Abstract

Milk thistle, *Silybum marianum* L. Gaertn., is a source of flavonoid taxifolin and flavonolignans – silymarin complex (silybin, silydianin, silycristin and isosilybin).

Milk thistle is usually obtained by field cultivation. Alternative way for getting the active components, is the use of *in vitro* cultures. But the production of secondary metabolites by the *in vitro* cultures is low in comparison with plant. One of the possibilites how to increase this produciton is the method of elicitation.

In this study, ethephon as the elicitor, in the concentrations of 500 μmol/l, 400 μmol/l, 200 μmol/l, 100 μmol/l and 50 μmol/l was used with the aim to increase secondary metabolite production in suspension and callus cultures. The effect of ethephon was compared to its inhibitor ($AgNO_3$, 120 μmol/l). The levels of flavonolignans and taxifolin were measured by the method of HPLC. The samples were taken 24, 48, 72, 96 and 168 hours after the ethephon application and inhibitor treatment. The nutrient medium of suspension culture was also tested for the possibity of secondary metabolites releasing into medium.

The highest content of flavonoid taxifolin was found in the suspension culture medium after 48 h treatment with ethephon in conc. of 400 μmol/l. The level of taxifolin was increased by 197-fold to 1,97 mg/100 ml, compared to control sample.

The statistically significant production of taxifolin in the callus culture was reached after 96 hours of treatment with ethephon in conc. of 50 μmol/l. (0,11 mg/g DW).

The statistically significant production of silybin A was reached in the nutrient medium 72 h after application of ethephon in conc. of 400 μmol/l (0,51 mg/100 ml).

The statistically significant positive effect of $AgNO_3$ as inhibitor was found in the case of taxifolin in the medium, 168 hours after application of ethephon in conc. of 400 μmol/l. Inhibitor increased taxifolin content by 58-fold to 0,58 mg/100 ml.

The statistically significant negative effect of inhibitor $AgNO_3$ was on silybin A content in medium, 168 hours after application of ethephon in conc. of 400 μmol/l. Inhibitor completely decreased the effect of ethephon.