

## **Application of the box-Behnken design to optimize the growth conditions and the secondary metabolites production of *Euphorbia peplus* cultivated in hydroponics.**

W. Ben Hmouda, F. Bafort, MH. Jijakli

Laboratory of Integrated and Urban Phytopathology, University of Liège, Gembloux Agro-Bio Tech, Passage des Déportés 2, 5030 Gembloux, Belgium

Plant secondary metabolites (PSM) are phytochemicals with great relevance to the pharmaceutical and food industries. The accumulation of PSM occurs often under stress as a protective and/or adaptive mechanism. Environmental factors such as temperature, relative humidity, fertilization, light intensity and atmospheric CO<sub>2</sub> concentration have all separately a significant impact on plant growth and PSM production. However, until now, there is a limited understanding of how combinations of factors can affect its production.

This study aims to determine the optimal conditions for the growth and biosynthesis of therapeutic molecules from *Euphorbia peplus* cultivated in hydroponics by examining the influence of a combination of three abiotic factors (t°, light intensity and CO<sub>2</sub> concentration).

A response surface methodology is being applied to the Box-Behnken model to examine the effects of three factors in a single block of 15 trials. Expert software was used to design the experiment and randomize the trials. Additionally, each of the three studied factors was divided into three levels (a minimum level, an intermediate level and a maximum level). So, the following values were chosen: temperature (18, 24 and 30 C), photosynthetic photon flux density (200, 350 and 500 mol/s/m<sup>2</sup>), and atmospheric CO<sub>2</sub> concentration (400, 950 and 1500 ppm). Experiments are conducted in shipping containers, which require intelligent piloting systems to control all climatic conditions.

Throughout the crop, agronomic measurements are carried out (plant height, identification of phenological stages, plant biomass...), as well as analyses of the molecules of interest via HPLC and UPLC (ingenol, ingenol mebutate, chlorogenic acid, kaempferol...).

By means of multiple regression, the results are analyzed and will be presented to fit a quadratic polynomial model expressed in the form of a second-order mathematical equation.

**Keywords :** PSM, Box-Behnken, abiotic stress, *Euphorbia peplus*, hydroponics.