

# EFFECT OF NATURAL PRODUCTS ON SOME PHYSIOLOGICAL PROCESSES OF SOYBEAN PLANTS (*GLYCINE MAX* L.)

## INFLUENȚA UNOR PRODUSE NATURALE ASUPRA UNOR PROCESE FIZIOLOGICE DIN PLANTELE DE SOIA (*GLYCINE MAX* L.)

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**Abstract.** *In this paper, are presented the results concerning the influence of deuterium depleted water (DDW) in combination with aqueous extract obtained from the spruce bark, on the growth and development of soybean plants. For this purpose, in the presence of distilled water (control), DDW, extract of spruce (SB) and spruce extract in combination with DDW, tests were performed for soybeans in vegetation pots. The influence of treatment was evaluated by gravimetric and biometric analysis, by quantifying photoassimilating pigments, photosynthetic activity, respiration intensity, transpiration intensity and substomatal carbon dioxide content. Results showed that in the presence of DDW and extract obtained from the spruce bark was a stimulation effect for biomass quantity and photoassimilating pigments synthesized. In the presence of spruce bark aqueous extract (26 mg GAE/100g) and spruce bark extract in combination with DDW photosynthetic and respiratory rate was high.*

**Key words:** *polyphenols, bioregulators, deuterium depleted water, photosynthesis, soybean.*

**Rezumat.** *În lucrare sunt prezentate rezultatele unui studiu în care s-a urmărit evidențierea rolului apei sărace în deuteriu (ASD) în combinație cu extractul apos obținut din coajă de molid în creșterea și dezvoltarea plantelor de soia. În acest scop au fost realizate teste în vase de vegetație în care s-au însămânțat semințele de soia în prezența apei distilate (martor), ASD, a extractului de molid și a extractului de molid în combinație cu ASD. Influența tratamentelor a fost apreciată prin efectuarea de analize biometrice și gravimetrice privind evoluția plantelor, precum și prin stabilirea conținutului în pigmenți fotoasimilatori, a activității fotosintetice, respiratorii, transpirației și conținutului substomatal în dioxid de carbon. Rezultatele au evidențiat că în prezența ASD și extractului obținut din coajă de molid se înregistrează o stimulare a cantității de biomasă acumulată dar și a pigmenților fotoasimilatori sintetizați. Prin determinarea ratei fotosintetice și respiratorii s-a constatat o intensificare a acestora în prezența extractului de molid cu concentrația 26 mg GAE/100g.*

**Cuvinte cheie:** *polifenoli, bioregulatori, apa săracă în deuteriu, fotosinteza, soia.*

### INTRODUCTION

Natural compounds and other plant interactions are part of allelopathy, commonly defined as any plant's or microorganism's direct or indirect, stimulatory or inhibitory effect toward other plants through various chemical

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compounds released into the environment. Allelochemicals are released into the environment through various mechanisms: volatilization from plant leaves, rhizosphere formation, bark extraction and residue decomposition (Rice, 1984). Studies on the mechanism by which polyphenolic compounds affect the metabolic processes of plants allow current research reorientation to an ecological agriculture (Lichtenthaler and Wellburn, 1983).

Thus, assessing the action of polyphenolic products of spruce bark extracts on tomato seeds germinated was a positive influence on the rate and capacity germination, seedlings growth and the amount of biomass accumulated. The stimulatory effect of the primary root elongation and hypocotyles, found in the presence of polyphenolic extracts at concentrations of 40, 200 mg / L in the growth medium, can be compared with those of auxins or cytokinins (Bălaș et al., 2005; Bălaș and Popa, 2007).

In the present study it was followed the physiological effect produced by polyphenolic extract from spruce bark in combination with deuterium depleted water on soybean plants that have developed in greenhouse conditions.

## MATERIAL AND METHOD

The experiment was conducted over a period of 60 days. Its beginning was to prepare the necessary materials following steps:

a) Imbibitions – soybeans seeds, were carefully selected to be without any deterioration. Then they were immersed in the test solution for 15 hours at a constant temperature of 25°C.

b) Sowing by 3 seeds / pot and 10 pots for each experimental variant.

c) Application of test solutions for their test solutions absorption was performed at intervals of 5 days, from the first day after sowing. This was done at radicular level. Experimental variants analyzed were organized into 5 experimental variants: deuterium depleted water (DDW) DDW mixed with spruce bark polyphenolic extract, with concentration of 26 mg GAE/100g (DDW + SB2), spruce bark polyphenolic extract with concentration 5 g / L (26 mg GAE/100g - SB1) and 10 g / L (52 mg GAE/100g - SB2) and control where water was applied.

To determine the intensity of photosynthesis, respiration, transpiration and substomatal CO<sub>2</sub> was used Non Dispersive Infrared (NDI). To determine these physiological processes of plants was used LCI device photosynthesis system (ADC BioScientific's).

Quantification of assimilating 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, and 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):

Chlorophyll a (µg/mL) = 12, 21 (A<sub>663</sub>) – 2, 81 (A<sub>646</sub>)

Chlorophyll b (µg/mL) = 20, 31 (A<sub>646</sub>) 5, 03 (A<sub>663</sub>)

Carotenoids (µg/mL) = (100· A<sub>470</sub> – 3,27 [chl a] – 104 [chl b])/22

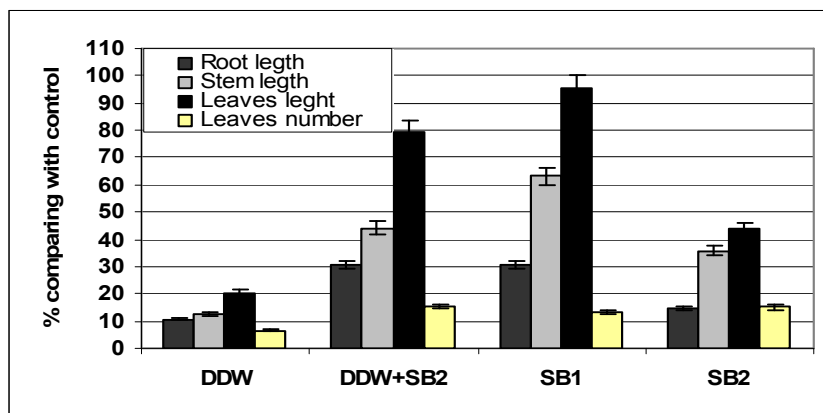
To determine the elongation and biomass accumulation ten plants/experimental version were separated into root, leaves and stems at 60 days after sowing. These parts of plants were oven dried at 65° C, and weighed. The results obtained as the average were reported (%) to the Control sample.

Our results are expressed as mean  $\pm$  standard error where  $n = 3$ . Comparison of the means was performed by the Fisher least significant difference (LSD) test ( $PB \leq 0.05$ ) 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

### *Vegetative organs elongation*

To determine the length differences between experimental variants for each organ vegetative biometric technique was applied. This was done after 60 days from sowing. The following indices were established: root length, stem length, length and number of leaves. The stimulation/inhibition effect was calculated in percentage compared with the control (this was for the case when distilled water was applied). Soybean plant roots in the DDW + SB2 and SB1 variants, has developed very well, with an increase in length growth of over 30 %. Analyzing the increase in length of the stem, it was observed significant stimulation in the SB1 (63.27%), SB2 (37.65%) and ASD + SB2 (43.2%) variants. For other variants, stimulating percentages are lower. Analyzing length and number of leaves, the stimulating effect was higher in the case SB1 variant (leaf length - 96.61%, leaf number - 12.76%). A significant stimulation of growth in length of leaves was observed for the plants developed in variant DDW + SB2 and DDW (20.21%) (Fig. 1).



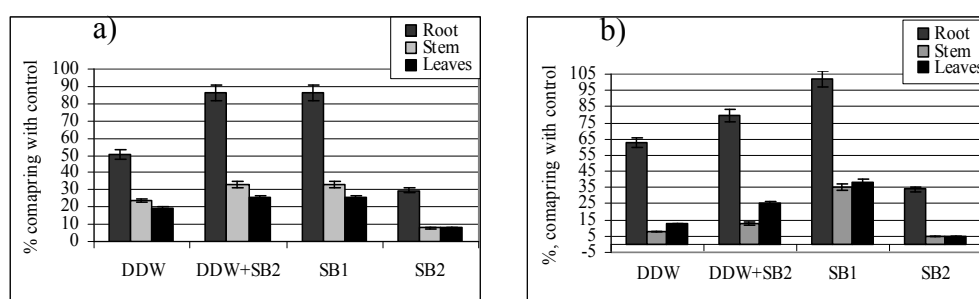
**Fig. 1** - The influence of DDW (deuterium depleted water), SB1 and SB2 (spruce bark aqueous extract) and SB aqueous extract in combination with DDW on soybean plant length growth

### *Biomass accumulation in vegetative organs*

Determination of fresh and dry weight plant biomass was performed by gravimetric method using analytical balance. These results underline once again allelopathic effect of deuterium depleted water and spruce bark aqueous extracts on soybean plants.

Analyzing Figure 2 we can observe a noticeable, increase in the amount of biomass from plants that have developed in the presence of spruce bark aqueous extract. The highest percentage of stimulation is obtained for the amount of biomass accumulated in roots of soybean plants for all experimental variants.

For the version with the lowest concentration in polyphenolic compounds (26 mg GAE/100g - SB1) were recorded the highest percentages of stimulation for accumulated amount of wet biomass (102.2% - root, 32.3% - strain, 33.12% - leaves) (fig. 2a). At a higher concentration of the extract (SB2), the percentage of stimulation is lower than SB1. The utilization of DDW + SB2 will increase the amount of biomass accumulated in all vegetative organs. The amount of dry biomass for this variant is higher than other alternatives (87.3% - root, 28.1% - stem, 20.1% - leaves) (Fig. 2b). The application of DDW increases the amount of biomass accumulated in all vegetative organs, but with lower percentages compared to variant DDW + SB2.



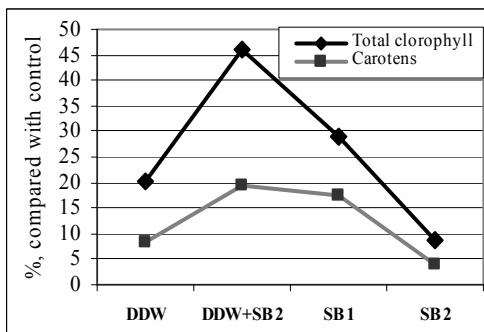
**Fig. 2** - The influence of DDW (deuterium depleted water) SB1 and SB2 (spruce bark aqueous extract) and SB aqueous extract in combination with DDW on soybean dry biomass accumulation

#### *Accumulation of photoassimilating pigments*

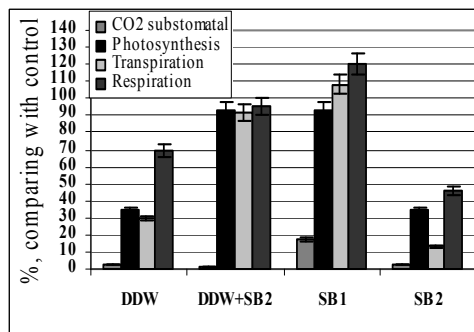
Analyzing the figure 3 we can observed stimulating effect on the amount of photoassimilating pigments accumulation in soybean plants, developed in case of SB1 and DDW + SB2 variants. The amount of total chlorophyll compared with the control, is higher for DDW + SB2 (46%) and SB1 (29.2%) variants. Polyphenolic extract with a concentration of 52 mg GAE / 100g, has a lower impact on the amount of pigments accumulation, compared with the values obtained for DDW, DDW + SB2 and SB1 variants.

#### *The intensity of photosynthesis, respiration, transpiration and CO<sub>2</sub> content*

By determining photosynthetic rate (Fig. 4) in plants subjected to treatment with various solutions there was a slight increase in all experimental variants. It is noteworthy result obtained for SB1 variant, where the percentage of photosynthetic intensity is with 92% higher compared with the control. Values recorded in variant DDW + SB2 are close to those recorded in SB1 version.



**Fig. 3** - Photosynthetic pigments content ( $\mu\text{g/g}$ ) for soybean plant grown under polyphenolic extracts and deuterium depleted water effect



**Fig. 4** - Physiological indices for soybean plant grown under polyphenolic extracts and deuterium depleted water effect

If the follow the obtained data, we can found an increase in the breathing process for soybean plants in all experimental variants with higher percentages for SB1, DDW + SB2 and DDW variants.

Noting the role of DDW and spruce bark aqueous extract on the intensity of transpiration, from the results, we find that the soybean plant to stimulate transpiration percentage for all experimental variants, especially for SB1 (120.1 %) and DDW + SB2 (94.2%) variants. We can see that the removal of carbon dioxide in the breath is correlated with the removal of water from the plant; these processes are more pronounced in plants treated with polyphenolic extracts obtained from spruce bark having 26 mg GAE/100g concentration. Regarding substomatal CO<sub>2</sub> content, there is a significant increase in soybean plants in case pf SB1 variant, compared with control.

## CONCLUSIONS

1. The aqueous extract obtained from the spruce bark, caused an increase in the amount of biomass, the amount of photosynthetic pigments as following due to increased photosinthehtical activity.
2. It is found that for soybean plants the percentage of stimulation transpiration intensity for all experimental variants is high, especially in the case of DDW + SB2 and SB1 variant.
3. It was observed that the intensity of respiration is higher in soybean plants that were treated with spruce bark aqueous extract with 26 mg GAE/100g concentration.
4. The information regarding the effects of polyphenolic compounds and deuterium depleted water on plant growth and development are important in determining their potential to be used in agriculture as natural growth bioregulators.

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