PLANT BIOSTIMULANTS ACTIVITY OF BIOENHANCED AND PELLETIZED SPENT PLEUROTUS SUBSTRATE (ENHANCEMENT OF BIOSTIMULANT ACTIVITY OF SPENT PLEUROTUS SUBSTRATE FOR SEEDLING PRODUCTION)

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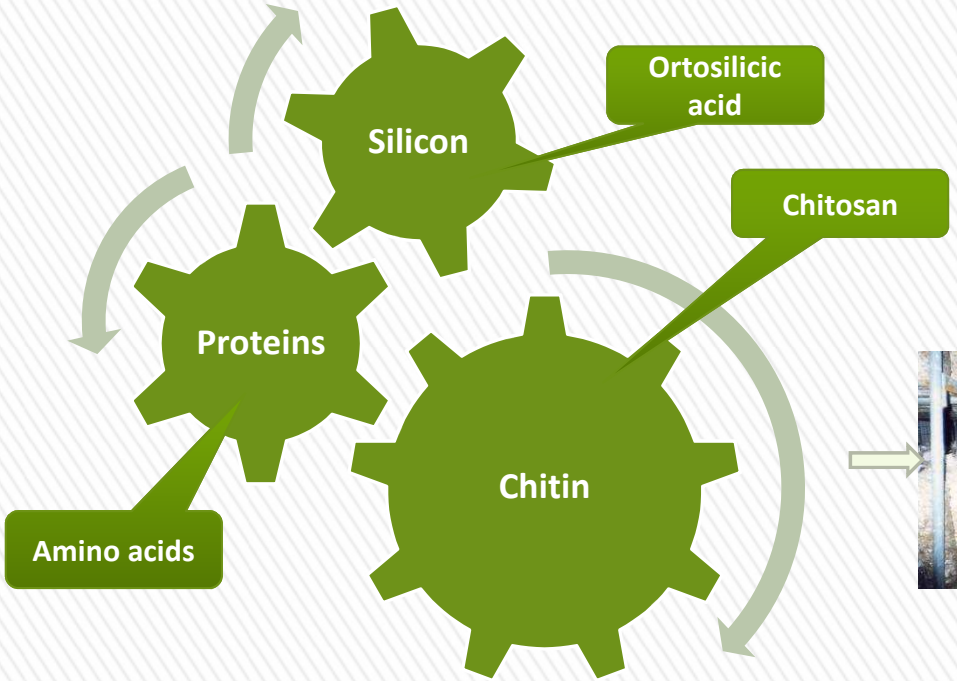
INTRODUCTION AND AIM



Substrate preparation

Substrate inoculation

Substrate incubation, mushroom harvesting

Spent *Pleurotus* Substrate

Plant biostimulants potential of SPS
(Spent *Pleurotus* substrate)
(Pre-biostimulant)

INTRODUCTION AND AIM

The aim of our study was to enhance the plant biostimulant activity of a spent *Pleurotus* substrate (SPS), resulted from mushroom cultivation on wheat straw, by a combination of treatment with a bacterial biostimulants strain and pelletizing. This enhancement of plant biostimulant activity aims also to improve nutrient bioavailability (including silicon) and to reduce osmotic stress to plant cultivated on growing media containing SPS (osmotic stress resulted from SPS salt content). The pelletized and spent *Pleurotus* substrate was tested for on seedling production, as an ingredient of growing media

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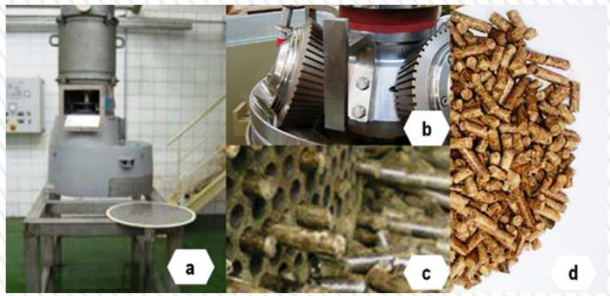
MATERIAL METHODS

Spent *Pleurotus* substrate (SPS) from a commercial mushrooms farm (Agrostar Srl, Filipești, Prahova, Romania) was dried in a hot air stream, around 75°C, until reaching 14% humidity. We assayed cellulose, hemicellulose, lignin and water extractible content of SPS according to standard NREL methods (Sluiter et al., 2005; Sluiter et al., 2010). Chitin content was estimated after deacetylation, with polyiodide method (Nitschke et al., 2011). The dried SPS was treated with a suspension of spores of a plant biostimulants *Brevibacillus parabrevis* B50 strain (NCAIM B 001413) (Oancea et al., 2014) in water, obtained by centrifugation from a 48 hours B50 bacteria culture on potato-dextrose broth. Final spore concentration was 10^7 cfu spores per g dried substrate.

B. parabrevis treated SPS was pelletized in horizontal laboratory pellet press, model Kahl 14-175 (Amandus Kahl, Reinbek, Hamburg, Germany), on 5 mm hole die, with a specific power of 1 kW for 0,015-0,02 m^2, forming pellets of 10-12 mm length.

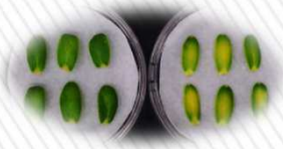


Pelletizing process of dried SPS treated with *B. parabrevis* B50 a) horizontal laboratory pellet press b) horizontal die aspects c) pellets forming on pellets die d) pellets with embedded *B. parabrevis* B50 .

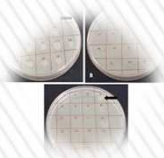
MATERIAL METHODS

Voges–Proskauer test

IAA production

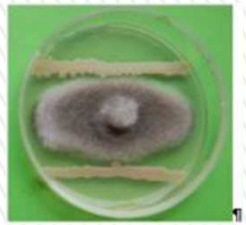


Cytochinin production



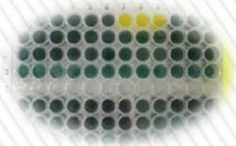
ACC-deaminase

Polyamine production – ornithine decarboxilase



Antagonism

B. parabrevis B50 selection



Nitrilase production

Phosphorus solubilization

Siderophore production

Cellulase production

Protease production

Seedling stimulation

MATERIAL METHODS

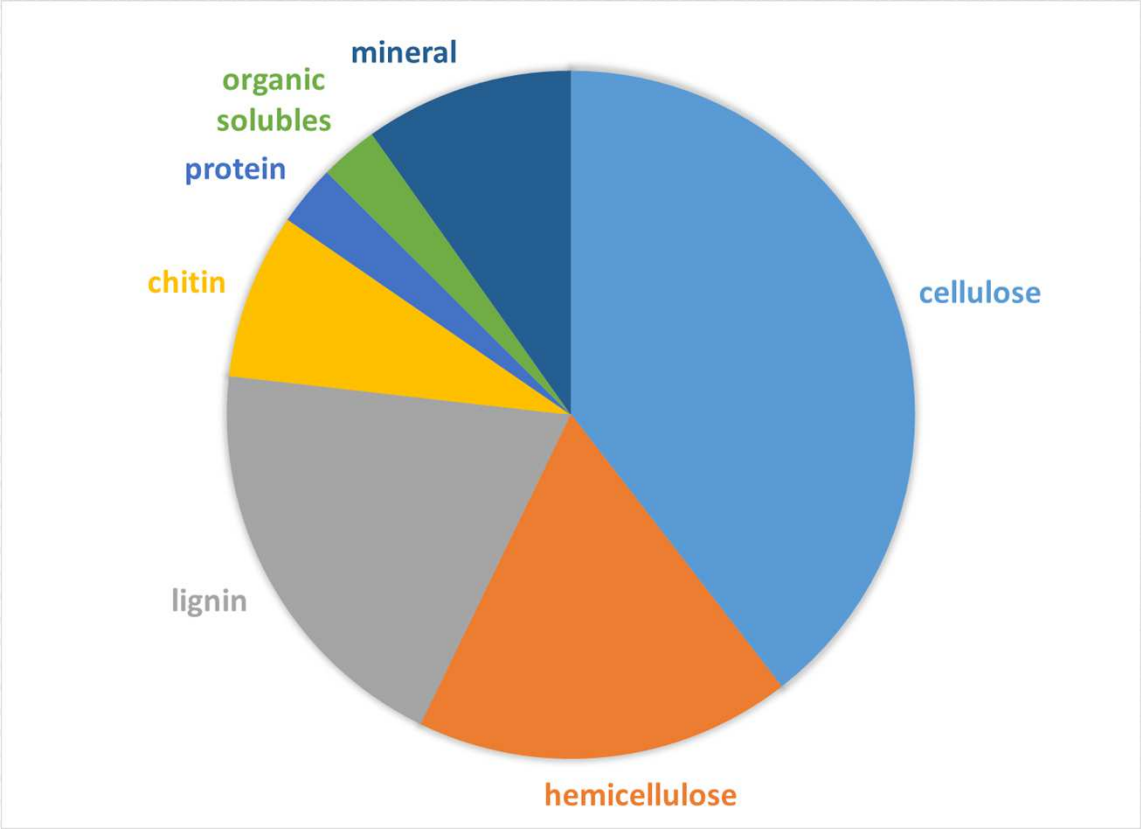
Polyamine and ortosilicic acid release

The resulted pellets were suspended in pure water (Milli-Q® Integral, Merck-Millipore, Darmstadt), in a ratio of 1 g pellets to 100 mL of pure water, on 500 mL Erlenmeyer flasks, covered with cotton wool. Pure water was incubated with suspended pellets on a rotary shaker, at 25°C and 5 rpm, for three weeks. Each two days 1 ml was sampled from the supernatant and analyzed the released soluble silicon, as ortosilicic acid, using silicomolybdic acid spectrophotometric method (Coradin et al., 2004), with a Merck kit (Merck Silicate Assay, 1.14794, Merck-Millipore), and the released polyamines, with a HPLC method (Taibi et al., 2000). Pelletized SPS (without bacterial spores) was used as control.

Effects on seedling

Bioenhanced and pelletized SPS was tested on germination and growth of two vegetables, tomato (*Solanum lycopersicum* L. cv. Menhir F1) and cucumber (*Cucumis sativus* L. cv. Karolina F1), in greenhouse conditions. The seeds of the tested plants, tomato and cucumber, were sown in the tested media, distributed in 1 L plastic pot, with one seed per pot. The tested vegetables were grown in 6 different media, obtained from bioenhanced and pelletized SPS, mixed in various ratios (2:1; 1:1; 1:2; 1:3; 1:4, v: v) with peat. Two controls were used: M1, peat, and M2, obtained from pelletized SPS (not bio-enhanced), mixed with peat (1:2, v: v). Experiment was conducted according to a completely randomized design, considering different growing media (with bioenhanced and pelletized SPS or controls) as treatments, with 3 repetitions (each repetitions consisting of 3 pots).

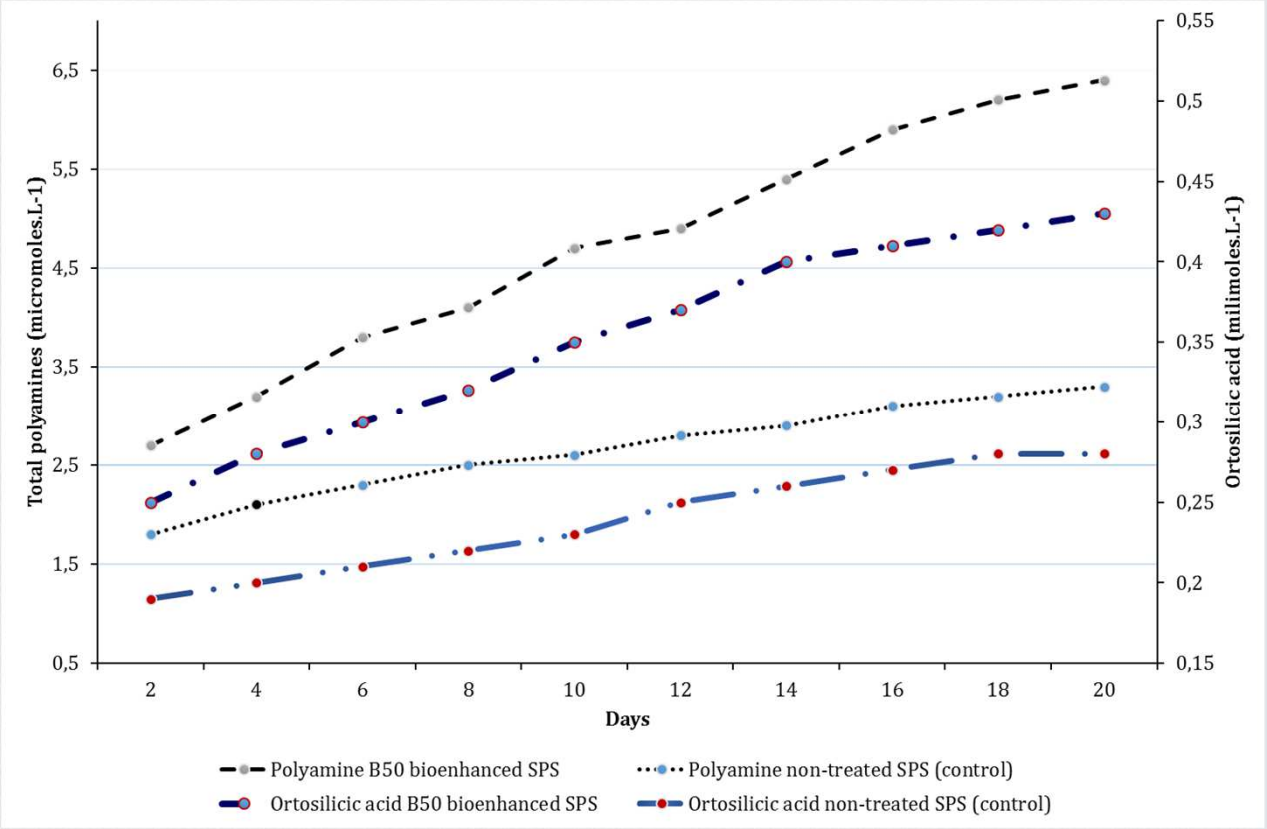
RESULTS



SPS plant biostimulant potential

- Extraction of components with biostimulant potential (chitin, protein, soluble organics – amino acids, oligo-saccharides, mineral – silicon)
- Bio-enhancement for a faster release of plant biostimulant compounds

RESULTS



Dynamic of soluble silicon (as ortosilicic acid) and total polyamines (sum of cadaverine, putrescine, spermidine and spermine), released into pure water, for B50 strain bioenhanced SPS pellets and SPS pellets not-treated with bacterial spores.

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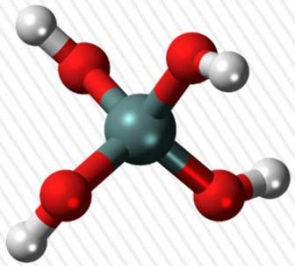
RESULTS

Dissolved silicon - plant biostimulant

Silicon (as ortosilicic acid, non-dissociated on soil pH, up-taken by plant roots) is a plant biostimulant, influencing nutrients uptake and nutrient use efficiency, delaying plant senescence and alleviating abiotic and biotic stress effects on plants (Savvas and Ntatsi, 2015), due to a broad spectrum activation of plant defense system (Van Bockhaven et al., 2013).



Ortosilicic acid
H_4SiO_4

Exogenous polyamines – plant biostimulant

Polyamines are ubiquitous endo- and exo-signals, involved into plant growth and development (Kusano et al., 2008), plant response to biotic (Jimenez-Bremont et al., 2014) and abiotic stress (Marco et al., 2011), and interactions between plants and beneficial microorganisms (Nassar et al., 2003; Perrig et al., 2007; Xie et al., 2014).

Putrescine
Tetra-methylenediamine

RESULTS

Growth substrate	Germination rate (%)	Height (cm)	Stem diameter (cm)	Fresh weight ($g.plant^{-1}$)	Dry weight ($mg.plant^{-1}$)	Water holding capacity ($mL.L^{-1}$)
Peat (control)	83x	8.24yz	0.272y	0.62y	5.5y	559x
Peat + NB-pSPS (2:1, v: v) (Control)	72xy	8.16yz	0.254yz	0.59y	4.7y	527y
Peat + B-pSPS (1:2, v: v)	62y	7.94z	0.232z	0.54y	4.5y	497z
Peat + B-pSPS (1:1, v: v)	74x	8.12z	0.246yz	0.75xy	6.4xy	515yz
Peat + B-pSPS (2:1, v: v)	76x	8.52xy	0.266y	0.79xy	6.7xy	527y
Peat + B-pSPS (3:1, v: v)	78x	8.78x	0.303x	0.86x	7.6x	539y
Peat + B-pSPS (4:1, v: v)	80x	8.92x	0.322x	0.88x	7.9x	537y
DL 5%	8	0.42	0.025	0.02	2.2	25.2

Effects of peat substitution with bioenhanced and pelletized SPS (B-pSPS) on tomato seed germination and seedling growth of the tomato.

Compared to controls, significant increase in plant height, stem diameter, fresh and dry weights were found in treatments with maximum 25% content of bioenhanced pelletized SPS (Peat + B-pSPS 3:1, v: v and 4:1, v: v, respectively, 25% and 20% peat replacement with bioenhanced and pelletized SPS)

RESULTS

Growth substrate	Germination (%)	Height (cm)	Stem diameter (cm)	Fresh weight (g.plant^{-1})	Dry weight (mg.plant^{-1})	Water holding capacity (mL.L-1)
Peat (control)	82x	12.64yz	0.492y	5.93y	38.3y	559x
Peat + NB-pSPS (2:1, v: v) (Control)	78xy	11.86yz	0.471xy	5.82y	36.5y	527y
Peat + B-pSPS (1:2, v: v)	68y	10.84z	0.422z	5.64y	35.8y	497z
Peat + B-pSPS (1:1, v: v)	78xy	11.08z	0.516y	5.85y	38.4y	515yz
Peat + B-pSPS (2:1, v: v)	82x	13.23y	0.508y	6.19xy	46.3xy	527y
Peat + B-pSPS (3:1, v: v)	84x	13.81x	0.546x	6.47x	49.6x	539y
Peat + B-pSPS (4:1, v: v)	86x	14.18x	0.557x	6.64x	51,7x	537y
DL 5%	12	0.86	0.047	0.36	4,2	25,2

Effects of peat substitution with bioenhanced and pelletized SPS (B-pSPS) in cucumber seed germination and seedling growth

More than 50% peat substitution, with pelletized and bioenhanced SPS, reduced biomass accumulation in both crops, as a result of a significant reduction of water holding capacity of the substrate. Bioenhanced and pelletized SPS could be used in proportion of maximum 25% in the growing substrate, additional components (e.g. fiber compost) being necessary in order to provide better physico-chemical characteristics of growing substrate, complementary to the plant biostimulant action of bioenhanced and pelletized SPS

CONCLUSION

Compared to controls, significant increase in plant height, stem diameter, fresh and dry weights were found on plants from treatments with maximum 25% content of bioenhanced pelletized SPS in growing media, in both vegetables. This seedling stimulation is probably due to an increased released of soluble silicon and polyamines from *Brevibacillus parabrevis* B50 bio-enhanced pelletized spent *Pleurotus* substrate.

Thank you for your attention

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