THE INFLUENCE OF NATURAL AROMATIC COMPOUNDS ON THE DEVELOPMENT OF *LYCOPERSICON ESCULENTUM* PLANTLETS

Anca Bălaş* and Valentin I. Popa

The influences of flax lignin and phenolic compounds obtained from spruce bark on the development of *Lycopersicon esculentum* plantlets were evaluated. Depending on the applied treatment and the concentrations used, the natural aromatic compounds had stimulatory effects on the germination capacity and the plantlets height and leaf area. The influence of lignin on *Lycopersicon esculentum* was lower compared to the phenolic extract.

Keywords: Spruce bark, Polyphenolic compounds, Flax lignin, Lycopersicon esculentum

*Contact information: Technical University "Gheorghe Asachi", Iasi, Bd. Mangeron 71, 700050, Romania, Department of Natural and Synthetic Polymers; *Corresponding author:* abalas@ch.tuiasi.ro

INTRODUCTION

In recent years, a special concern has been manifested towards "green chemistry." Some of the effort has been based on the use of new waste sources, with the aim to obtain biologically active compounds which can be applied in different fields. These natural compounds are compatible with the environment and could provide the sources for specialty chemicals (Popa et al. 2006). In this context wood bark, which represents an important part of the tree, resulting in large amounts after the processing of logs for the pulp and paper industry, could be taken into account as a valuable raw material. The majority of this raw material is used as fuel, since its processing is expensive due to transport and maintenance costs (Hemingway 1997). Based on the present knowledge of the bark components, this raw material can be used as a source of various separated extractives with important application potential. Thus we proposed a procedure which allows turning to good account the wood bark after a scheme of complex processing (Simionescu et al. 1987) assuming the separation of each component. In the first stage, it was possible to separate polyphenols, which can be used as substitutes for phenols or phenolic resins in the wood processing industry (Simionescu et al. 1988a). Lignocelluloses resulting from extraction may represent products which can be introduced in composite structures, after different treatments (Simionescu et al. 1988b). We have also demonstrated that crude spruce extract may have a positive influence on seed germination and plants growth, manifesting similar effects to the endogenous hormones auxins and cytokinins (Simionescu et. al. 1991, Popa et al. 1996, Popa et al. 2002, Bălaş et al. 2005).

On the other hand, lignin resulting from the chemical processing of wood or from lignocelluloses after polyphenols extraction is known to be involved in a series of chemical transformations modifying the soil fertility. In this process, the soil microorganisms have important roles in these processes, due to their capability to modify and metabolize these compounds, with the formation of humic acids (Popa 1983). We observed that the introduction of lignin in sandy soil had a favorable influence on the growth of *Phaseolus vulgaris* plantlets, stimulating the growth speed and the quantity of green and dry biomass, as well as the productivity of obtained seeds (Dumitru et al. 2003).

In this paper, the results concerning the influence of spruce bark polyphenols and flax lignin on *Lycopersicon esculentum* plantlets are presented. The effects of polyphenolic extract were tested using different addition times: at the beginning of the experiment and in constant doses. The percentages of established plantlets, the variation of height, and leaf area during the experimental time were evaluated.

EXPERIMENTAL

Obtainment of Phenolic Compounds

The phenolic extract was obtained according to Simionescu et al. (1989). Spruce bark, provided by a Romanian pulp and paper company, was air-dried and ground in a mill. The lipophilic compounds were extracted in a Soxhlet apparatus with ethyl ether. The phenolic compounds were separated with 1.5% NaOH, for three hours at 90°C, and at a liquor-to-wood ratio of 10. The liquid fraction was passed over an ion-exchange resin (Vionit SR3) in order to obtain a neutral pH for the extract and to eliminate the sodium ions. After filtration, the neutralized extract was dried under vacuum at 40°C, obtaining a brown colored powder.

Lignin used in the experiments was obtained from flax alkaline delignification and contained 3.07 % hemicelluloses (Granit - Lignin, Switzerland, type PF 30-35 Flax Soda Pulping).

Bioassay

Seeds of *Lycopersicon esculentum* L. cv. A106/25, collected during 2004, were commercially available products. The seeds were planted in vegetable pots in sand, 20 variants per sample. The plantlets growth was followed using aqueous solutions of the spruce extract at different concentrations: 0.04, 0.08, 0.16, and 0.32 g/L and diverse additions of bioactive compounds:

- Single treatment with crude extract and every 5 days with distilled water.
- Repeated treatment with phenolic extract.

The influence of lignin on plantlets growth was tested at different dosages: 100, 150, 200, and 250 kg/ha. In both cases, control tests were performed under the same conditions using distilled water.

Plantlets development was observed for 30 days from seedling, and the number of established plants, their height, and leaf area were followed. The leaf area was determined according to Schwarz et al. (2001).

In the graphics, data are reported as percentage differences from control. Thus, zero represents the control; positive values represent stimulation of the parameter studied, and negative values represent inhibition. The characteristics for the control samples are presented in table 1.

Table 1. Parameters of the control samples

Period (days)	15	20	25	30
Height (cm)	2.29	2.65	3.23	3.53
Leaf area(cm^2)	0.05	0.09	0.14	0.15

Statistical Analysis

Statistical analysis was performed using ANOVA one way test and the level of significance was set at $P < 0.05$.

RESULTS AND DISCUSSION

The Influence of Spruce Extract on Plant Growth

The global extract had different influences on the *L. esculentum* plantlets, depending on the concentrations of bioactive compounds and the treatment applied during the experimental period.

Single treatment with spruce extract at a concentration of 0.04 g/L stimulated the number of established plants by 10% (Fig. 1 A). The concentration of 0.08 g/L induced a strong inhibitory effect on the number of established plantlets, but increasing the concentration of bioactive compounds reduced the studied characteristic.

Repeated treatment with phenolic extract had lower influence on the germination capacity in comparison to the first experimental lot (Fig. 1 B). In this case, the increase of concentration was correlated with the inhibition of the established plantlets.

The single treatment with phenolic compounds at concentration 0.04 g/L increased the plantlets' height by a factor of 20 %, while the other concentrations tested had slight effects (Fig. 2 A). The continuous treatment with spruce extract had strong stimulatory effects at concentrations 0.04 g/L and 0.08 g/L, and inhibitory influence at a concentration 0.16 g/L (Fig. 2 B).

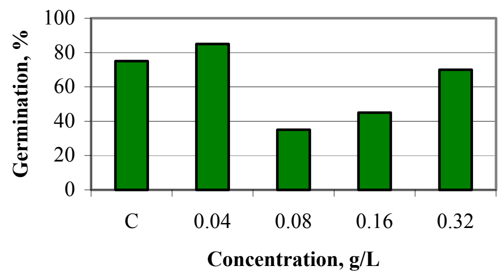
The leaf area was estimated using a mathematic model, without destruction of the plant. In the first experimental group, the leaf area was stimulated at a concentration of 0.08 g/L, while the other concentrations tested had slight effects of the studied feature (Fig. 3 A). The repeated treatment with solutions containing 0.04 g/L and 0.32 g/L spruce extract had stimulatory effects on leaf area (Fig. 3 B). The concentration of 0.16 g/L in bioactive compounds induced a strong inhibitory effect.

The single treatment with phenolic extract had a positive influence on the established plantlets of *L. esculentum*, while the repeated use of polyphenols had better influences on plantlets' height and leaf area.

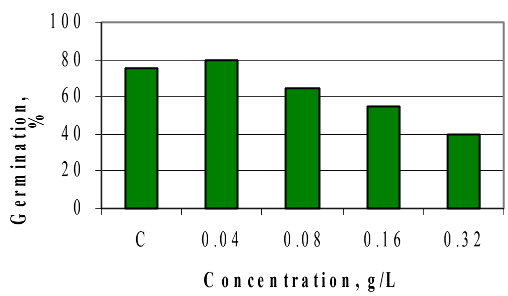
Due to the complex composition of the spruce bark extract, the growth processes can be influenced by many factors, including the synergism of polyphenols or the presence in small amounts of compounds with strong bioactivity. However, the influence of every compound from spruce bark has not been established, making it difficult at this moment to elucidate the mechanism in which the growing processes in plants are modified.

Our previous data (Simionescu et al. 1991) showed that polyphenols extracted from spruce bark have some effects similar to endogenous plan hormones, depending on the dosage used in the experiments of cells tissue culture. At present we have obtained information about the composition pf polyphenols existing in crude spruce bark extracts (Bălaş A. unpublished data). At the same time, some experiments are developed to establish a correlation between the structure of polyphenols and their capacity to influence plant development and different enzymatic system biosynthesized by plants.

Therefore, it is possible for polyphenols to interact or interfere with hormones produced by plants, influencing thus growth process. One cannot exclude the possibility that polyphenols may be transformed or destroyed at the soil level during plant development.



A.



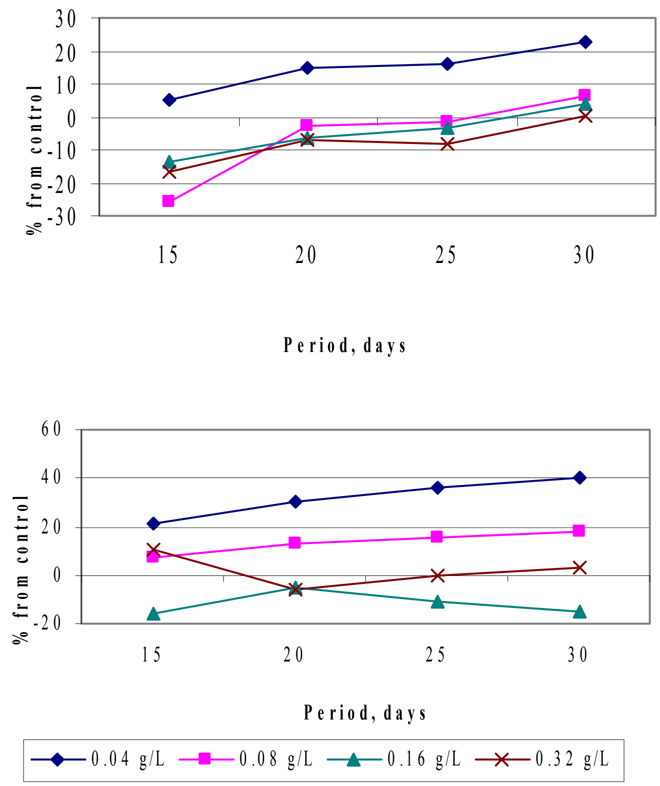
B.

Concentrations	0.04 g/L	0.08 g/L	0.16 g/L	0.32 g/L
A	P<0.01	0.01<P<0.05	0.01<P<0.05	P>00.5
B	P>00.5	0.01<P<0.05	0.01<P<0.05	P<0.01

Figure 1. The influence of spruce extract on established plantlets at different treatment: single (A) and repeated (B). F values for each concentrations used and treatments.

The Influence of Lignin on Plant Growth

The presence of lignin at dosages of 100 kg/ha and 200 kg/ha had stimulatory effects on the number of established plants (Fig. 4). The growth speed of *L. esculentum* plantlets was accelerated in the first 20 days of the experiments, but reduced in the next 10 days (Fig. 5). After 30 days, the plantlets' height had increased by 10 % at dosage 150 kg/ha and with 5 % for concentrations 100 kg/ha and 200 kg/ha. The addition of 250 kg/ha of lignin had a slight inhibitory effect on the plantlets' height. The most significant influences on leaf area were observed at concentrations 100 kg/ha and 200 kg/ha. These results are in concordance with those previously obtained in the case on bean cultivation (Dumitru et al. 2005). In this case, some observations have been made concerning the accumulation and developing of some microorganisms in sandy soil treated with lignin.



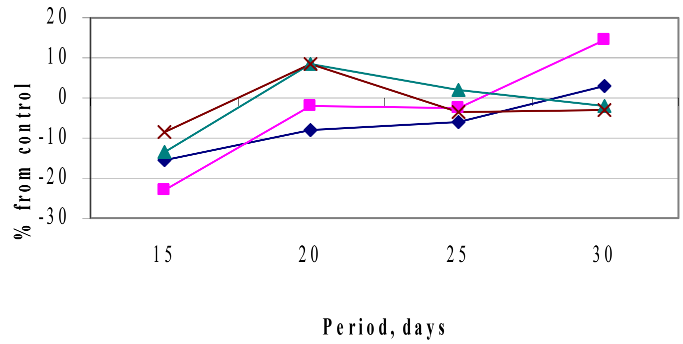
A.

B.

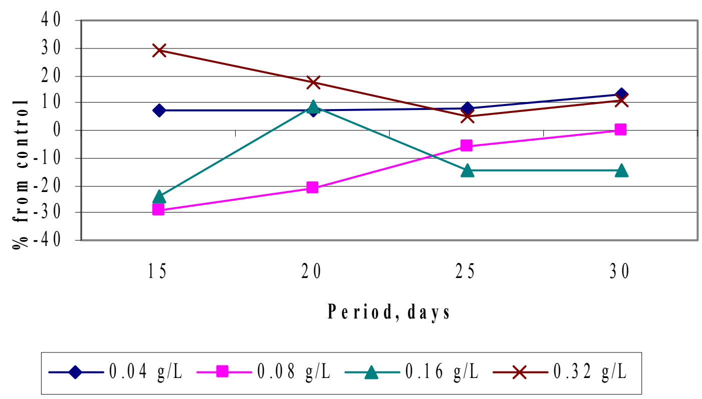
Figure 2. Variation of plantlets' height compared to control for single (A) and repeated (B) treated with spruce extract. Values are presented as percentage differences from control. P<0.01 (A) and 0.01<P<0.05.

At the same time cultivation of the plants and following the whole vegetation cycle allowed us to obtain information regarding the productivity. Evidence of an interesting phenomenon, from genetic point of view, was observed, concerning the regulation of cell division.

The influence of lignin on plants' development was reduced in comparison to the polyphenolic extract, and these effects can be correlated with its low accessibility in medium. The modifications of the studied parameters can be explained by the structural lignin modification under the influence of soil microorganisms, which can lead to the formation of degradation products with stimulatory effects on plant growth. Previous data showed that the presence of lignin has a beneficial influence on plantlets development even after a short period of time, (Dumitru et al. 2003; Popa et al. 2005). Also, the plantlets' growth may be influenced by the hemicelluloses content of the lignin extract, which can be degraded by soil microorganism, thus contributing to the fertilization.

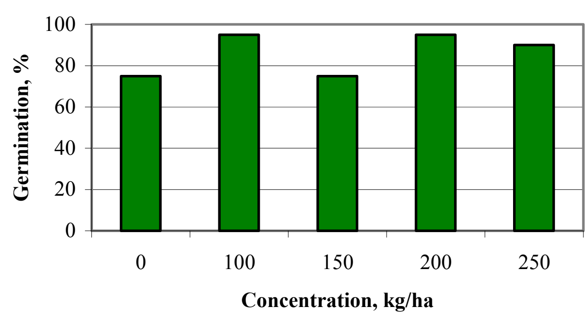


A.



B.

Figure 3. Variation of leaf area compared to control for single (A) and repeated (B) treatment with spruce extract. Values are presented as percentage differences from control. $P<0.01$



Concentrations	150 kg/ha	150 kg/ha	200 kg/ha	250 kg/ha
F values	$P<0.01$	$P>0.05$	$P<0.01$	$P<0.01$

Figure 4. The influence of lignin on established plants of *L. esculentum*. F values for each concentrations used and treatments.

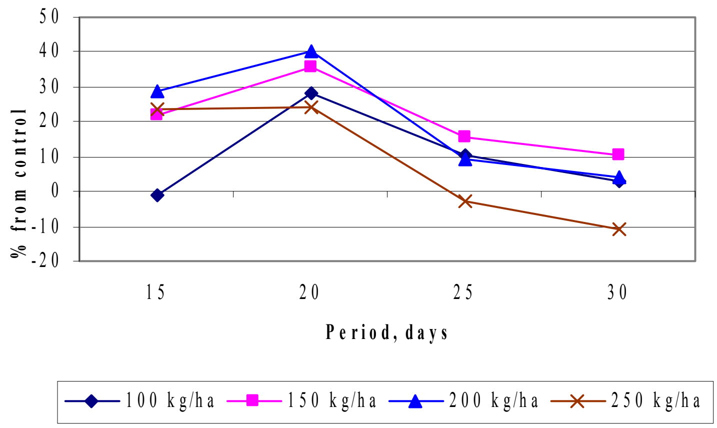


Figure 5. Variation of plantlets' height under the influence of lignin. Values are presented as percentage differences from control. P<0.05.

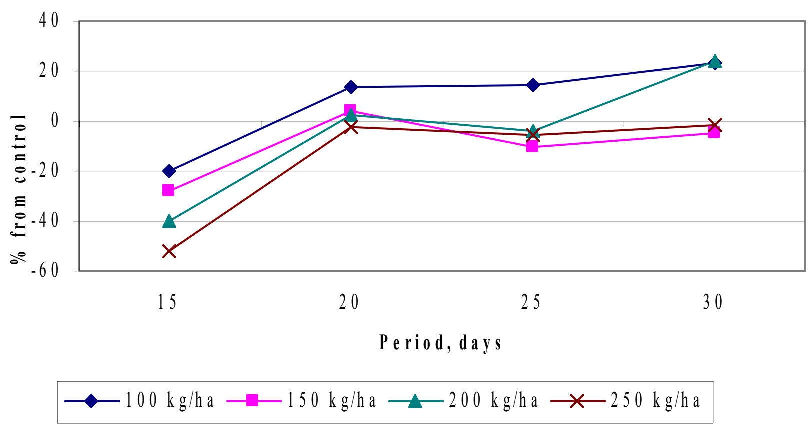


Figure 6. Variation of leaf area under the influence of lignin. Values are presented as percentage differences from control. P<0.05.

CONCLUSIONS

1. The single treatment with spruce bark extract had positive effects on the number of established *L. esculentum* plantlets.
2. Repeated treatment with polyphenols had a beneficial influence on the plantlets' height and leaf area.
3. In both cases, at concentrations of 0.04 g/L the bioactive compounds induced better development of *L. esculentum* plantlets compared to the control.
4. Lignin had a positive influence on the plantlets growth at 100 kg/ha and 200 kg /ha, but lower compared to spruce extract.
5. The obtained results will be further developed with the aim of establishing the role of polyphenols in biosynthesis processes and their influence on genetic characteristics. A correlation of these parameters with structure of polyphenols will be investigated.

REFERENCES CITED

Bălaş, A., Dănăila, M., Popa, V. I., and Anghel, N. (2005). "On the behavior of natural polyphenolic products as plant growth regulators," *Buletinul I. P., Seria Chimie şi Inginerie Chimică*, LI (LV), 3-4, 121-125.

Dumitru, M., Popa, V. I., Obreja, I., and Campeanu, M. M. (2003). "The influence of some lignin products on the metabolic processes of plants," *Buletinul I. P.*, XLIX (LIII), 1-2, 109-118.

Hemingway, R. W. (1997). "Wood residues into revenue," *Residual Wood Conference Proceeding*, Richmond, 80-85.

Popa, V. I. (1983). "Technologies of lignin upgrading" (in Romanian), I.P.I., 91-93.

Popa, V. I., Beleca, C., Popa, M., Bodarlau, R., Bara, I. I., and Truta E. (1996). "Studies regarding the behaviour of some individual biological products action," *Bol. Soc. Brot.*, 67 (2), 245 -255.

Popa, V. I., Agache, C., Beleca, C., and Popa, M. (2002). "Polyphenols from spruce bark as plant growth regulator," *Crop Res.*, 24 (2), 398-406.

Popa V. I., Volf I., Anghel N., and Dumitru M. (2005). "Lignin and polyphenols as allelochemicals," *Proceedings of Seventh Forum of International Lignin Institute*, Barcelona, 69-70.

Popa, V. I., and Volf, I. (2006). "Green chemistry and sustainable development," *Enviromental Engineering and Management Journal* 5(4), 545-558.

Schwarz, D., and Kläring, H. S. (2001). "Allelometry to estimate leaf area of tomato," *J. Plant. Nutr.*, 8(24), 1291-1309.

Simionescu, Cr. I., Bulacovschi, J., Petrovici, V., Popa, V. I., Chiş, E., Nicolau, F., and Rusan, V. (1984). "Researches dans le domaine de la mise en valeur de l'écorce de bois. L'extraction alkaline," Bulletin de Liaison no. 12 du Groupe Polyphenols. Narbonne, France, Compte-rendu des Journées Internationales d'Etude et de L'assemble Générale, Plovdiv – Bulgarie.

Simionescu, Cr. I., Rusan, V., and Popa V. I. (1987). "Options concerning phytomass valorification," *Cellulose Chem. Technol.* 21(1), 3-16.

Simionescu, Cr. I., Bulacovschi, J., Popa, V. I., Popa, M., Nuta, V., and Rusan V. (1988a). "New possibilities of using alkaline extracts form vegetable biomass in adhesive systems for wood industry," *Holzforschung u. Holzverwertung* 40 (6), 136-140.

Simionescu, Cr. I., Popa, V. I., Rusan, M., Vitalaru C., and Rusan V. (1988b). "Biodegradation of vegetal materials under the action of some mixed culture of microorganisms," *Cellulose Chem. Technol.* 22(3), 293-301.

Simionescu, Cr. I., Popa, V. I., Rusan, V., Colf, V., Zanoaga, C. V., Marinescu, C., Bara I. I., and Vantu S. (1991). "Study on the action of some wood bark polyphenolic compounds upon plant growth," *Cellulose Chem. Technol.* 24(3-4), 189-197.

Article submitted: April 27, 2007; First round of reviewing completed: May 28, 2007; Revised article received: June 18, 2007; Article accepted: June 18, 2007; Published: June 18, 2007