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Study Regarding the Potential Use of a Spent Microbial Biomass in Fertilizer Manufacturing

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Abstract: A spent biomass, which results from the biopharma industry, is stabilized and functionalized by biosorption with microelements. The efficiency of this new biomaterial was tested in two experiments: (1) In a mixture with soil to determine its effects of the germination capacity of cereals and vegetables, and (2) in a formulation of mixed fertilizers to determine its influence on the development and production of the two types of vegetables. The results obtained during germination experiments performed in pots showed that at a biomass concentration less than 20%, the germination output was greater than 95% and the germination index was almost 1. The experiments performed in land on vegetables (including *Solanum lycopersicum* and *Capsicum annuum*) featured six types of fertilizers formulated with new biomaterials. The obtained results indicated that two types of fertilizers (N 10:0:0 and NP 5:5:0), which were formulated with functionalized biomass and featured the microelements Co, Cu, Fe, Mn, and Zn, exhibited significant effects when compared with vegetables cultivated on unfertilized soil surfaces (the untreated variant). The studies regarding the effect of the new fertilizers obtained based on spent biomass from biopharma industry indicate the following: (a) This material, even if it is stabilized and functionalized, cannot be used as such as a germination substrate for vegetables; in addition, it cannot be introduced into soil together with cereals seeds (during the autumn work), because the germination can be affected negatively; (b) the functionalized biomass can be used in the formulation of different types of fertilizers; if these fertilizers are introduced into soil with the autumn plowing, then they may have a positive influence on the yield of some species of vegetable, such as *Solanum lycopersicum* and *Capsicum annuum*. The new fertilizers have a major environmental impact due to: (1) Removal of waste, which results from pharmaceutical biosyntheses, with significant impact on soil pollution, due to its storage in the form of waste dumps, on the soil; (2) recovery and reinsertion into the natural circuit of nutrients like C, N,

P, K, Mg, and Ca contained in spent biomass, by their reuse in agriculture; and (3) high content of compounds with C from spent biomass can improve in time the content of fulvic and humic acids in soil, with a positive effect on soil characteristics from an agronomic point of view.

Keywords: biopharma spent biomass; functionalized biomass; fertilizer efficiency

1. Introduction

Byproducts from different industries have been introduced in the economic circuit and currently serve as alternatives that have positive effects on environmental protection. Thus, valuable products for agriculture can be obtained using oat shells, which, by pyrolysis at 300 °C, yields an active carbon that can retain fertilizers, such as urea, in its pores. This product (biochar) can be used as such or it can be encapsulated with cellulose acetate, ethyl cellulose, or alginates to obtain biochar, in which the nutrients (N) are time released in the soil [1]. Wheat straws can be used to produce cellulose-based hydrogel products, which can incorporate nitrogen (N) and phosphorus (P) into fertilizer compounds, which, once introduced into the soil, can release these nutrients over time [2]. Other researchers have used rice husk to obtain silica compounds, which were then encapsulated in nanocomposites based on methyl sulfonate cellulose, acrylic acid, and chemical fertilizer-type NPK, to obtain controlled-release fertilizers [3]. Further, Prunomo et al. [4] incorporated NKS-type fertilizers in a matrix that contains fly ash, which is a waste product obtained from sugarcane processing, using molasses as a binder. The resulting product was pelleted into cylindrical shapes with a diameter of 6 mm and a length of 5–8 mm, and can be used as a fertilizer. Mineral clays, such as kaolin ($\text{Al}_2\text{Si}_2\text{O}_5(\text{OH})_4$) or nanotubes of halloysite, can be used as a natural filler to obtain granulated NPK, type 25:75:0; 50:50:0, or 75:25:0 [5], or to obtain nitrogen fertilizers featuring controlled release and high water retention capacity [6,7]. In other studies, the silica obtained from rice husks, together with biodegradable polymers, charcoal, and organic fertilizers, can be used to obtain biodegradable mulch [8]. The solid waste (microbial biomass) that resulted from pharmaceutical biosynthesis represents a valuable product that can be used in agriculture following stabilization and functionalization; can be used in agriculture for numerous applications [9–11], following stabilization and functionalization; and it also can incorporate microelements into fertilizer formulations.

In the actual context, the pharmaceutical biotechnologies, in particular the production of antibiotics, generate environmental problems, due to the fact that they generate large quantities of microbial biomass, which are stored on the soil in waste dumps. The European environmental legislation in force (e.g., Directive 2008/98/EC on waste) obliged pharmaceutical companies to look for profitable ways of exploiting this waste, for example, as products intended for the amendment and fertilization of unproductive soils.

The present study aimed to reintroduce the biomass resulting from pharmaceutical biosynthesis into the economic circuit, by using it in fertilizer formulations. The microbial biomass was previously processed according to the approach detailed by Radu et al. [10,11] to obtain the functionalized biomass [12]. These biomaterials have two advantages when compared with other materials like clinoptilolite or other filler materials: 1) They can act as precursors of humic and fulvic compounds, which can improve soil fertility over time; and 2) due to its capacity to functionalize with cations like Co, Cu, Fe, Mn, and Zn, they represent a source of organic microelements [9,13].

2. Materials and Methods

2.1. Chemical Analysis

Biomass analysis was performed as follows:

- (1) The carbon (C) and nitrogen (N) content was determined using a CHNS/O Analyzer (Perkin Elmer 2400 Series II, with a thermal conductivity detector and the following analytical range: C: 0.01–3.6 mg (max 0.1%); N: 0.001–1.0 mg (max 0.03%)); each measurement was performed in triplicate.
- (2) The content of metallic cations, boron (B), silicon (Si), and phosphorus (P) (Figure 1) was determined with inductively coupled plasma atomic emission spectroscopy, using a spectrometer (ICP-AES VARIAN Liberty 110 with following detection limits: B: 10 µg/L; Ca: 0.5 µg/L; Co: 5 µg/L; Cu: 55 µg/L; Fe: 5 µg/L; K: 100 µg/L; Mg: 0.5 µg/L; Na: 50 µg/L; Mn: 1 µg/L; Mo: 10 µg/L; Si: 20 µg/L; Zn: 5 µg/L). Solubilization was performed with 65% HNO₃ and 30% H₂O₂ (Sigma Aldrich Co., St. Louis, MO, USA), using a Berghoff Digester. The results represent the average value obtained for three samples; for each element, the device was programmed to make three measurements. The standard deviation (STDEV) for the elemental analysis was 5%.

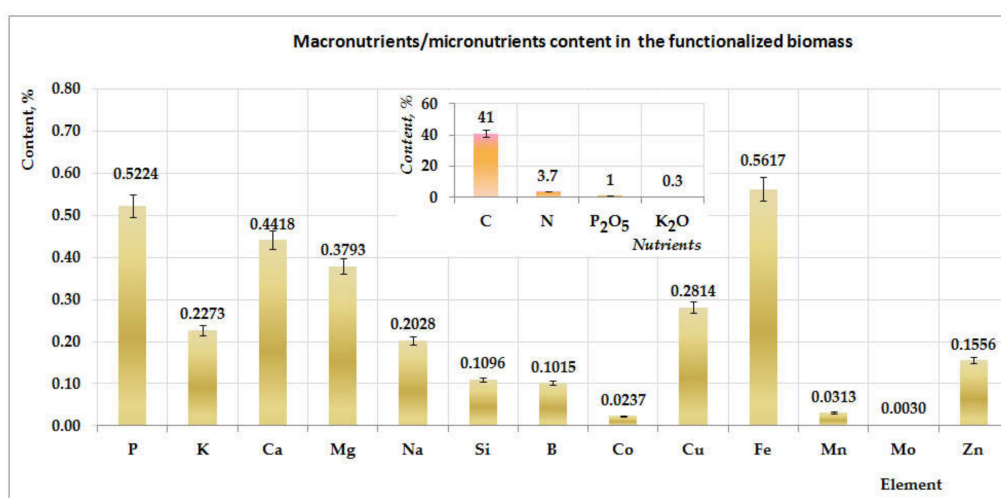


Figure 1. Functionalized biomass composition.

2.2. Cultivation Substrate for Germination

The spent microbial biomass of *Streptomyces noursei* resulted from antibiotic biosynthesis, and was stabilized and functionalized with microelements [10,11]. The composition (presented in Figure 1) was used in a mixture with sterilized soil to obtain the germination substrate. To achieve this aim, different proportions of microbial biomass and soil were used to obtain mixtures that contained 5.9% functionalized biomass (weight %), 11% functionalized biomass, and 20% functionalized biomass, respectively. The mixture of biomass and soil was obtained by manual mixing after weighing each component. The functionalized biomass was used alone (without soil) in order to test its ability to initiate germination (100% biomass). As a reference for germination (control or untreated variant), we used soil (the same soil used to obtain the previous mixture with the microbial biomass).

2.3. Germination Trials

The microbial biomass functionalized with microelements (FM), as well as mixtures of red-brown forest soil and soil, were manually introduced into each pot. In all, 400 counted seeds of different cereals (*Zea mays*, *Triticum aestivum*) and vegetables (*Beta vulgaris*, *Capsicum annuum*, *Lactuca sativa*, *Brassica oleracea*) were placed into pots (one seed in each pot) and covered with 5 cm³ of soil mixture. We chose to perform the germination tests on cereals like wheat and corn in order to evaluate in vitro the effects of these materials for the case in which these will be introduced into the soil, together with cereals seeds. The aim of the germination tests made on vegetables was to evaluate the possibility of using the functionalized biomass as a culture substrate for vegetable seedlings. For each germination test, 4 trays were used, each with 100 plastic pots, and which were introduced in 4 Easy Green

Auto-sprouter devices (Seed Grain Tech. USA Ltd.). The tray dimensions were as follows: L 350 mm × 1 290 mm × H 45 mm; pot dimensions: L 25 mm × 1 30 mm × H 45 mm; the volume of each pot was 18 mL. The germinated seeds were counted manually, and the results were reported as the average of 4 experiments; the standard deviation was determined. The germination index (GI) was calculated with the following formula:

$$GI = \frac{GP_n \times 100}{GP_o} \quad (1)$$

where GP_n represents the germination percent for different contents of biomass in soil and GP_o represents the germination percent in normal conditions, without biomass in soil [14].

2.4. Cultivation Trials

2.4.1. Fertilizers with Microbial Biomass

The fertilizers with the microbial biomass used in the experiments were mixed fertilizers, and were prepared according to the methodology of Radu et al. [10–12]. The active compound sources for each type of fertilizer used in these experiments were as follows: Urea (46.6% N), diammonium hydrogen phosphate (21.21% N, 53.78% P_2O_5), calcium phosphates (38.76% P_2O_5), potassium sulphate (54% K_2O), and functionalized biomass (3.7% N, 1% P_2O_5 , 0.3% K_2O).

2.4.2. Soil Surface Preparation (Plot Preparation)

A total of 14 plots, each with a surface of 2 m², were used in the experiment and were previously prearranged in the autumn, at the end of November. For each plot, each fertilizer with the microbial biomass was manually incorporated under the soil, in autumn, after plowing.

The fertilization norm used was 100 kg of the active compound/ha (0.02 kg active compound/for each plot surface, respectively). The active compounds were quantified as the total sum between major nutrients (N + P_2O_5 + K_2O).

2.4.3. Fertilizer Influence on Vegetable

In order to establish the influence of the fertilizer on vegetable development and yield, two types of vegetables (*Solanum lycopersicum* and *Capsicum annum*) were used. For these experiments, 7 fertilizer types were prepared (Table 1) and were used in the fertilization of 14 plots; each plot had the following dimensions: length = 2 m; width = 1 m. Only one type of plant was cultivated in each plot with a surface of 2 m²; this was done in the experimental land of the Academy of Agricultural and Forestry Sciences of Bucharest, located in Baneasa.

Solanum lycopersicum (Moneymaker variety) was planted in the middle of May at a distance of 60 cm × 70 cm. After planting, the seedlings were watered with 1.5 L of water for each plant and a wooden rod 1.5 m long was fixed near each plant. In all, 11 seedlings (plants)/plot of *Solanum lycopersicum* were used. The plants were tied to this rod with rope during the vegetation period to prevent them from falling and to protect the fruit from making contact with the soil. At the first flowering, measurements were taken regarding the height and total number of leaves for each plant. The results were reported as the average height and average number of leaves for each plant from the plot, respectively. Variance analysis was performed while considering the average number for each fertilization variant and plant. Tomato harvesting was done starting in August and continued until the middle of October. The total production obtained per plot was calculated; the variance analysis was again performed for each fertilization variant (each plot).

Table 1. Fertilization variants used in trials.

| Fertilization variant | Fertilizer Type (Major Macro-Nutrients from Chemical Compounds; Micro-Elements Sources: FM) | Source of Macro-Nutrients/ Micro-Nutrients | Content of FM in Fertilizers, % (w/w) | Active Compound (from Chemical Compounds and FM), %(w/w) | Microelements Content %(w/w) |
|-----------------------|--|---|--|---|--|
| 1 | 2 | 3 | 4 | 5 | 6 |
| V1 | NPK 11:11:11 (33% active compound) Total microelements content: 0.63% | CO(NH ₂) ₂ (NH ₄) ₂ HPO ₄ K ₂ SO ₄ , FM | 54.43 | 35.72 | B = 0.055; Co = 0.013; Cu = 0.153; Fe = 0.306; Mn = 0.017; Mo = 0.002; Zn = 0.085 |
| V2 | N 10:0:0 (10% active compound) Total microelements content: 0.91% | CO(NH ₂) ₂ FM | 78.54 | 13.93 | B = 0.08; Co = 0.019; Cu = 0.221; Fe = 0.441; Mn = 0.025; Mo = 0.002; Zn = 0.122 |
| V3 | NP 5:5:0 (10% active compound) Total microelements content: 0.85% | CO(NH ₂) ₂ Calcium phosphates ^a , FM | 73.37 | 13.67 | B = 0.074; Co = 0.017; Cu = 0.206; Fe = 0.412; Mn = 0.023; Mo = 0.002; Zn = 0.114 |
| V4 | NK 5:0:5 (10% active compound) Total microelements content: 0.93% | CO(NH ₂) ₂ K ₂ SO ₄ , FM | 80 | 14 | B = 0.081; Co = 0.019; Cu = 0.225; Fe = 0.449; Mn = 0.025; Mo = 0.002; Zn = 0.124 |
| V5 | PK 0:5:5 (10% active compound) Total microelements content: 0.9% | calcium phosphates, K ₂ SO ₄ FM | 77.84 | 13.9 | B = 0.079; Co = 0.018; Cu = 0.219; Fe = 0.437; Mn = 0.024; Mo = 0.002; Zn = 0.1241 |
| V6 | N 3.7:1:0.3 (5% active compound) Total microelements content: 1.16% | FM | 100 | 5 | B = 0.102; Co = 0.024; Cu = 0.281; Fe = 0.562; Mn = 0.03; Mo = 0.003; Zn = 0.156 |
| V7 | Control (untreated variant) | | | | |

^a The calcium phosphates represent a source of phosphorus which contain: 2.65% Ca(H₂PO₄)₂; 16.76% CaHPO₄; 61.98% Ca₃(PO₄)₃.

Capsicum annuum (California Wonder variety) was planted in the middle of May at a distance of 30 cm × 30 cm. The number of *Capsicum annuum* seedlings used was 32 plants/plot. Watering was applied after planting (1 L for each plant). At the first flowering, the plant height and number of leaves were measured for each plant. The results are reported as the average height and average number of leaves for each plot, respectively. Variance analysis was performed while considering the average number of each fertilization variant and plant. Harvesting was completed gradually starting from August and extended until the beginning of November. The total production was reported for each fertilization variant (each plot); variance analysis was performed for each fertilization variant.

2.5. Statistics

In the case of the chemical analyses and germination, Microsoft Excel was used to calculate the standard deviation (STDEV). For the results obtained for the crops cultivated on each plot, we used *t*-tests for analyses of significance between the average values of differences (*d*) from the fertilized variant and control (untreated variant) using the probabilities of usual transgression: 5%, 1%, and 0.1%. This was done using Microsoft Excel. For the usual probabilities of transgressions (5%, 1%, and 0.1%), the limit differences (DL5%, DL1%, DL0.1%) were calculated.

Notations:

$d < DL\ 5\%$: insignificant differences;

$DL\ 5\% < d \leq DL\ 1\%$: significant differences between the fertilization variant and control variant; notation: *;

$DL\ 1\% < d \leq DL\ 0.1\%$: distinct significant differences between the fertilization variant and control variant; notation: **; and

$d > DL0.1\%$: highly significant differences between the fertilization variant and control variant; notation: ***.

3. Results

3.1. Studies Regarding the Influence of Functionalized Biomass on Seed Germination

In the case where only the functionalized biomass was used as a substrate, germination did not occur for the plant species used in this study (plants for cereals; leaves or fruit; Figure 2). When the cultivation substrate contained 20% functionalized biomass, the germination capacity (*G*) of all studied plants was below 51%. The lowest values were obtained for *Solanum lycopersicum* (*G* = 6%; *GI* = 0.08), *Capsicum annuum* (*G* = 10%; *GI* = 0.1), and *Lactuca sativa* (*G* = 25%; *GI* = 0.26; Figures 2 and 3). The highest values in this case were found for *Brassica oleracea* (*G* = 51%; *GI* = 0.51), *Triticum aestivum* (*G* = 48.5; *GI* = 0.49), *Beta vulgaris* (*G* = 42.5; *GI* = 0.43), and *Zea mays* (*G* = 40%; *GI* = 0.47). The value of the germination index for this concentration was below 0.51, which clearly shows that the culture substrate with 20% functionalized biomass negatively affected germination.

For the concentrations of 11% functionalized biomass in the cultivation substrate, the germination capacity for most plant species involved in the study was between 92.5% and 98.5%, except for *Zea mays* (*G* = 70%; *GI* = 0.82) and *Solanum lycopersicum* (*G* = 43.5%; *GI* = 0.58). The germination index generally ranged from 0.93 ÷ 1, except for *Solanum lycopersicum* (*GI* = 0.62) and *Zea mays* (*GI* = 0.82; Figures 2 and 3). With 5.9% biomass content in the substrate, the germination capacity of most plant species was between 95% and 99%, except for *Zea mays* (*G* = 77.5%) and *Solanum lycopersicum* (*G* = 46.5%). The germination index ranged from 0.91 ÷ 1.03, with a slightly stimulating effects following germination of the seeds of *Lactuca sativa* (*GI* = 1.03) and *Triticum aestivum* (*GI* = 1.01). In this case, the lowest germination index was obtained for *Solanum lycopersicum* (*GI* = 0.62; Figures 2 and 3).

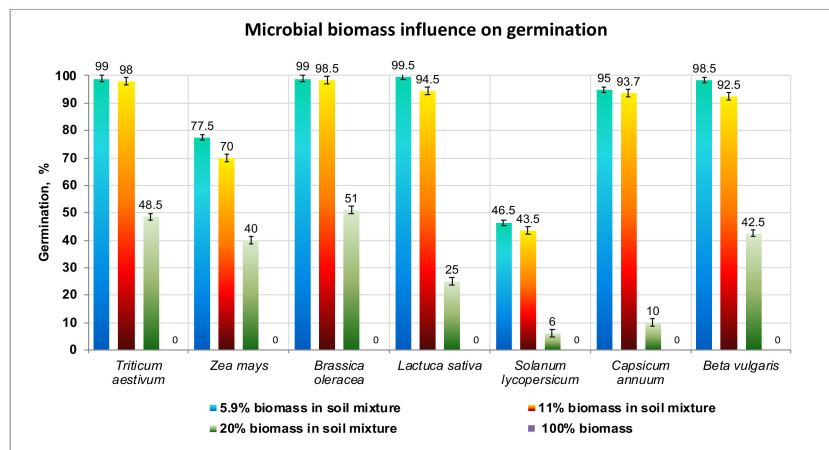


Figure 2. Microbial biomass influence on seed germination in pots (0 represent the germination % for 100% biomass in pot).

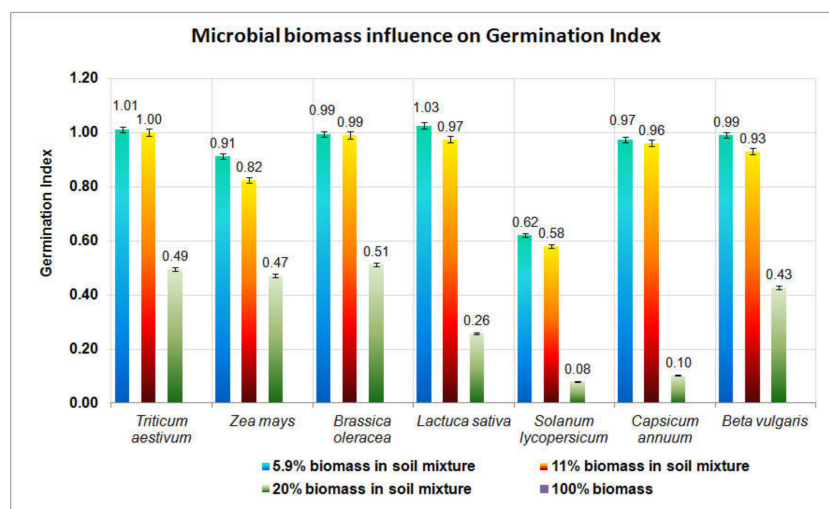


Figure 3. Microbial biomass influence on GI.

3.2. Studies on the Influence of Fertilizers with Functionalized Biomass on the Development and Production of *Solanum lycopersicum* and *Capsicum annuum* Crops

For *Solanum lycopersicum*, there are important differences in terms of plant height. For the V2 fertilization variant (urea + functionalized biomass), the average plant height was 109.8 cm and it was similar for the V3 variant (NP + functionalized biomass), with a plant height of 102.8 cm. Plant heights were lower for the fertilization variants V1 (NPK + functionalized biomass; average plant height of 97.5 cm) and V4 (NK+ functionalized biomass; average plant height of 99.8 cm; Figure 4). The height was further reduced when fertilization was carried out only with the functionalized biomass (average plant height = 86.5 cm). Regarding the number of leaves, significant differences between the fertilization variant and the control were obtained only in the case of V2 (urea + functionalized biomass; Figure 5). The fertilization variants V2 (urea + functionalized biomass) and V3 (NP + functionalized biomass) led to yields of 19.230 and 15.328 t/ha, respectively. The recorded statistical differences between the untreated variant (11.69 t/ha) and the abovementioned variants V2 and V3 were highly significant (Figure 6) in this case.

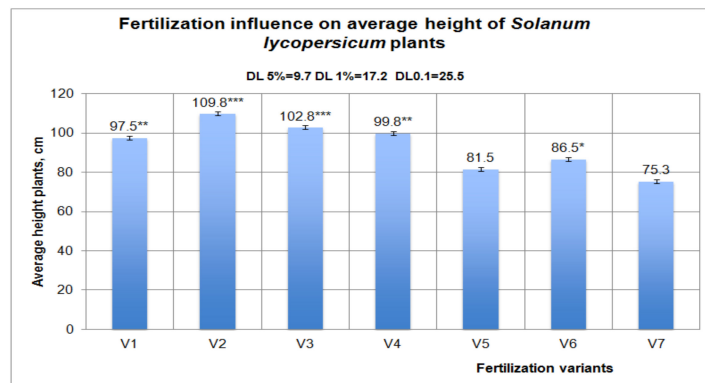


Figure 4. Fertilization influence on the average height of *Solanum lycopersicum* plants.

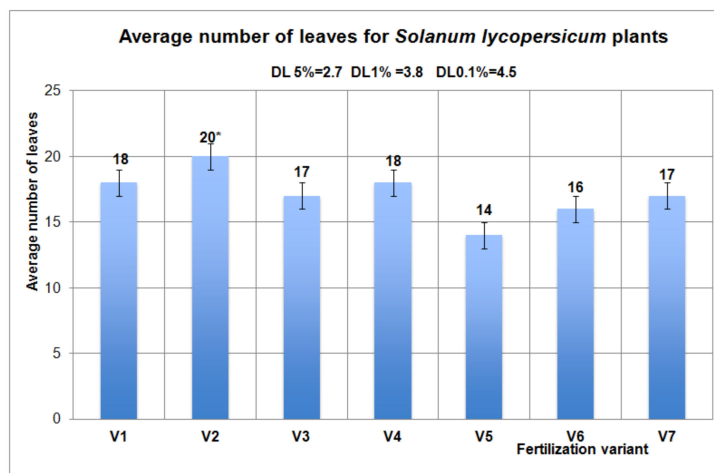


Figure 5. Fertilization influence on the average number of leaves for *Solanum lycopersicum* plants.

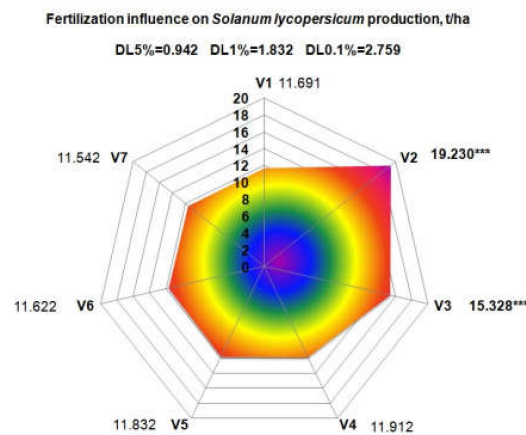


Figure 6. Fertilization influence on *Solanum lycopersicum* production.

In the case of *Capsicum annuum*, the average plant height was 35 cm (for fertilization variant V2) and 27.25 cm (for fertilization variant V3), respectively; plant height was significantly different when compared with the height of untreated plants (Figure 7). Significant differences were observed in comparison with the control in the case of the V4 fertilization variant (NK + functionalized biomass; when plants have an average height of 28.12 cm) and V1 (NPK with functionalized biomass; with an average plant height of 27.15 cm). Another important parameter for the cultivation of *Capsicum annuum* is the number of leaves per plant. There are significant differences when the control (untreated plant) is compared with fertilization variants V2 (urea + functionalized biomass; with an average of

32 leaves per plant) and V3 (NP + functionalized biomass; with an average of 26 leaves per plant; Figure 8). Similar results regarding the number of leaves per plant were obtained for fertilization variants V1 (NPK + functionalized biomass; with an average of 28 leaves) and V4 (NK + functionalized biomass; with average of 20 leaves per plant). For fertilization variant V2 (urea + functionalized biomass), the average fruit yield was 10.521 t/h; the obtained value was much higher than the control (unfertilized variant) yields of 8.255 t/h (Figure 9). Significant yield differences were also obtained in the case of the V3 fertilization variant (NP + functionalized biomass).

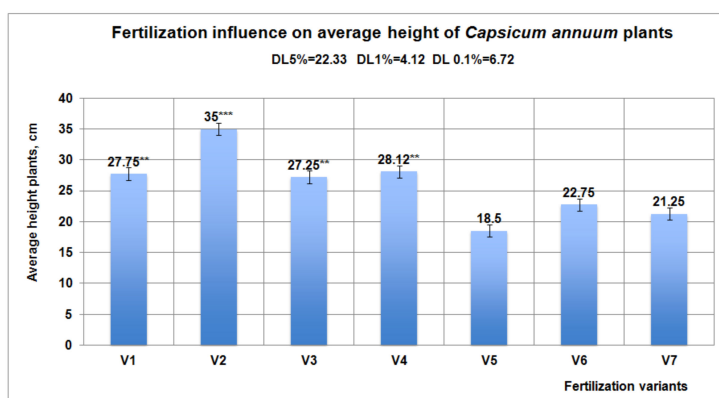


Figure 7. Fertilization influence on the average height of *Capsicum annuum* plants.

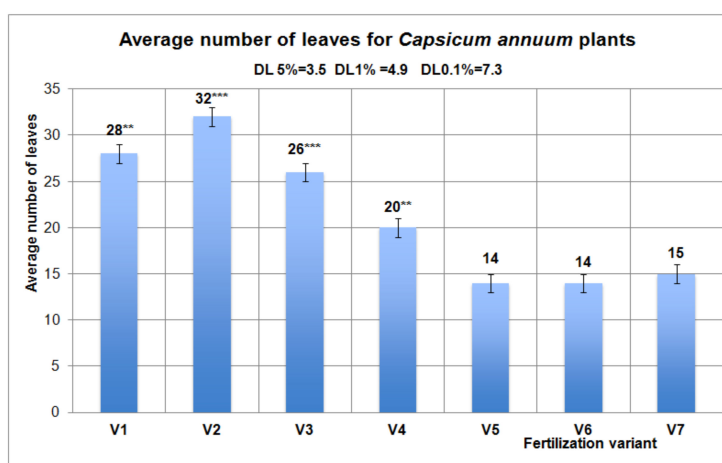


Figure 8. Fertilization influence on the average number of leaves for *Capsicum annuum* plants.

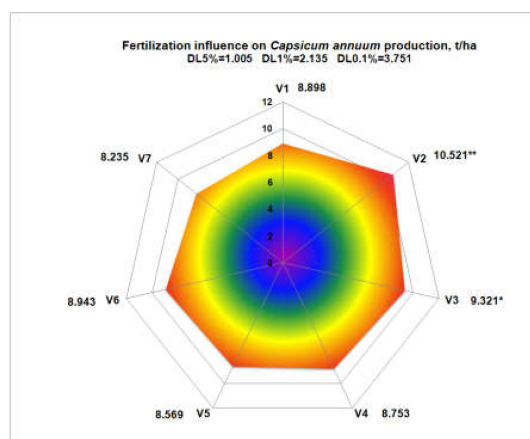


Figure 9. Fertilization influence on *Capsicum annuum* production.

4. Discussion

Germination is inhibited if the functionalized biomass is used as a cultivation substrate without other additions. This may be due to the high concentration of cations (cations of microelements adsorbed on the biomass and cations already existing in the biomass structure) due to diffusion from the biomass in the watering solution [14]. These, and likely other components of the biomass structure, obstruct the hydrolysis of reserve nutrients from the seeds [15], and inhibit the expression of signaling pathways responsible for regulating the level of gibberellin and abscisic acid, which results in total germination inhibition [16].

Regarding the development of *Solanum lycopersicum* plants, highly significant differences in plant height were observed in the case of fertilization variants with urea (V2), and/or with urea and ammonium phosphates + urea in the presence of the functionalized biomass, particularly when compared with the control.

Significant differences were obtained when fertilization was performed with NPK and/or NK. This fact is confirmed by Yeboah et al. [17], who found that if the fertilization of *Solanum lycopersicum* (Petomech variety) was done with NPK 15:15:15 and with fertilizers containing 24% N and 6% S, respectively, then plants had the highest average height. The best results in terms of plant height were obtained by Ewulo et al. [18] for fertilization variants based on urea and bird manure. Martins et al. [19] and Ashraf et al. [20] obtained similar results for foliar fertilization variants, which were based on mono-ammoniacal phosphates or urea and boric acid. In contrast, in hydroponic crops of *Solanum lycopersicum*, the best results regarding plant height were obtained in the case of fertilization with $\text{Ca}(\text{NO}_3)_2$ [21].

Degefa et al. [22] showed that for a fertilization norm of 99 kg N/ha, the plant height of *Solanum lycopersicum* was comparable with that of unfertilized plants; the differences obtained were statistically insignificant.

In terms of fruit production for *Solanum lycopersicum*, Fandi et al. [23] obtained high yields for NPK 20:6:45 and 30:6:30 fertilization variants. Other scientists [24] obtained high yields per hectare in the case of fertilization with chemical products (44–46.6 t/ha) and when using an exhausted compost of macromycetes (43.3–42.5t/ha), respectively.

In another study, Hozhbryan [25] found that fertilization with urea at a norm of 150 mg N/kg soil resulted in yields of 344 g fruits per plant. Ogundare et al. [26] showed that a fertilization rate of 108.6 kg $\text{CO}(\text{NH}_2)_2$ /ha led to significant production differences for the culture of *Solanum lycopersicum* when compared to the unfertilized variant. Ewulo et al. [18] found that by combining $\text{CO}(\text{NH}_2)_2$ with the manure from poultry farms to fertilization norms of 100 kg N from $\text{CO}(\text{NH}_2)_2$ + 4t manure, significant differences were obtained in terms of the average number of fruits/plant (29 fruits/plant) and the average amount of fruit harvested from each plant (724.24 g fruits/plant), when compared to the variant when only urea (100 kg N/ha) was used (616.07 g of fruits/plant).

Fertilization with materials formulated with vermicompost and mineral fertilizers of NPKS, which contain $\text{CO}(\text{NH}_2)_2 + (\text{NH}_4)_2\text{HPO}_4 + \text{K}_2\text{SO}_4$ and that are supplied in amounts of 8 t vermicompost/ha + 50% of the fertilizer requirement, resulted in greater yield than the control [27], with an average yield of 15.47. Fertilization with nitrogen, at a norm of 99 kg/ha, led to significant differences regarding the average number of fruits per plant [22], when the average number of 28.16 fruits per plant was obtained with an average production of 33.2 t/ha.

Concerning the culture of *Capsicum annuum*, after Stagnari and Pisante [28], the cultivation substrate must have an electroconductivity of 0.6–0.8 dS/m. Good fertilization results can be reached with N, P, K, and microelements. Regarding chemical fertilizers, the plant can tolerate N and P from $(\text{NH}_4)_2\text{HPO}_4$, $\text{NH}_4\text{H}_2\text{HPO}_4$, NH_4NO_3 , and $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ obtained by the Norsk Hydro process. This may be the reason why the height and average number of leaves per plant obtained in our experiments had distinct, significant values for fertilization variants V1 (NPK) and V3 (NP) when compared to the untreated variant.

Highly statistically significant results regarding plant height and the average number of leaves/plant were obtained with fertilization variant V2 (N from $\text{CO}(\text{NH}_2)_2$ + N from functionalized biomass) compared with the untreated version. This result is in agreement with the observations of Malek et al. [29], who obtained measurements from plants grown in pots; the greatest numbers of leaves in that study were obtained using fertilization variants featuring zeolite and urea (3 g zeolite + 2 g urea/pot).

In experiments performed on the soil surface, Islam et al. [30] obtained higher results with respect to the number of leaves per plant when using fertilization variants containing urea and manure. The average number of leaves/plant in these cases was 16.6, which was compared to fertilization variants featuring NPK chemical fertilizers, which were based on $\text{CO}(\text{NH}_2)_2$ + $\text{Ca}(\text{H}_2\text{PO}_4)_2$ + KCl; the average number of leaves per plant in this case was 14.2.

The application of slow-release fertilizer materials, as based on cellulose and polyacrylamide, and which contained fertilizer elements, such as 14.3% nanohydroxyapatite, 8.6% $(\text{NH}_4)_2\text{HPO}_4$, 14.3% $\text{CO}(\text{NH}_2)_2$, and 5.7% K_2SO_4 , can lead to crops characterized by the highest average height and the average number of leaves per plant [31].

Currently, on the fertilizer market is commercialized a product called Biosol, developed by the pharmaceutical company Sandoz GmbH Biochemie, Austria, and which in a first form was used for the fertilization of mountain ski areas. The product is obtained by fermenting the residual microbial biomass resulting from the manufacture of penicillin (contains spent biomass of *Penicillium* sp.), wastes that are initially stabilized, in order to remove the residual penicillin [32].

The process is important because current environmental legislation [33,34] does not accept the introduction of even small amounts of antibiotics into the soil, in order to discourage the development of antibiotic-resistant bacteria. Another commercialized fertilizer, obtained from spent biomass of *Penicillium* sp., is called Biomeal [35] and contains enzymes, hormones, organic acids, and proteins. The product also contains a) degrading microorganisms of the genera *Aspergillus*, *Cladosporium*, *Sterptomyces*, antagonistic microorganisms (*Trichoderma* sp.); phosphate-solubilizing microorganisms (*Penicillium* sp., *Bacillus* sp.), and N-fixing microorganisms (*Azotobacter* sp.). Biomeal improves soil structure and increases production by 20%–30%.

The fertilizers formulated in our study with functionalized microbial biomass can be used for basic soil fertilization in controlled environments or in fields. In the first phase of vegetation, the plants use the available nutrients (like urea or phosphates from soluble calcium salts), followed by the stage of vegetation, when the biomass and insoluble calcium phosphates from this type of fertilizers are mineralized into the soil. Finally, these processes lead to the accumulation of nutrients and humic compounds into the soil that will be used by plants during vegetation, which was demonstrated in the present study through the significant productions obtained for peppers and tomatoes.

5. Conclusions

The solid byproducts resulting from pharmaceutical biosynthesis represent an important bioresource that can be used in agriculture following stabilization and functionalization with microelements. Such a bioresource, after functionalization, can be applied to formulate different assortments of mixed fertilizers, which are advantageous, as once introduced into the soil, they represent a source of progressively available N (functionalized biomass contains 3.7% N) and acts as a precursor for humic and fulvic compounds. The functionalized biomass represents a source of microelements that are gradually released into the soil when the concentration drops below a certain level. In quantities $\leq 11\%$, the functionalized biomass can be mixed with soil and can be used as a germination substrate for most leguminous plants grown for leaves (*Lactuca sativa*, *Brassica oleracea*) or fruits (*Solanum lycopersicum*, *Capsicum annuum*, *Beta vulgaris*).

Regarding crop yields, the best results were obtained during the autumn fertilization of vegetables with products containing urea and functionalized biomass, which can be granulated together or embedded as such under the furrow. In these cases, a highly significant production increase can be obtained for crops of *Solanum lycopersicum* and *Capsicum annuum*, respectively. There are also NP fertilizer types that contain a functionalized biomass for autumn fertilization, and which facilitate the significant and distinct production of *Solanum lycopersicum* and *Capsicum annuum*, respectively.

In comparison with the products obtained from waste of *Penicillium* sp., as Biomeal and Biosol, the biofertilizers obtained from the spent biomass of *Streptomyces* sp. represents an original variant to obtain bioproducts for agriculture, which was demonstrated by the tests in surface soils. The spent biomass, stabilized and functionalized with microelements, can be used in the formulations of mixed NP or NPK fertilizer types and the use of these products in the autumn fertilization brings significant production enhancements in the case of tomatoes and red pepper crops.

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