THE EFFECT OF SPRUCE BARK POLYPHENOLS EXTRACT IN COMBINATION WITH DEUTERIUM DEPLETED WATER (DDW) ON *GLYCINE MAX L.* AND *HELIANTHUS ANNUUS L.* DEVELOPMENT

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Keywords: deuterium depleted water (DDW), soybean, spruce bark, sunflower, polyphenolic compounds.

Abstract: The aim of this study was to evaluate the effect of spruce bark aqueous extract and deuterium depleted water (DDW) as bioregulators on the plant growth *Glycine max L*. and *Helianthus annuus.* The following specific parameteres were closely monitorised: germination energy and germination capacity, plants vegetative organelles growth and development and photoassimilatory pigments concentrations. The results have shown that DDW presents different effects depending on tested plant species. In the case of soybean, DDW presented stimulatory effects on both germination energy and capacity, radicles elongation, primary leaves growth and development but inhibitory effects on photoassimilatory pigments. Spruce bark extract reduced the germination capacity of soybean seeds, but accelerated the germination process of sunflower seeds and present stimulatory effects on plantlets biomass accumulation. The combination of DDW with *Picea abies* polyphenolic extract promoted soybean plantlet elongation, especially the rootlets ones and stimulated green biomass accumulation for both soybean and sunflower plantlets. Analyzing the photoassimilatory pigments concentration for sunflower, it can be observed an increasing trend (almost 100% comparing with control) when introduce into the growth medium DDW and *P. abies* polyphenolic extract. DDW and *P. abies* bark extract have shown an important role in plant growth and development, improving photoassimiliation process.

INTRODUCTION

Polyphenolic compounds are the most important classes of secondary metabolites that play an important role in the biosynthesis process. Natural bioactive compounds have a broad spectrum of both the plant as a whole and on tissues and organs, interfering in the metabolic processes (Anghel et al., 2001). Through, the characteristic biological activity, natural polyphenols are essential compounds in the stimulation of plants growth and development. The stimulation or inhibition capacities on the plant growth and development is closely correlated with concentrations of polyphenolic compounds applied. Thus, in some cases the presence of these compounds in low concentrations can have a beneficial effect on the plants development and in other cases, when concentrations are high there is an inhibition phenomenon (Popa et. al., 2007). The aim of this study was to establish the effect of polyphenolic extract from the spruce bark, the DDW and the mixture of polyphenolic extract and DDW, on some physiological processes occurring in plants. The researches undertaken in recent years, which were aimed at finding new biostimulating products compatible with the ambient environment, drew the attention on the possible involvement of aromatic natural products, isolated from biomass to reagent chemicals, in metabolic processes of plant. It was established that spruce bark aqueous extract, rich in polyphenolic compounds, has a stimulating effect in the processes of germination, growth and development of seedlings of rape and soybean (Stîngu et al., 2010, Ignat et al., 2009).

On the other hand, deuterium depleted water or light water is a distilled water microbiologically pure, with an isotopic concentration of 25 ppm, obtained by isotopic distillation, in vacuum, of natural water with an isotopic concentration of 145 ppm D / (D + H) (Somlyai., 2001). In Romania DDW is obtained in two centers: the heavy water plant at Halanga, where daily discharge as waste tons at the DDW and INCDTCI of Ramnicu Valcea where is obtained in special installation get built. Recent research has shown that DDW has a great influence on living organisms, namely in developing cells and tissues and changes that occur in normal water features lead to significant changes in fundamental processes of cells. Some of the main properties of DDW in living organisms are: influence on the development and multiplication of cells, influence on cellular transport, DNA synthesis and also has antioxidant properties (Somlyai., 1993, Olariu et al., 2007). Since the studies on the influence of DDW in plant systems are not representative, it was necessary to develop this subject.

In this context, we analysed the effects of DDW and in combination of it with P. abies extract on bean and sunflower plant growth and development. The following specific parameteres were closely monitorised: germination energy and germination capacity, plants vegetative organelles growth and development and photosynthesizing pigments concentrations.

MATERIALS AND METHODS

Deuterium depleted water was purcheased from INCDTCI Râmnicu Vâlcea, Romania. To obtain an aqueous polyphenolic extract the spruce bark of industrial origin was used as a vegetable raw material. After drying at room temperature and under conditions of normal aeration, the bark was ground, followed by a new stage of drying.

1. Extraction. Ground spruce bark was subjected to extraction using procedure properly on aqueous extraction, namely: 5 g dried vegetal material is brought into a 250 mL Erlenmeyer flask in which there were 125 mL distilled water. Erlenmeyer flask was covered with a watch glass and heated on a water bath so that the temperature in the vessel to be 85-90 ° C. Leave it at that temperature for 45 min., shaking from time to time. The material is allowed to settle and passed the clear solution through a crucible of glass or porcelain funnel. This operation was repeated 3-4 times until a colorless extract was obtained. All extracts are cumulated in a 500 mL volumetric flask and make up to volume mark with distilled water (Rozmarin et al., 1984). Polyphenolic extract was used in two concentrations: 0.5 and 1 g of plant material in 100 ml distilled water. The polyphenolic aqueous extract it was characterized in terms polyphenols total contents. Thus, for 1 g vegetal material in 100 ml distilled water was recorded 130 mg / L total content in polyphenols (Stingu et al., 2010).

2. Germination tests were carried out going through a standard procedure, using a number of 5 Petri dishes for each solution studied (distilled water - control, DDW, extract of spruce, and spruce bark extract in combination with distilled water / DDW). On a filter paper were placed every five soybean seeds, carefully selected to no present major damage. For starters, the vegetal material has undergone a process presterilization, which consisted of submerged seed absolute ethanol for 10 seconds, following the sterilization in the presence of sodium hypochlorite 10% for 20-30 minutes (Cachita et al., 2004). The volume of solution added was 10 mL / dishes. Petri dishes thus prepared were incubated in the dark in a thermostat set at 27 º C. After a period of seven days, Petri dishes were kept in daylight for 3 days to allow the seedlings to synthesize assimilatory pigments. Finally, the biometric and quantitative measurements on components of seedlings (root, stem, primary leaves) and spectrophotometric measurements were carried out to determine the concentration of photoassimilatory pigments.

3. 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, 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 663) - 2.81 (A 646)
Chlorophyll b (µg / mL) = 20.31 (A 646) 5.03 (A 663)
Carotenoids (µg / mL) = (100 • A 470-3.27 [chl a] - 104 [chl b]) / 22

RESULTS AND DISCUSSIONS

After measurements made on seeds and seedlings of soybean and sunflower it can see the different influence of test solutions according to plant species subjected to experiment. Thus, form resulting energy and capacity germination of soybeans it was observed stimulation of these samples where tested solution was deuterium depleted water. When added to the germination medium, spruce bark aqueous extract or in combination with deuterium depleted water is found a reduction in germination energy and no influence on capacity germination (Fig. 1 and 2).

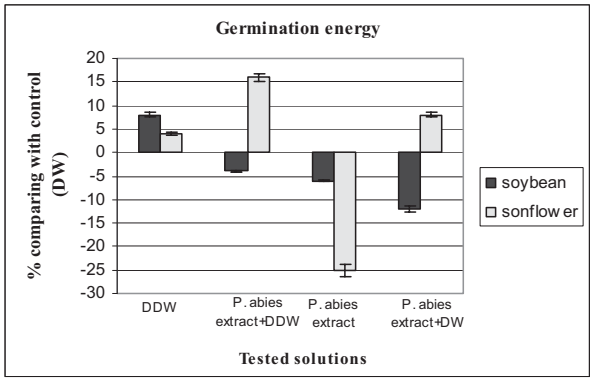

Fig. 1.The influence of DDW and *P. abies* aqueous extract in combination with DDW on seed germination energy of *Glycine max L.* and *Helianthus annuus L.* plants

For sunflower seed there was little different situation. Thus, it was observed that the mixture of DDW and spruce bark aqueous extract (1:1) accelerates seed germination up to 16% and increases the number of seeds germinated by 15% compared to control. Also, stimulation of germination energy and capacity is recorded, and when applied in the germination medium, the spruce bark aqueous extract with 0.5% concentration. When the extract concentration is higher (1%), germination energy and capacity is reduced by 25 and 10 percent for the samples where distilled water was added (Fig. 1 and 2).

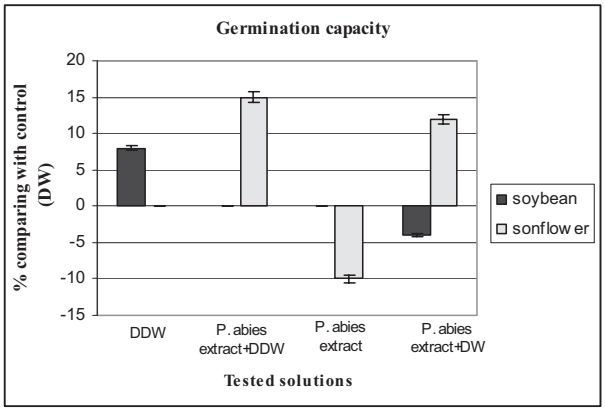

Fig. 2.The influence of DDW and *P. abies* aqueous extract in combination with DDW on seed germination capacity of *Glycine max L.* and *Helianthus annuus L.* plants

Analyzing the influence of test solutions on vegetative organs elongation, there is a stimulating for both plant species considering for experiments. The most significant influence, as shown in figure 4, there was registered for the sample treated with 1% spruce bark aqueous extract. Thus, the percentage of root and stem elongation stimulation of sunflower seedlings, increases to 54% and 31%. In this case, must highlighted differences in the growth of sunflower seedlings were observed, depending on the concentration of polyphenolic extract applied. Therefore, a concentration of 1% of polyphenolic extract, triggers a stimulation of growth process, compared with a concentration of 0.5% for which the influence where reduced, or even more, weak inhibitors was observed. A positive influence on this index was recorded when the distilled water of growth medium, was substituted with DDW or mixed with spruce bark aqueous extract. In this case it was observed a significant stimulation effect for all vegetative organs of sunflower seedlings growth, especially radicle and primary leaves (Fig. 3).

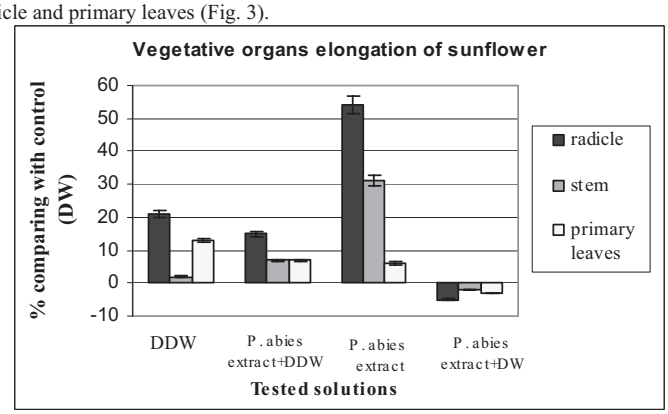

Fig. 3.The influence of DDW and *P. abies* aqueous extract in combination with DDW on vegetative organs elongation of *Helianthus annuus L.* plant.

Spuce bark aqueous extract in combination with deuterium depleted water, increases significantly soybean seedlings elongation, especially radicle (38%). A positive influence is recorded for separate application at the two

solutions, but it is lower than the application in the mixture. Noting the increase in length of soybean seedlings, there is a difference not of it depending on the concentration spruce extract applied samples (Fig. 4).

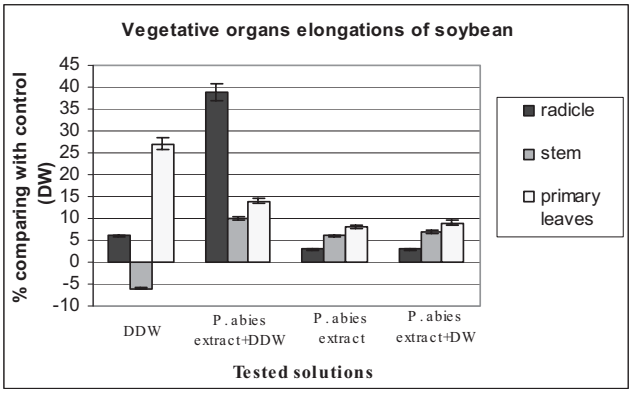

Fig. 4.The influence of DDW and *P. abies* aqueous extract in combination with DDW on vegetative organs elongation of *Glycine max L.* plant.

The observations outlined in the analysis results in increasing the length of vegetative organs which are also confirmed by the results obtained in the case of vegetal biomass for soybean and sunflower seedlings. Analyzing figure 6 it could be observed that the vegetal biomass of sunflower seedlings was significantly higher in the presence of polyphenolic extract, comparing with control (68% - radicle, 30% - stem, 67% - primary leaves). Also, in this case, the difference in concentration of polyphenolic extract is followed by differences in growth and development of sunflower seedlings. The incentive effect is recorded, when DDW, respectively DDW and polyphenolic extract mixture is applying into the growth medium. Spruce bark aqueous extract in combination with deuterium depleted water, increases vegetal biomass accumulation in all vegetative organs of soybean seedlings (17% - radicle and stem, 19% - primary leaves). Soybean seedlings, which were developed in the presence of deuterium depleted water, have accumulated a high amount of primary leaves vegetal biomass with 27% more than the amount accumulated in control seedlings (Fig. 5). The effect of polyphenolic extract, on vegetal biomass accumulation was lower in soybean seedlings. It diminishes proportionally with decreasing polyphenolic extract concetration.

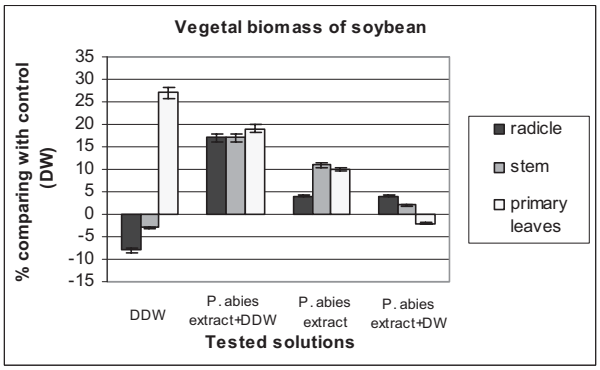

Fig. 5.The influence of DDW and *P. abies* aqueous extract in combination with DDW on vegetal biomass accumulation of *Glycine max L.* plant.

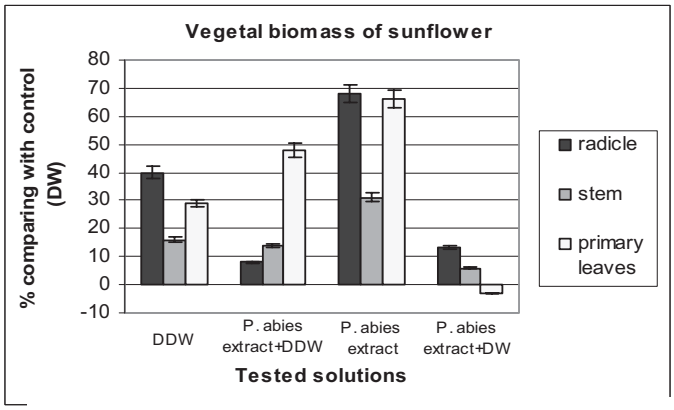

Fig. 6.The influence of DDW and *P. abies* aqueous extract in combination with DDW on vegetal biomass accumulation of *Helianthus annuus L.* plant.

Analyzing photoassimilating pigments synthesis, it was found that it was reduced in the the presence of DDW (Fig. 7). On contrary to sunflower seedlings, deuterium depleted water stimulates the photoassimilating pigments synthesis in a high percentage (45% - chlorophyll "a", 98% - chlorophyll "b", 68% - carotenoids). Following the influence of polyphenolic extract, it was registered a lower stimulation effects for the photoassimilating pigments of synthesis in soybean seedlings (Fig. 7), but a high stimulation effects in case of sunflower seedlings (Fig. 8). For all three tested solutions, charactherized by the presence of spruce bark aqueous extract, which was applied in sunflower seedlings growth medium, it was found a clear increase in photoassimilating pigments content. As it could be observed in figures 7 and 8, the supplementation of soybean and sunflower seedlings growth medium with spruce bark polyphenolic extracts stimulates clorophyll and carotenoids pigments biosynthesis process.

A concentration of 0.5% spruce bark extract into the growth medium increases the amount of chlorophyll 'a' with 54%, chlorophyll "b" with 121% and 55% the total carotenoid pigments concentrations.

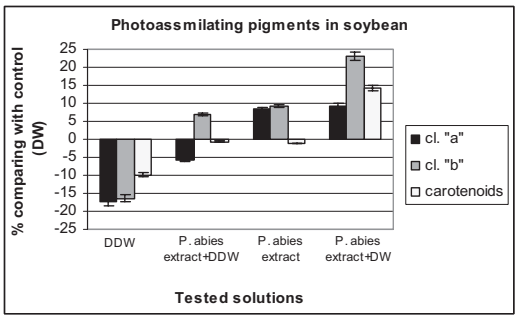

Fig. 7.The influence of DDW and *P. abies* aqueous extract in combination with DDW on photoassimilating pigments accumulation of *Glycine max L.* plant

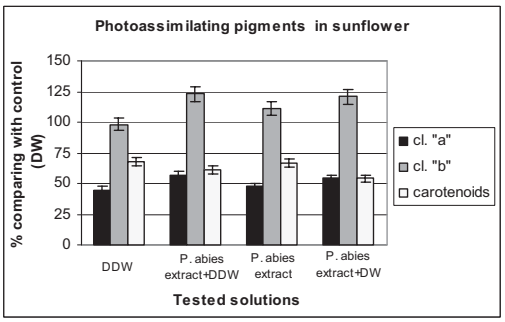

Fig. 8.The influence of DDW and *P. abies* aqueous extract in combination with DDW on photoassimilating pigments accumulation of *Helianthus annuus L.* plant

CONCLUSIONS

The obtained data shown that the deuterium depleted water, stimulates germination energy and capacity, radicle and primary leaves elongation, vegetal biomass accumulation and inhibits photoassimilating pigment synthesis. In

the case of sunflower it was found that DDW stimulates the elongation of all vegetative organs, vegetal biomass accumulation and photoassimilating pigments synthesis.

Spruce bark aqueous extract, reduce germination energy and capacity of soybeans seeds, stimulates accumulation of biomass in soybean seedlings, stimulates elongation for all vegetative organs, accumulation of biomass and photoassimilating pigments synthesis for sunflower seedlings.

Spruce bark aqueous extract in combination with deuterium depleted water stimulates the elongation of all vegetative organs and accumulation of biomass for soybean seedlings. In the case of sunflower seedlings it was observed stimulatory effects on biomass accumulation, photoassimilating pigments synthesis, germination energy and capacity.

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