Contract 160/2014
Scientific Report – Phase I/2014
Abstract

In the framework of the project there were performed three experimental models for the selection of *Trichoderma* consortia strains with multifunctional activity on plants, protective activity against phytopathogenic agents from soil or inducing diseases in plants during vegetation, stimulative activity on plant development, improving activity of nutrition (especially by biodisponibility of some nutrients) and activation of induced mechanisms of plant defense. These 3 experimental models consists in: a) Printing by micro-contact using inox replicators with 96 pins for quick identification of *in vitro* multiple action; b) Sandwich-technique of microplates separated by a gas-permeable film for determining antagonism by producing volatile microbiocides and cellulose activity and c) determination of ACC-deaminase activity by cultivation on liquid minimal media with ACC and reaction with ninhidrine.

There were drawn up 2 categories of experimental models designated to test multifunctional products in the field experiments: (i) experimental models for rapid testing of biological activity of (bio)products on the 15 m^2 plots and (ii) experimental model for integration of multifunctional products in the sustainable crop practices, which will be used in the experimental field of Hofigal Company.

Preliminary tests were conducted for the development and implementation of experimental models for multifunctional products based on mesoporous silica from a natural source (sodium silicate) and oleic acid (OLA) derived from saponified vegetable oils. We have analyzed the conditions under which stable aqueous dispersions containing silica nanoparticles can be obtained, stabilized by the oleic acid/sodium oleate complex. In aqueous media, the OLA/OLANa (acid / soap) system generates, depending on the concentration, pH and temperature, stable liposome like structures. Through the domains of associated hydrophobic chains, these vesicular aggregates are able to solubilize various bioactive compounds, aimed for the treatment of the nutraceutical plants. Hydrophobic interactions between octadecyl chains grafted onto silica and the OLA/OLANa mixture increases system stability and allows solubilization of oleophilic additives. Stability of the systems and their homogeneity was monitored using olive oil as oleophilic component, colored with a hydrophobic commercial dye (Solvent Red). All the synthesis led to stable and homogeneous dispersions, in which the colored olive oil is completely embedded in the hydrophobic areas.

Preliminary studies for evaluation of biological activity plant extracts from *Momordica charantia* and *Passiflora incarnata*, newly cultivated in Romania, were performed by alternative *in vitro* tests: (i) the cellular viability was determined by the method with Neutral Red, (ii) analysis of cellular morphology using optic microscopy after Giemsa coloration and (iii) identification of the plant extract concentrations which allow the conservation of cellular viability and their proliferation ability, normal morphology, as well as the concentrations with citotoxic activity, alterating the cellular morphology. There were elaborated the qualitative and quantitative analysis methodes for the main biological active compounds from *M. charantia* and *P. incarnata* extracts and it has been drawn up a study for identification and dosing these plant compounds.

To produce ceramic materials for multifunctional products, the functionalization of the porous ceramic granules with amino groups is considered. By insertion of amino functions on the ceramic granules surface are generated anchoring sites for humic acids. These are able to bind

the nutritive elements used for the treatment of nutraceutical plants, which will be gradually released. Preliminary tests were conducted for the selection of ceramic components and ceramic materials characterization for multifunctional products, focused on the possibility of obtaining mesoporous silica containing amino functional groups by the sol-gel of aminopropyltriethoxysilane (APTES) on the preformed silica particles. These are the experimental model for the synthesis of functionalized porous ceramics with amino groups. The materials obtained are characterized in terms of the effect of the type co-precursors and sol-gel system components ratio on the structure and morphology of the hybrid silica network and on particles size. All hybrids TEOS/APTES supports showed a very good capacity of coupling both types of humic acids, maximum efficiency being recorded for the carriers who had grafted onto the silica surface, beside the amino functions, also spacing mono- or di-methyl groups.

There were tested and screened, *in vitro* and *in vivo*, nine plants extracts manufactured by Hofigal S.A. against *Botrytis cinerea* (strain B.c. 27). The highest antibotrytis *in vitro* activity was obtained using the following extracts: *Hyssopus officinalis* (at 20%, 10% and 5%), *Satureja hortensis*, *Allium sativum*, *Tagetes patula* (at 20% and 10%) and *Mentha* sp. (at 20%). A moderate antibotrytis activity has been noticed for *Mentha* sp. (at 10% and 5%), *Satureja hortensis*, *Allium sativum* and *Tagetes patula* (at 5%) extracts. The lowest antibotrytis activity or no efficiency was noticed using extracts obtained from *Achillea millefolium*, *Artemisia dracunculus* '*sativa'*, *Rosmarinus officinalis* and *Valeriana officinalis* even applied at 20%. Based on results obtained in *in vitro* tests, six plant extracts were tested and screened *in vivo*, under field conditions at Hofigal S.A. Bucharest. *Satureja hortensis*, *Allium sativum*, *Hyssopus officinalis*, *Mentha* sp. and *Tagetes patula* extracts have been efficient in limiting gray mold severity in blackcurrant applied at 10% compared to untreated control. No *in vivo* activity was registered for *Valeriana officinalis* extract. Plant extracts with high antibotrytis activity will be used in the future research for the next project activities.