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INFLUENCE OF CHEMICAL FERTILIZERS AND BIOFERTILIZERS ON THE DYNAMICS OF SOME MICROBIAL GROUPS (HETEROTROPHIC BACTERIA, FREE NITROGEN-FIXING BACTERIA) IN CHERNOZEM SOIL OF DOBROGEA (CUMPĂNA, VALU LUI TRAIAN)

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ABSTRACT

The aim of the current paper was to observe the effect of the application of organic and inorganic amendments on the dynamics of heterotrophic bacteria and free nitrogen-fixing bacteria abundance. Research, done at two locations in Dobrogea, Romania, showed that the average total number of culturable heterotrophic bacteria ranged between 21.89-116.32x10^6CFU/g dry soil (at Cumpăna), and 19.29-104.94x10^6CFU/g dry soil (at Valu lui Traian). The average total number of free nitrogen-fixing bacteria ranged between 14.9-123.18x10^5CFU/g dry soil (at Cumpăna) and 135.2-238.29x10^5CFU/g dry soil (at Valu lui Traian). Bacterial density increased significantly following the administration of specific biofertilizers (Biovin, BactoFil Professional, Mycos Green). Inorganic fertilizers did not have a positive effect on microbial density values, which were more or less similar to those reported for the control.

Key words: humus, bio-activators, fertility, biodiversity, heterotrophic bacteria, free nitrogen-fixing bacteria.

INTRODUCTION

The research was conducted in Cumpăna and Valu lui Traian, in Dobrogea, aiming to establish a pattern of chernozem soil biological reconstruction by applying various amendments for stimulating bacterial abundance, with the following objectives:

- degradation and decomposition of organic matter;
- restoration of soil structure;
- recovery of soil humus stock;
- atmospheric N_2 fixation in soil.

MATERIAL AND METHODS

Experimental plots consisted of 7 hectares of arable land outside Cumpăna and 7 hectares of arable land outside Valu lui Traian in Constanța County. Variants were labelled according to the technological inputs used, each variant being given a different type of fertilizer, in different quantities and at different times (Table 1). Winter wheat cultivar Josef was used.

Experimentation period was the 2009-2010 agricultural year (at Cumpăna) and 2010-2011 (at Valu lui Traian), both in different phases of the wheat growth season and after harvest, studying microbial growth for each variant.

Biovin is produced through a special technological process from grape kernels. 12 years of research proved that it aerates the soil, improves it (it contains up to 70% humus producers – 8x10^7CFU/g), enriches the soil with microorganisms that create humus (8x10^9 aerobic microorganisms per gram), supplies the plants with nutrients and biostimulators, increases the number of radicles and root hairs (www.Biovin.intertrest.com).

BactoFil Professional is a product for improving the soil biological quality and contains nitrogen-fixing bacteria 5.2x10^9CFU/ml (*Azospirillum brasiliense, Azotobacter vinelandi* (associative nitrogen-fixing system to 100-110 kg_ha), phosphate-solubilization bacteria and heterotrophic bacteria (*Bacillus megaterium, Bacillus polymyxa,*

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Pseudomonas fluorescens, Streptomyces albus) that stimulate the decomposition of organic matter and increase the bioavailability of phosphorus and potassium (Berca, 2008).

Mycos Green is a product containing arbuscular mycorrhizal fungi and a number of alginates, polysaccharides, enzymes and hormons that stimulate the establishment of mycorrhizal symbiosis, improving the soil quality for up to 20 years (Berca, 2008).

The soil was sampled at a depth of approximately 15cm, to perform a quantitative analysis of free nitrogen-fixing bacteria and heterotrophic bacteria.

Table 1. Fertilizers applied

Experi-mental plots	Fertilizers applied
V_1	$N_{15}P_{25}K_{15}$ – 100 kg_ha^{-1} in autumn NH_4NO_3 – 150 kg_ha^{-1} at the beginning of spring
V_2	Biovin – 400 kg_ha^{-1} Biovin – 30 l_ha^{-1}: – ½ at herbicide application – ½ at flowering stage
V_3	Manure – 15t_ha^{-1} in autumn;
V_4	Biovin – 30l_ha^{-1}: – ½ at herbicide application – ½ at flowering stage
V_5	Biovin – 150kg_ha^{-1} – administered during sowing NH_4NO_3 – 150kg_ha^{-1}: – 40kg_ha^{-1} at the beginning of spring – 50kg_ha^{-1} at herbicide application – 60kg_ha^{-1} at flowering stage
V_6	Biovin – 375kg_ha^{-1}; Biovin – 30l_ha^{-1}: – ½ at herbicide application – ½ at flowering stage Green Mycos – 1mc_ha^{-1}; BactoFil Professional – 1l_ha^{-1}.
V_7	Control – without applied amendments

Quantitative determination of microbial abundance was done by decimal dilutions of soil followed by inoculation of known quantities on solid nutrient media (Clark, 1965; Florenzano, 1983). For this purpose, after weighing, the samples were inoculated on culture media with a specific composition (Buchanan et al., 1975).

To determine the number of total culturable free N_2-fixing bacteria (*Azotobacter* species and Cyanobacteria), Ashbey's Nitrogen-Free Agar medium was used (Mannitol 15g; K_2HPO_4 0.2g; $MgSO_4 \cdot 7H_2O$ 0.2g; $CaCl_2 \cdot 2H_2O$ 0.2g; MoO_3 (10% solution) 0.1ml; $FeCl_3$ (10% solution) 0.05ml; agar 17-20g; pH 7.2+/- 0.2; sterilized 30 min at 121^{o}C). Three Petri plates were inoculated for each variant.

To determine the number of total culturable heterotrophic bacteria, nutrient-agar medium was used (pulvis yeast extract 2.5g, peptone 0.2g, agar 17-20g; sterilized 20 min at 120^{o}C). Three Petri plates were inoculated for each variant (Dumitru et al., 2005).

The total number of bacteria per gram of soil was calculated using the following formula:

No. bacteria = X colonies x dilution x 10 x 100/100-U

where: X = average of colonies grown on culture medium; 10 = balancing coefficient of 0.1ml; inoculum to which soil dilution is reported; U% = soil moisture (Papacostea, 1976).

RESULTS AND DISCUSSION

In the experimental field at Cumpăna (2009-2010), the average abundance of free N_2-fixing bacteria varied from a minimum of 14.9x10^{5}CFU/g dry soil, in the control variant to a maximum of 123.18x10^{5}CFU/g dry soil, in the V_3 (manure), followed closely by V_6 (Biovin, BactoFil, Mycos), with 90.11x10^{5}CFU/g dry soil (Figure 1).

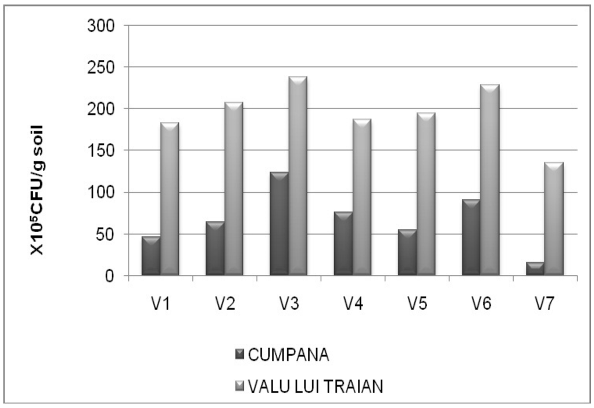

Figure 1. Annual average variation in the dynamics of free nitrogen-fixing bacteria, depending on technological inputs

Variants which received Biovin compost, V_4, with $75.3x10^5$CFU/g dry soil, and V_2, with $63.68x10^5$CFU/g dry soil (at Cumpăna), showed similar values, higher than both the control variant and the variants with chemical fertilizers (Figure 1).

Variants V_1 with ($46.41x10^5$CFU/g dry soil) and V_5 ($54.6x10^5$CFU/g dry soil), at Cumpăna, where NH_4NO_3 was applied, showed the lowest abundance of free nitrogen-fixing bacteria, their growth being inhibited by the high nitrogen quantity in soil (Figure 1).

During the 2010-2011 agricultural year, (at Valu lui Traian), the lowest abundance of free nitrogen-fixing bacteria was observed in the control variant ($135.2x10^5$CFU/g dry soil). The highest was observed in V_3 (manure) – $238.29x10^5$CFU/g dry soil; closely followed by V_6 (Biovin, BactoFil Professional, Mycos Green), with $228.25x10^5$CFU/g dry soil and V_2 (solid and liquid Biovin), with $207.55x10^5$CFU/g dry soil (Figure 1).

Variants $V_{1,}$ (chemical fertilizers), V_4, (liquid Biovin) and V_5, (ammonium nitrate, Biovin compost), showed higher, but not significantly higher values of microbial abundance, than those in the control variant (Figure 1).

Regarding the abundance of free nitrogen-fixing bacteria, compared with the control plot, different variants showed different degrees of growth:

- highest growth -V_3 (manure) and V_6 (Biovin, BactoFil Professional; Mycos Green);
- medium growth - V_4 and V_2 (Biovin compost);
- insignificant growth - V_5 (Biovin, ammonium nitrate) and V_1 (chemical) (Figure 1).

Comparing the annual dynamics of heterotrophic bacteria, it can be observed that the values obtained at Valu lui Traian were higher than those at Cumpăna, but the increase was insignificant, considering the different local trophic and meteorological conditions in the two years (Figure 2).

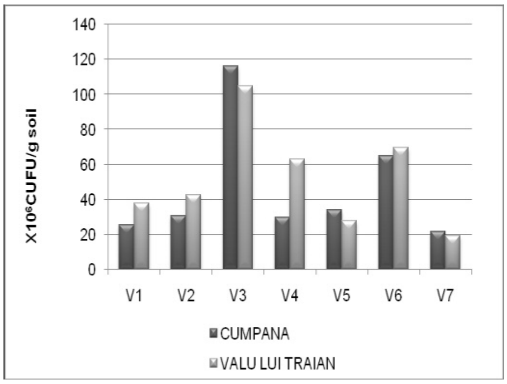

Figure 2. Comparison between average annual abundances of heterotrophic bacteria, depending on technological inputs

At both locations, the inputs that maintained and stimulated the abundance of heterotrophic bacterial communities were the following:

- V_3 (manure) – $116.32x10^6$CFU/g dry soil (Cumpăna); $104.94x10^6$CFU/g dry soil (Valu lui Traian);
- V_6 (Biovin, BactoFil Professional; Mycos Green) – $64.99x10^6$CFU/g dry soil (Cumpăna); $69.73x10^6$CFU/g dry soil (Valu lui Traian);
- V_5 (Biovin, ammonium nitrate) - $34.01x10^6$CFU/g dry soil (Cumpăna);
- V_4 (liquid Biovin) – $63.03x10^6$CFU/g dry soil (at Valu lui Traian), on the third place due to trophic conditions; the first data in the autumn of 2010 were superior to those in other variants;
- V_2 (solid, liquid Biovin) – $42.66x10^6$CFU/g dry soil (Valu lui Traian);
- V_1 (chemical fertilizers) – $25.66x10^6$CFU/g dry soil (Cumpăna), $37.95x10^6$CFU/g dry soil (Valu lui Traian), showed insignificant growth, values being close to the control variant (Figure 2).

CONCLUSION

In order to increase the abundance of free nitrogen-fixing bacteria, we recommend the technology used in the following variants:

- V_3 (manure);
- V_6 (Biovin, BactoFil Professional; Mycos Green);
- V_2 (solid and liquid Biovin).

In both experimental plots, the technological inputs that maintained and stimulated the abundance of heterotrophic bacterial communities were the following:

- V_3 (manure);
- V_6 (Biovin, BactoFil Professional; Mycos Green);
- V_5 (solid Biovin, ammonium nitrate).

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