



СЕМИНАР ПО ЕКОЛОГИЯ – 2011

28 - 29 април

**ПРОГРАМА / PROGRAM
АБСТРАКТИ / ABSTRACTS**

гр. София

RELATIONS BETWEEN POLYPHENOLICS LEVELS AND ANTIOXIDANT ACTIVITY AND PHYSIOLOGICAL STATUS IN *HYPERICUM RUMELIACUM* SHOOT CULTURES

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The Balkan endemic *Hypericum rumeliacum* belongs to the *Drosocarpium* section of the genus. Though species of this section are considered as hypericin highly productive, biotechnological research on hypericin biosynthesis has been conducted on any of its representatives so far. This work studies the hypericin/pseudohypericin and phenolics/flavonoids production in relation to antioxidant activity, growth characteristics and physiological status of *H. rumeliacum* shoot cultures with modification of vitamin supplementation to the culture media. Hypericin/pseudohypericin amounts were studied by a RP HPLC method; phenolics/flavonoids, DPPH radical scavenging activity and malondialdehyde/hydrogen peroxide amounts were determined spectrophotometrically. Leaf area and dark glands density were determined by light microscopy.

Our results indicate that high phenolics/flavonoids amounts were positively related to increased radical scavenging activity, reduced growth index and predominance of leaf biomass of the shoots. High hypericin content, on the contrary was related to increased growth index, reduced leaf area, high dark glands density and elevated levels of hydrogen peroxide and malondialdehyde, implying of possible autotoxic effect of hypericin *in vitro*. We have established amounts of 0.29mg DW and 1.47mg/gDW for hypericin and pseudohypericin, respectively, which are higher than other *Hypericum* shoot cultures, reported in literature so far.

Acknowledgements: We are thankful to Seventh Framework Programme, Marie Curie Actions, People International Research Staff Exchange Scheme, PIRSES-GA-2009-247548 and Operational Programme "Human resources development" co-financed by the European Union through the European Social Fund ISUN BG051P0001-3.3.04/42.

ANALYSIS OF ANTIOXIDANT PROPERTIES OF BULGARIAN BEE PRODUCTS

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Aim: The aim of this study was to determine the antioxidant activity of Bulgarian bee products: propolis and honeybee, using yeast cells *Saccharomyces cerevisiae*.

Materials and Methods: Yeast cells *S. cerevisiae*- DG1141ts1, MAT α , *ura3*, *his3 Δ 200:TymHis* *sec53* were used. Quantitative Ty1 retrotransposition test, method for quantitative measurement of superoxide anions in cells *S. cerevisiae* and determination the antioxidant activity of Bulgarian propolis and honey were used.

Results: The antioxidant activity of propolis and honey were measured in yeast cells *S. cerevisiae* DG1141ts1 by quantitative determination of Ty1 retrotransposition, caused by increased levels of reactive oxygen species, induced by carcinogens. ROS levels, respectively the frequency of transposition were increased tenfold in comparison with the control, and decreased by preliminary treating of the yeast culture with propolis and honey. The antioxidant properties of bee products

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FACTORS AFFECTING POLYPHENOLICS BIOSYNTHESIS AND GROWTH PARAMETERS IN *PULSATILLA HALLERI* AND *P. SLAVIANKA* IN VITRO

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Species of the *Pulsatilla* genus, Ranunculaceae, are widely utilized in Eastern traditional medicine for the treatment of enteritis, as well as for their anti-inflammatory, spasmolytic and antitumor properties. Nevertheless, the *in vitro* biosynthesis of pharmaceutically active secondary metabolites has not been investigated for this genus so far. In our previous research we investigated the relations between benzyl adenine and indole-3-butyric acid supplementation to the culture media with polyphenolics and chlorophylls levels for *P. montana* ssp. *balcana*, *P. halleri* ssp. *rhodopea* and *P. slaviankae*. In continuation of this experiment, here we present a broader study of growth parameters, spectrophotometric assay of polyphenolics and photosynthetic pigments levels of *P. halleri* ssp. *rhodopea* and *P. slaviankae*. We have experimented on 11 different media variants based on variation of benzyl adenine and auxins (indole-3-butyric acid and naphthylacetic acid).

We have established that each species has a different response to the growth regulators supplementation. As a result of the study, media variants were selected for *P. halleri* and *P. slaviankae*, favorable for either maximal multiplication or polyphenolics biosynthesis.

Acknowledgements: We are thankful to Seventh Framework Programme, Marie Curie Actions, People, International Research Staff Exchange Scheme, PIRSES-GA-2009-247548 and Operational Programme "Human resources development" co-financed by the European Union through the European Social Fund ISUN BG051P0001-3.3.04/42.

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IMPACT OF GROWTH REGULATORS ON THE ESSENTIAL OIL PROFILES OF *IN VITRO* CULTURED *ARTEMISIA ALBA* TURRA

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Essential oil of *Artemisia alba* has been shown to possess strong spasmolytic and antimicrobial activities. The great variability of the essential oil composition, however, is a drawback for large scale cultivation and yield of standardized plant biomass. It has been disputed whether this variability is due to the impact of genetic or environmental factors on the plants. Biotechnological development of selected plant cultivars is an approach to avoid environmental factors and study secondary metabolites biosynthesis in controlled conditions. We have established shoot cultures of the aerial parts of field grown commercial cultivar of *A. alba*, and studied the essential oil composition in media varying in the supplementation of indole-3-butyric acid and benzyl adenine to the basic Murashige and Skoog culture medium. The essential oils were analyzed by GC-MS. Up to 24 different components were identified, consisting of mono- and sesquiterpenoids. Both structural and quantitative variability in the terpenoid composition was established between the media variants, indicating the decisive role of environmental factors for the quality of *A. alba* oils.

Acknowledgements: We are thankful to the Operational Programme "Human resources development" co-financed by the European Union through the European Social Fund ISUN BG051P0001-3.3.04/42.