ON THE POSSIBILITIES OF USING NATURAL BIOACTIVE COMPOUNDS AS PLANT GROWTH REGULATOR

EVALUAREA UNOR COMPUŞI NATURALI CU ACTIVITATE BIOLOGICĂ ÎN REGLAREA CREŞTERII PLANTELOR

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Abstract. *The aim of this study is to examine the possibility of using polyphenolic compounds as rape plant growth regulators. The natural bioactive compounds were obtained by successive hot aqueous extraction of different raw material: spruce bark, grape seeds, chestnuts shell, Asclepias syriaca plant. The aqueous extracts were characterized in terms of total polyphenolic content by using Folin Ciocalteu method. The influence of polyphenolic compounds on rape plantlet, growth and development was followed in hydroponic culture and germination tests. Biometric measurements of rootlets, hypocotyls and cotyledons of rape plantlet, quantitative determination of fresh biomass and the assay of assimilatory pigments content (chlorophyll* ***a*** *and* ***b****) allowed the evaluation of polyphenolic compounds role in plant growth and development. It was observed similar phytohormones effects, stimulating or inhibiting effects on rape plant development, depending on the type of aqueous extract administered during the experiment.*

Key words: *Brassica napus,* polyphenols, total polyphenolic content, chlorophyll a, b.

Rezumat. *Studiul îşi propune evaluarea unor compuşi polifenolici ca bioregulatori de creştere a plantelor de rapiţă. Compuşii polifenolici au fost obtinuţi prin extracţia apoasă succesivă, la cald, a diferitelor surse de biomasă vegetală: coaja de molid, seminţele de struguri, coaja fructelor de castan, planta Asclepias syriaca. Extractele au fost caracterizate din punct de vedere al conţinutului total de polifenoli, utilizând metoda Folin Ciocalteau. Influenţa extractelor polifenolice asupra creşterii şi dezvoltării plantulelor de rapiţă a fost monitorizată prin intermediul unor teste de germinare şi culturi hidroponice. Analizele biometrice privind alungirea radiculelor, hipocotilelor şi cotiledoanelor plantulelor de rapiţă, alături de determinările cantitative de biomasă verde şi conţinut în pigmenţi asimilatori (clorofila* ***a*** *şi* ***b)*** *au permis evaluarea comparativă a rolului compuşilor polifenolici în creşterea şi dezvoltarea plantelor. Rezultatele obţinute au evidenţiat efecte similare fitohormonilor, de stimulare sau inhibare a dezvoltării plantulelor de rapiţă, în funcţie de natura extractului apos administrat pe parcursul experimentului.*

Cuvinte cheie: *Brassica napus,* compuşi polifenolici, conţinut total de polifenoli, clorofila a, b.

INTRODUCTION

The polyphenols represent one of the most important classes of the plants secondary metabolites which play an important role in biosynthesis process. Natural growth regulators or synthetic ones influenced and controlled the growth

and development of roots, stems, shoots, buds, seed gemination processes, etc. Natural bioactive compounds present a large spectrum of action both on whole plant and only on certain tissues and organs intervening also in the regulation of important metabolic processes. Normal plant growth largely depends on the balance that is estabilished between the content of phytohormones and inhibitors (Anghel et al., 2001).

The previous studies have shown the growth regulator potential of chestnuts shell polyphenolic extracts on oat and rape plants. Also, it have been studied the influence of natural bioactive compounds separated from spruce bark, grape seeds and *Aslepias syriaca* plant, by using different technics, on bean plant growth and development (Stingu et al., 2009). Inhibitory or stimulatory effects depends on extract nature, total polyphenolic concentration and also on tested plant species.

This study presents the influence of different polyphenolic extracts on rape plant growth and development. The rape plant response was monitorised in germination test and hydroponic culture experiments.

MATERIAL AND METHOD

Aqueous extraction. Vegetable raw materials, carefully selected, were ground with a mill to obtain a fine powder (0.5mm). 10 g of dry material were succesively extracted (three times) in 125 mL distilled water on a water bath at 80-90°C for 45 min. The aqueous filtrates were collected and brought to a final volum of 500 mL with distilled water (Rozmarin et al., 1984).The tested solution were suggestively noted (spruce bark extract – SB, chestnuts shell extract – CS, grape seeds extract – GS, *Asclepias syriaca* extract - AS) in correlation with the type of extracted vegetal raw material.

Total polyphenolic content determination. Folin Ciocalteu method was used to determine the total polyphenolic content (TPC). The principle of this colorimetrically method is based on the reducing properties of phenolic compounds in contact with Folin Ciocalteau reactiv which present a dark blue coloration with a maximum absorbance at 750nm. The results were expressed in mg gallic acid/L (Bao et al., 2005).

Germination tests. Germination tests were carried out in Petri dishes in fivereplicates for each sample. Each one contained ten rape seeds, carefully selected to do not present major visible damage, placed on a filter paper and 10 mL of tested polyphenolic extract. The control sample was represented by tap water. Petri dishes were placed in a thermostat at 25°C for a period of seven days, when the rape seedlings were analyzed.

Hydroponic culture. Rape seedlings obtained after seven days of germination were transfered in plastic containers containing 150 mL Hoagland nutrients solution (1mM KH_2PO_4, 5mM KNO_3, 5mM Ca $(NO_3)_2$ $4H_2O$, 2mM $MgSO_4 \cdot 7H_2O$, 11.8μM $MnSO_4$ H_2O, 0.7μM $ZnSO_4$ $7H_2O$, 0.32μM $CuSO_4$ $5H_2O$, 0.16μM $(NH_4)_6Mo_7O_{24}$ $4H_2O$, 46.3μM H_3BO_3, 5μM Fe) and 10 mL of each tested polyphenolic aqueous extracts. The influece of natural bioactive compounds separated from different raw material on rape plantlets growth and development was tested in four replicates (four plantlets/containers) for three weeks. Nutrient solutions were changed every five days.

Plantlet analyses. The concentrations of assimilatory pigments, extracted from rape plantlet fresh leaves in 80% acetone, were tested spectrophotometrically by

reading the absorbance values at fixed wavelengths 470, 646, 663nm. The chlorophyll **a** and **b**, carotene content (expressed in µg/g of green material) were established by using the characteristics extinctions coefficients proposed by Lichtenthaler and Wellburn (1983).

RESULTS AND DISCUSSIONS

The results shows that the aqueous extract obtained from *Aslepias syriaca* plant present the highest values regarding the dry matter and organic matter content and the lowest content in total polyphenols (72mg/L extract). Spruce bark and grape seeds polyphenolic extracts are characterized by the highest values for total polyphenolic content (table 1). The most significant values for total polyphenolic content were registered for spruce bark extract (191mg/L) even if the dry matter content and organic matter content registered the lowest values compared with the other extracts.

Table1

Polyphenolic extracts characterization

Extract type	Dry matter content (g/L extract)	Organic matter content (g/L extract)	Total polyphenolic content (mg/L)
SB (spruce bark)	1.004	0.964	191
CS (chestnuts shell)	1.606	1.602	165
GS (grape seeds)	1.832	1.536	188
AS (*Asclepias syriaca*)	1.852	1.668	72

Germination tests results shown that all four tested polyphenolic extracts stimulates pigments assimilations (chlorophyll **a** and **b**,carotene) in the seedlings of *Brassica napus.*The highest values for chlorophyll **a** and **b** concentrations were registered under SB and GS extract treatments. The carotene bioaccumulation is also stimulated by the presence of grape seeds aqueous extract in the growth medium (table 2). Biometric measurements evidenced the stimulatory effects of CS and GS extracts on radicles elongation (fig.1).

Table 2

Assimilatory pigments concentrations ((µg/g) in rape seedling germination tests

Extract type	Chl a	Chl b	Carotene	Chl a+b	Chl a/b
Control	57.91	92.13	20.30	150.50	0.62
SB	64.88	102.70	30.16	167.58	0.63
CS	61.08	96.27	30.87	157.35	0.63
GS	64.88	102.70	52.14	167.58	0.63
AS	58.08	91.96	35.47	150.62	0.63

Hypocotyls length was stimulated under AS aqueous extract treatment. The growth index for rape plantlets recorded higher values, comparing with control, in

the presence of polyphenolic extract obtained from *Asclepias syriaca* plant (fig.2). This situation could be explained by the presence of different individual polyphenolic compounds, in different concentrations in the aqueous extracts, varying with the nature of vegetal raw material extracted.

The natural bioactive compounds extracted from spruce bark, depending on their nature and concentrations, may develop similar action as auxin and cytokinin phytohormones (Simionescu et al., 1991). On the other side, we should take into account the interactions that can be estabilished between plant phytohornones and polyphenolic compounds from the extracts, which can promote stimulatory or inhibitory effects on some biosynthesis process.

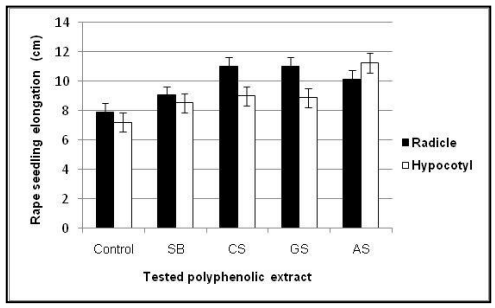

Fig. 1. Radicles and hypocotyls elongation in the presence of different polyphenolic extracts of rape seedling in germination tests

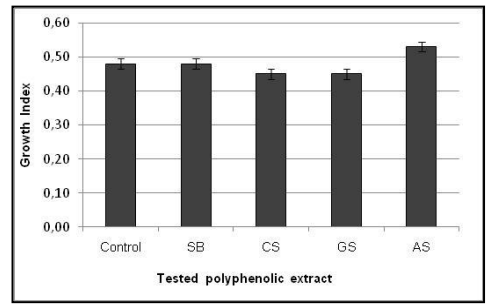

Fig. 2. Rape seedling Growth index registered in germination tests

Green biomass accumulation was stimulated by the presence of polyphenolic extracts in the growth medium, mostly under grape seeds extract treatment.Inhibitory effect on rootlets biomass was registered for rape plantlet in the presence of spruce bark and *Asclepias syriaca* polyphenolic extracts (fig.3).

The presence of aqueous polyphenolic extracts in the rape plantlet growth medium stimulates the chlorophyll **a** and **b** biosynthesis process. Grape seeds polyphenolic extracts present major stimulatory effect in pigments assimilation (chlorophyll **a** and **b**, carotene). *Asclepias syriaca* and spruce bark extracts inhibit carotene assimilation in *Brassica napus* cotyledons (table 3).

Table 3

Assimilatory pigments concentrations (µg/g) in rape plantlet hydroponic culture

Extract type	Chl a	Chl b	Carotene	Chl a+b	Chl a/b
Control	438.76	128.39	113.59	567.166	3.41
SB	570.49	151.66	108.66	722.16	3.76
CS	584.89	153.42	122.85	738.321	3.81
GS	704.87	184.60	138.26	889.47	3.81
AS	505.87	132.99	96.90	638.86	3.80

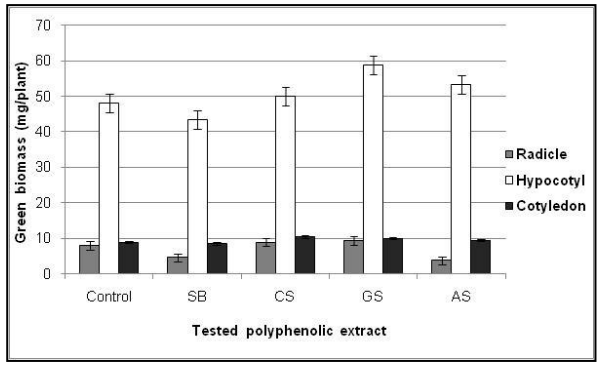

Fig. 3. Variation of green biomass accumulation in rape seedling under polyphenolic extracts treatments in germination tests

In hydroponic culture experiment roots elongation was stimulated in the presence of grape seeds extract, mean while the hypocotyls length was significantly stimulated under SB and AS extracts treatment (fig.4). The highest value of growth index of rape plantlet was registered in the presence of spruce bark aqueous extract (fig.5).

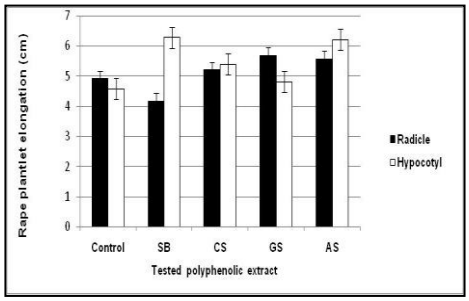

Fig. 4. Radicles and hypocotyls elongation in the presence of different polyphenolic extracts of rape plantlet in hydroponic culture

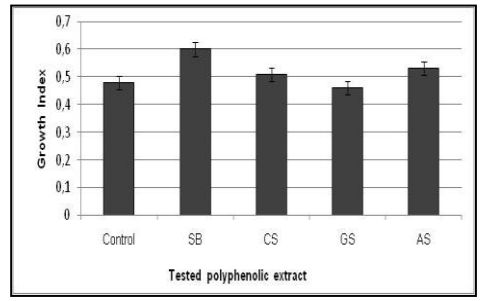

Fig. 5. Rape plantlet Growth index registered in hydroponic culture

Green biomass accumulation in hypocotyls was stimulated by AS extract. Not the same thing could be said regarding cotyledons green biomass accumulation, which seems to be inhibited by all four tested polyphenolic extracts (fig.6).

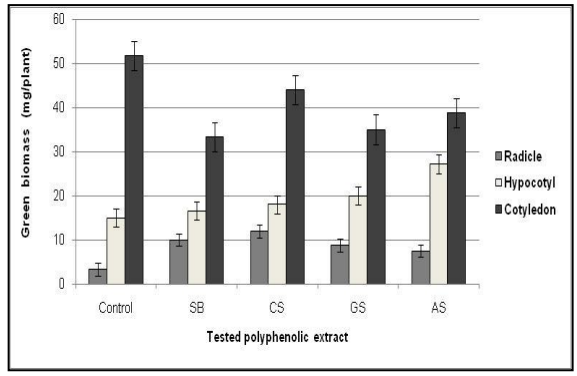

Fig. 6. Variation of green biomass accumulation in rape plantlets under polyphenolic extracts treatments in hydroponic culture

CONCLUSIONS

1. The aqueous polyphenolic extracts obtained from spruce bark, chestnuts shell, grape seeds and *Asclepias syriaca* plant present stimulatory effects on rape plant growth and development, assimilatory pigments biosynthesis (chlorophyll **a** and **b**, carotene) and green biomass accumulation.

2. These processes could be correlated with the nature of tested polyphenolic extract (concentration, composition, total polyphenolic content) and also with the growth medium conditions. From all of four tested extracts, the one obtained from grape seeds present the major stimulatory effect on rape plant growth and development and also on pigments assimilation (both chlorophyll and carotene).

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