ALLELOPATHIC EFFECTS OF SPRUCE BARK EXTRACTIVES ON SEED GERMINATION AND DEVELOPMENT OF *LYCOPERSICON ESCULENTUM* PLANTLETS

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The allelophatic effects of spruce bark extract on seed germination and development of ***Lycopersicon esculentum*** *plantlets were examined. Spruce bark, provided by a Romanian pulp and paper company, was air-dried and ground in a mill. The phenolic compounds were separated with NaOH 1.5%, for three hours at 90°C, at a liquor-to-wood ratio of 10, with a preliminary extraction of lipophilic compounds with ethyl ether. After treatment with ion-exchange resins and filtration, the neutralized extract was dried, obtaining a brown colored powder. The experimental work included seed germination in Petri dishes with various concentrations of bioactive compounds. The biotests used aqueous solution of polyphenolic extract in concentrations of 0.12, 0.16, 0.2, 0.24, 0.28, 0.32 and 0.36 mg/L. The influence of the extractives was expressed through germination percentage, elongations of roots and shoots. The effects of spruce bark on plantlets growth were evaluated in laboratory conditions using vegetations pots with sand. In the experiments a global extract was used at the concentrations of 0.04, 0.08, 0.16 and 0.32 g/L. The tomato plantlets were treated with different additions in bioactive compounds: initial treatment with aqueous solutions of spruce extract, then every 5 days with distilled water and repeated treatment with aqueous solutions of spruce extracts every 5 days. The influence of phenolic compounds on* ***L. esculentum*** *plantlets was expressed through the number of established plants, their height and leaf area. The natural phenolic compounds influenced the growth of* ***L. esculentum*** *plantlets depending on the concentrations used and the experimental design.*

Keywords*: spruce bark, Lycopersicon esculentum, seed germination, plantlets development, leaf area*

Allelopathy is an important phenomenon concerned with the effects that chemicals of plant or microbial origin have on the growth and development of others plants or microorganisms in natural communities or agriculture systems [2]. New studies are based on the ways to enhance crop production and develop more sustainable agriculture, adapting allelochemicals as herbicides, pesticides and growth stimulants. Bark is a significant part of the tree and it is produced in large amounts after logs processing in pulp and paper industry. Based on the present knowledge of the bark components, this raw material can be used to separate various extractives with important application potential. Different studies demonstrated that global extract from spruce bark might have a positive influence on seed germination and plants growth, manifesting similar effects to the endogenous hormones auxins and cytokinins [1, 3, 6]

In this paper, the results concerning the influence of spruce bark extract on seed germination and *Lycopersicon esculentum* plantlets are presented. The influence of the extractives on seed germination was expressed through germination percentage, elongations of roots and shoots. The effects on plantlets development were tested using different addition times: at the beginning of the experiment and in constant doses during the experiment. The percentages of established plantlets, the variation of height and leaf area during the experimental time were evaluated.

MATERIALS AND METHODS

Plant extract The phenolic extract was obtained according to Simionescu et al. [5]. 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 solution of NaOH 1.5%, 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.

Bioassays Seeds of *Lycopersicon esculentum* L. cv. A106/25, collected during 2004, were commercially available products. For the experimental part, we used Petri dishes in 90 mm diameter with one sheet of filter paper as support. Germination and growth were conducted in aqueous solutions of the global extract of different concentrations: 0.12, 0.16, 0.2, 0.24, 0.28, 0.32 and 0.36 g/L. After the addition of 10 seeds and 5 mL of test solutions, Petri dishes were placed in a thermostat at 25 °C in the dark according to literature protocols [7]. Controls tests used the same number of seeds in distilled water.

L. esculentum seeds were planted on 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 during the experiment every 5 days.

Plantlets development was pursued 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. [4].

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. Germination rates of control samples in Petri dishes were 48 %, root lengths 6.75 cm and shoot lengths 5.01 cm. The characteristics for the control samples growth in vegetable pots are presented in table 1.

Table 1

Parameters of the control samples in vegetable pots

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

RESULTS AND DISCUSSIONS

The biological activity of the spruce extract was evaluated in bioassay on *L. esculentum* seeds. The influence of the global extract was pursued through germination capacity of the seeds, roots and shoots lengths. The results obtained showed that these compounds have different levels of activity depending on the concentrations used. The majority of the concentrations tested had inhibitory effects on seed germination, especially at 0.32 g/L in bioactive compounds (figure 1). The global extract had different influences on the roots and shoots elongations. The global extract had strong inhibitory influences on the roots length at higher concentrations with a maximum of 30 % at concentrations 0.36 g/L (figure 2). The shoot elongation under the action of spruce extract was stimulated, in all the studied cases. At concentration 0.28 g/L, the characteristic was with 15 % higher than control sample.

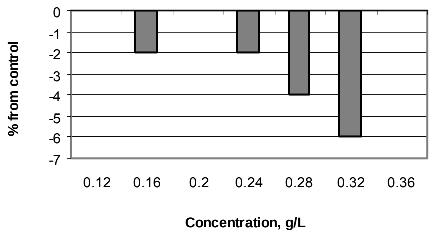


Figure 1. **The influence of the global extract on germination of *L. esculentum* seeds. Values are presented as percentage differences from control**

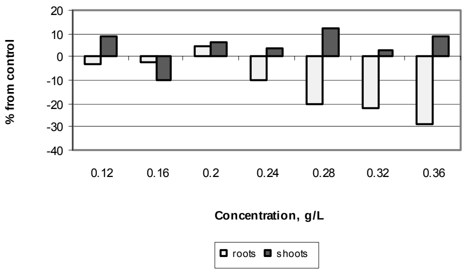
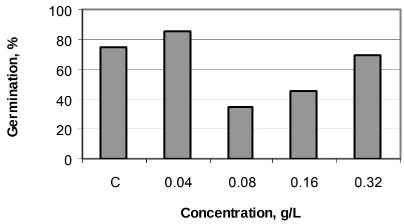


Figure 2. **The influence of the compounds extracted from spruce bark on roots and shoots elongation of *L. esculentum*. Values are presented as percentage differences from control**

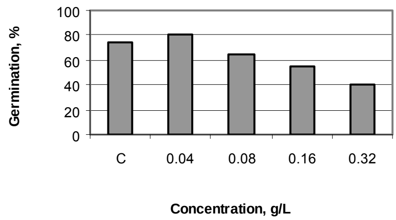
The global extract had influenced the *L. esculentum* plantlets depending on the concentration in bioactive compounds and the treatment applied during the experimental period.

Single treatment with spruce extract at a concentration of 0.04 g/L stimulated with 10 % the number of established plants (Fig. 3A). 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 reduces the inhibitions.

Repeated treatment with phenolic extract had lower influence on the germination capacity in comparison to the first experimental lot (Fig. 3B). In this case, the increase of concentration was correlated with the inhibition of the established plantlets.



A.

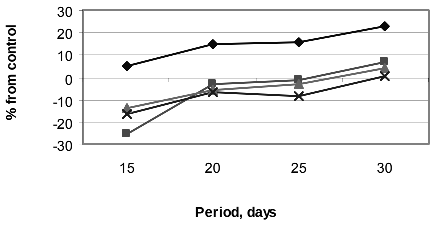


B.

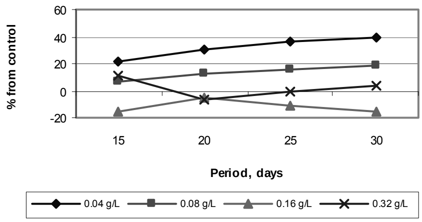
Figure 3. **The influence of spruce extract on established plantlets at different treatment: single (A) and repeated (B)**

The single treatment with phenolic compounds at concentration 0.04 g/L determined the growth of plantlets' height with 20 %, while the others concentrations tested had slightly effects (Fig. 4A). The continuous treatment with spruce extract had strong stimulatory effects at concentration 0.04 g/L and 0.08 g/L, and an inhibitory influence at concentration 0.16 g/L (figure 4B).

The leaf area was estimated using a mathematic model, without destruction the plant. In the first experimental group, the leaf area development was stimulated at a concentration of 0.08 g/L, while the others concentrations tested had slight effects on the studied feature (Fig. 5A). The repeated treatment with solutions containing 0.04 g/L and 0.32 g/L spruce extract had stimulatory effects on leaf area (Fig. 5B). The concentration of 0.16 g/L in bioactive compounds induced a strong inhibitory effect.

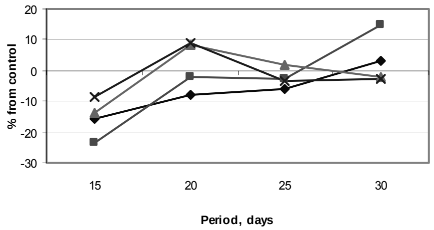


A.

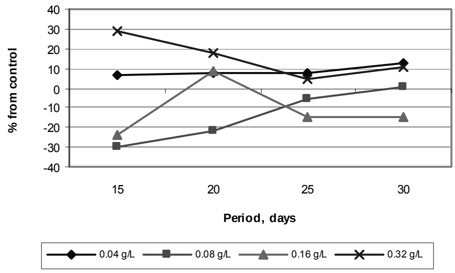


B.

Figure 4. **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**



A.



B.

Figure 5. **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**

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, it is not certain the influence of every compound from spruce bark, making difficult at this moment to elucidate the mechanism in which the growing processes in plants are modified. A possibility is that polyphenols can interact or interfere with hormones produced by plants, influencing thus growth process.

CONCLUSIONS

The global extract had slight or inhibitory effects on the germination of *L. esculentum* seeds. The global extract had strong inhibitory influences on the roots length at higher concentrations, while the shoots length was stimulated at the majority of used concentrations. The recommended concentration is 0.2 g/L bioactive compounds, taking into account the germination capacity and the roots and shoots elongations.

The single treatment with spruce bark extract had positive effects on the number of established *L. esculentum* plantlets. Repeated treatment with polyphenols had benefic influence on the plantlets height and leaf area. In both cases, at concentrations of 0.04 g/L in bioactive compounds induced better development of *L. esculentum* plantlets comparing to control.

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