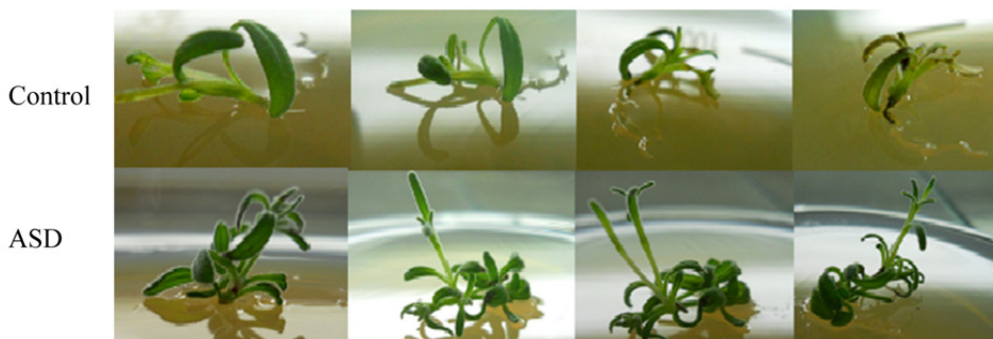
$$\text{Chlorophyll b } (\mu\text{g} / \text{mL}) = 20.31 (A_{646}) - 5.03 (A_{663})$$

$$\text{Carotenoids } (\mu\text{g} / \text{mL}) = (100 \cdot A_{470} - 3.27 [\text{chl a}] - 104 [\text{chl b}]) / 22$$

RESULTS AND DISCUSSIONS

Phenomenon of shoots, number of leaves formed and main stem elongation

Thus, the observations carried out at predetermined intervals, on the stems explants of control samples, revealed no growth and development (any reactions) culminating with partial necrosis explant at 30 days after inoculation (Fig.1)



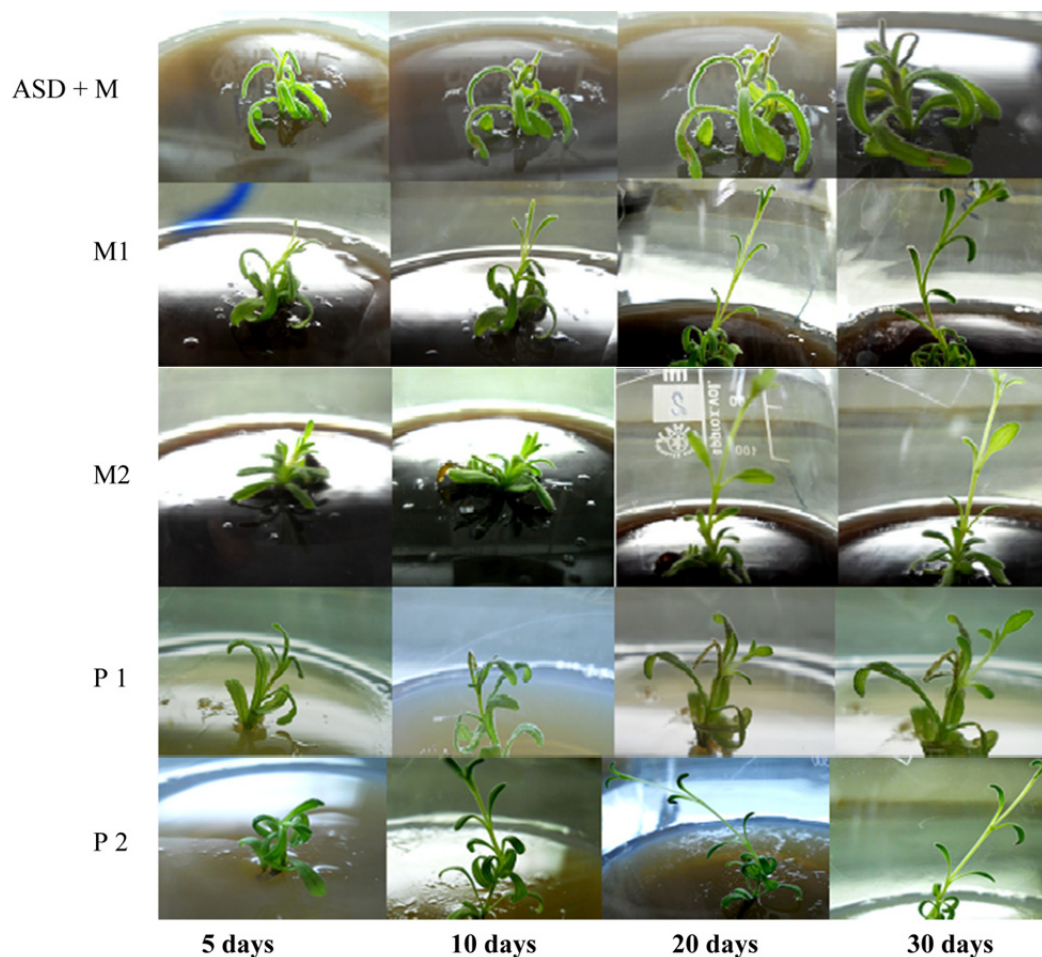


Figure 1 - Aspects of the experimental variants at the analyzing time

In case of DDW version, there was the onset of a process of multiple shoots after 10 days of inoculation, associated with a slight increase in the number of newly formed leaves. In contrast, the variant ASD + M1 were not significant differences in the evolution of the initial explant (Fig. 1).

At meristematic explants that were developed on medium supplemented with spruce bark polyphenolic extract (M2) was found the stimulate growth and shoot elongation after 5 days after inoculation. This process continued spectacular main stem elongation together with the formation of new leaves (Fig. 2) . It is also found a multiple shoots (Fig. 3) . M1 variant showed the same previous sample in the elongation of the main spindle. However, there is a difference in the number of leaves which is formed which is smaller in the variant mentioned above (Fig. 1).

When the growth medium was supplemented with hemp shives aqueous extract (P1) shows a high number of leaves formed after 5 days from the inoculation, the process is slightly

decrease after 20 days. High frequency of the newly formed leaves (Fig. 2) is associated with the emergence of new shoots after 10 days after inoculation (Fig. 1).

In the variant P2 there is a progressive increase of the main rod together with the emergence of new leaves, predominantly in the range 5-10 days after inoculation. After 10 days from the inoculation, the shoots are observed for P2 variant (Fig. 4).

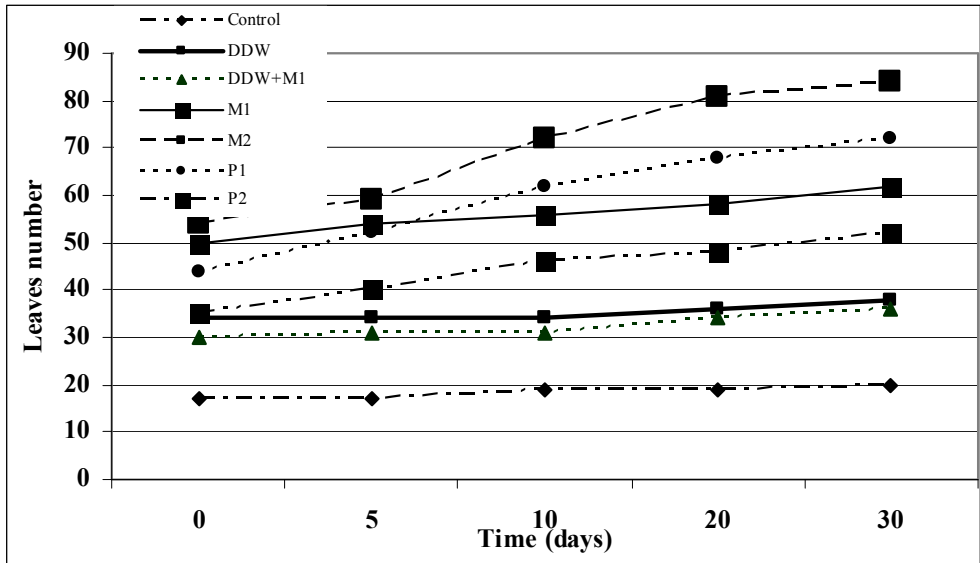


Figure 2 - Influence of polyphenolic extracts and DDW on the number of leaves formed

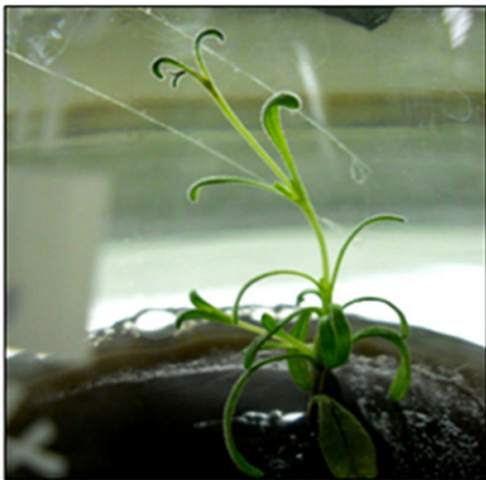


Figure 3 - Regenerative capacity of shoots (Variant M2)

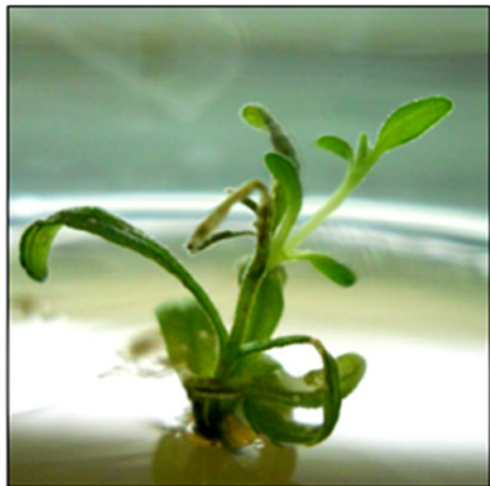


Figure 4 - Regenerative capacity of shoots (variant P1)

The influence of the tested solutions on the photoassimilating pigments content. By analyzing the photoassimilating pigments content at 30 days after inoculation (Table 2), it is found that it is much higher in explants that have developed on the culture medium supplemented with the spruce bark aqueous extract at a concentration of 130 mg / L (M2) and 191 mg / L (M1). At a higher concentration of the extract applied, the percentage of the stimulation is decreased (for spruce bark aqueous extract - M2 and hemp shives extract - P2).

Thus, the growth medium supplemented with hemp shives aqueous extract (in two concentrations) triggers an obvious stimulation for photoassimilating pigments content. Deuterium depleted water increases the the amount of pigments compared with control, but much less than other experimental variants. Moreover and visual observation, direct, there is a depigmentation (relating to the day of inoculation) leaves of explants that developed in control variants, culminating even a start foliar tissue necrosis.

Table 2 - The amount of photoassimilating pigments after 30 days after inoculation

	Chl a $\mu\text{g/g}$	Chl b $\mu\text{g/g}$	Carotens $\mu\text{g/g}$	Chl a+b	Chl a/b
Control	129.81	26.05	36.38	155.86	4.9
DDW	239.95	47.12	49.02	287.07	8
DDW+M1	362.21	67.97	80.05	430.18	5.3
M1	1019.11	191.27	322.86	1210.38	3
M2	1590.05	277.99	372.64	1868.04	5.7
P1	889.38	162.47	235.22	1051.85	2
P2	1550.32	274.04	301.64	1824.36	5.4
					7
					6

CONCLUSIONS

It was found that the addition of spruce bark aqueous extract with concentration of 130 mg GAE / L, in the growth of explants of *Lavandula angustifolia* Mill, an increase in the elongation of the main stem, number of leaves formed, the amount of photoassimilating pigments and causes the phenomenon of shoots. At a higher concentration of the extract (26 mgGAE/100g) values are lower.

The hemp shives aqueous extract has a stimulating effect, in particular, the phenomenon of, the shoots, the amount of photoassimilating pigments and the number of leaves formed. Also a higher concentration values of consequently decreases. Deuterium depleted water increases the the amount of pigments compared with control

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¹ “Gheorghe Asachi” Technical University, Faculty of Chemical Engineering and Environmental Protection, 73 Prof. Dr. Doc. Dimitrie Mangeron Street, 700050, Iasi, Romania

² “Al. I. Cuza” University, Faculty of Biology, Vegetal Biology Department, Bulevardul Carol I, Nr. 11, 700506, Iasi, Romania

* tanase.corneliu@yahoo.com