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SOIL QUALITY ASSESSMENT BASED ON C:N RATIO IN AN ALLUVIAL SOIL TREATED WITH MICROBIAL INOCULANTS

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Abstract

Carbon and nitrogen are two of the most important elements found in soil structure and the relationship between them has a special relevance on soil characterization. This relationship is known as carbon-nitrogen ratio (C/N ratio) and indicates the rate of decomposition of organic matter. Degradation of organic matter will certainly modify the C/N ratio, and this modification is related to the existing microorganisms in soil. The present study follows the evolution of the C:N ratio in a calcaric alluvial soil from Buzau county used for organic tomato cultivation and subjected to a microbial treatment based on Beauveria bassiana inoculants. The experimental scheme includes three variants: (1) untreated soil, uncultivated (control); (2) untreated soil, cultivated with Florina 44 tomato variety; (3) soil treated with microbial inoculants, cultivated with Florina 44 tomato variety. Each variant was observed on two different lots and in two moments (autumn 2019 and autumn 2020). The soil samples were taken from topsoil, dried at room temperature, and analysed using the CHNS elemental analyser for C and N determinations. Regarding the C:N ratio, the results pointed out that there are some differences that correlate with the use of microbial inoculants in tested variants.

***Key words**: C:N ratio, alluvial soils, microbial inoculants, CHNS elemental analysis, organic system*

INTRODUCTION

Lately, the idea of sustainability became the way forward for more and more farmers due to increasing demand for organic agricultural products. To deal with this challenge, the farmers must follow practices that help maintain a balance in natural systems. These practices cannot guarantee zero risk products but can minimize other problems such as air, water, and soil pollution (Klonsky, 2000).

The long-term soil organic management practices affect microorganisms and microbiological processes and change the quality and quantity of plant wastes, which are the main source of soil organic matter (Micuți et al., 2020). Between organic farming practices, the use of microbial inoculant for crop production gains more and more attention.

Although the first microbial inoculant was developed more than one hundred years ago, the true progress of this type of input was achieved in the last decades, regarding both production and the use of it (Santos et al., 2019). Microbial inoculants are natural products, and they are used for pest control, for quality improvement of soils or crops, and they can help reduce chemical fertilizer applications. Microbial inoculants could include bacteria, fungi, and algae (Alori & Babalola, 2018). There are a small number of studies that have investigated the use of microorganisms for enhancing the soil fertility. Application of entomopathogenic fungi was tested with many species as *Metarhizium anisopliae sensu lato, Beauveria bassiana, Isaria farinosa, Isaria fumosorosea, Aspergillus flavus and Lecanicillium lecanii* by Jaworska, in 1979 and 1981 and the studies about these fungal species were initiated continuously. Many studies focused on pest and disease management using fungias an alternative to chemical inputs in the organic agricultural systems (Sicuia et al., 2014; Canhilal, 2016). Also, the addition of these fungal endophytes in composting materials could improve the decomposing process resulting in materials that have better properties such as high nutrient content, high population of beneficial microbes and low toxicity (Nchu, 2020). Elson et al. (1998) studied various effects of carbon concentrations, C:N ratios, and amino acids to *Helminthosporium solani* growth on different culture mediums. Similar work has been conducted by Gao et al. (2007), when the effects of several fungi were observed on carbon concentration and C:N ratio. The ratio of total organic carbon and total nitrogen indicates the rate of decomposition of organic matter. Recently, the amount of soil nitrogen and C:N ratio are used as indicators of soil carbon sequestration (Edu et al., 2012). Carbon is the main energyproducing element, while nitrogen helps building cell tissues. A C:N ratio of 20 indicates that there are 20 grams of C for each 1 gram of N. When organic matter decomposes, the carbon content decreases faster than nitrogen, reducing the C:N ratio (Miller, 2000). When the C: N ratio gets lower, nitrogen rapidly will be released into the soil for instantaneous crop use. When an organic substrate has a C:N ratio lower than 15, rapid mineralization takes place, with release of N, which is available for plant uptake. A C:N ratio bigger than 35 results in nitrogen immobilization. A ratio of 20-30 consists in equilibrium between mineralization and immobilization (Figure 1) (Brust, 2019).

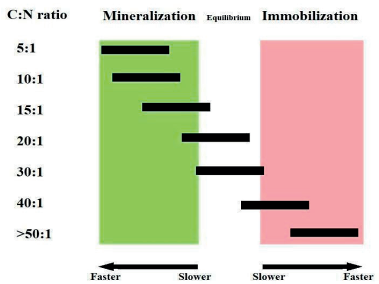

Figure 1. Nitrogen availability by C:N ratio variations. (After Brust, 2019)

Mineralization and immobilization are two soil processes related to nitrogen cycle; during mineralizationnitrogen is released for plant uptake while during immobilization the microbes utilize and tie up nitrogen.

During microbial degradation, the organic matter is used as a source of carbon by the soil microbial populations to fuel considerable population growth. These native microbial populations represent a natural antagonist to fungi, and they are one of the most active factors in determining the persistence of fungi in these environments (Jaronski, 2010). In an experiment about soil application of *Beauveria bassiana*, Swiergiel et al. (2016) noticed that the fungal density was maintained above the upper natural background level for about one season but came back to normal background levels within a year. A special attention must be paid to pesticide management, especially to fungicide use, because these treatments can influence or even stop the effects of *B. bassiana* soil inoculant (Soares et al., 2017). This study follows the evolution of the C:N ratio of the soil when a microbial inoculant is used in a tomato crop. The results are compared with two control variants: the soil untreated with no crop and the soil untreated but cultivated with the same tomato variety.

MATERIALS AND METHODS

Experimental field

The experiment was conducted between 2019 and 2020 at the Vegetable Research and Development Station Buzău (S.C.D.L Buzău). The study aims to determine the modification of soil C:N ratio after using a microbial inoculant based on *Beauveria bassiana* ('Bbmi') fungus, on a tomato crop. As biological material, tomato Florina 44 variety was used. The soil was characterized in 2018 as a calcaric alluvial soil, founded on fluvial deposits, on a meadow region, with the groundwater lower than 3 m (Musat et al., 2018). Experimental design consists in two different plots, each one holding three variants: V1- untreated soil, uncultivated (as control); V2 - untreated soil, tomato crop; V3 -'Bbmi' treatment, tomato crop. The experimental scheme is represented in Table 1.
The soil samples were taken in autumn 2019 and in autumn 2020, to find out if Bbmi treatment will keep his effects over time. The soil samples were collected from the surface layer of the experimental fields (20 cm), of the three variants (Figure 2).

Figure 2. Aspects from the experimental fields.
V1 - uncultivated lot; V2 - untreated soil, tomato crop lot; V3 - Bbmi treatment, tomato crop lot

Analysis of C and N content

The analyses were performed at the Laboratory of Agrochemistry, of the Research Center for Studies of Food Quality and Agricultural Products, University of Agronomic Sciences and Veterinary Medicine of Bucharest.

All soil samples were air dried, ground with the laboratory soil grinder and sieved through a sieve of 250 microns. The samples were kept in a dry environment until analysed.

Table 1. Experimental scheme

Plot	Variant	Sample collection time
Plot A	V1 untreated soil, uncultivated	October 2019
	V2 untreated soil, tomato crop	October 2019
	V3 Bbmi* treatment, tomato crop	October 2019
	V1 untreated soil, uncultivated	September 2020
	V2 untreated soil, tomato crop	September 2020
	V3 Bbmi treatment, tomato crop	September 2020
Plot B	V1 untreated soil, uncultivated	September 2020
	V2 untreated soil, tomato crop	September 2020
	V3 Bbmi treatment, tomato crop	September 2020

*Bbmi - microbial inoculants based on *Beauveria bassiana.*

An amount of 5-10 mg of soil sample was used to determine the total nitrogen and carbon content. The analysis was performed using the CHNS elemental analyzer (EuroVector EA3100 Elemental Analyzer). Cystine was used as standard reference material. All determinations were performed in three repetitions.

Statistical analysis

The obtained values were processed using IBM SPSS (Version 27.0) statistical software. Duncan test (Alpha = 0.05) was used for multiple comparison between the three variants used in the experiment.

RESULTS AND DISCUSSIONS

Taking the plots each one separately, the effects of applied inoculants compared with the variants without microbial treatments differ from one year to another.

On plot A in 2019, the C:N ratio on untreated and uncultivated soil (V1) was 11.02. On cultivated soil, untreated (V2) and on cultivated and treated soil (V3) the C:N ratio was significantly higher, 11.83 and 12.00, respectively (Figure 3). This change may occur due to tomato crop which may influence the soil microbiology or due to nitrogen uptake by the plants which led to a decrease of nitrogen in soil. Even so, the modification of C:N ratio must be much higher to have a visible effect on the plants. At this value, the nitrogen content is adequate and there is no risk of immobilization of this element.

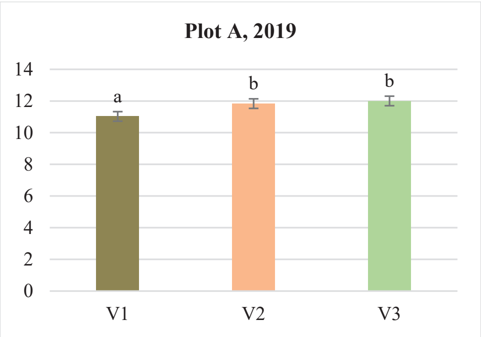

Figure 3. C:N ratio between V1, V2, V3 variants on Plot A in 2019

On the same plot (Plot A), in 2020, a significant increase of C:N ratio is observed on all variants, compared with the previous year. (Figure 4).

The modification of this indicator, even on the control variant may be explained by either the loss of nitrogen on this area or the increase of carbon content. It is also possible that the lower outside temperature at the first sample collection (October 2019) influenced the microbial activity compared to the second sample collection (September 2020), leading to this increase on C:N ratio. The influence of temperature on C:N ratio was studied by Kai et al. (1969), leading to the same response, that at lower temperatures, the nitrogen immobilisation is higher, resulting in a reduction of C:N ratio.

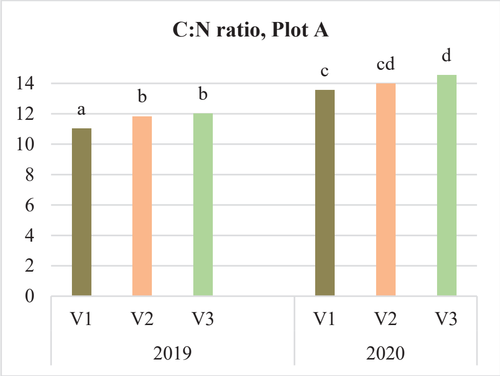

Figure 4. The evolution of C:N ratio over a year (2019 and 2020), on the same plot

Also, regarding the difference between variants on 2020 samples, it can be noticed that the MI treated variant (V3) had a significant increase of C:N ratio compared to untreated and uncultivated variant (V1) (Figure 5). This also may be due to soil treatment with microbial inoculation. The increase of this indicator, even far to the optimal level, can be beneficial for the crops on this treated soil.

Figure 5. C:N ratio between V1, V2, V3 variants on Plot A in 2020

Considering the plot B, no difference was observed on C:N ratio in any variant, for soil samples collected in the same year as the inoculation (Figure 5).

Comparing the two plots studied in 2020, the only significant difference was observed on

Bbmi treated variant on the plot A, where the treatment was 2 years old.

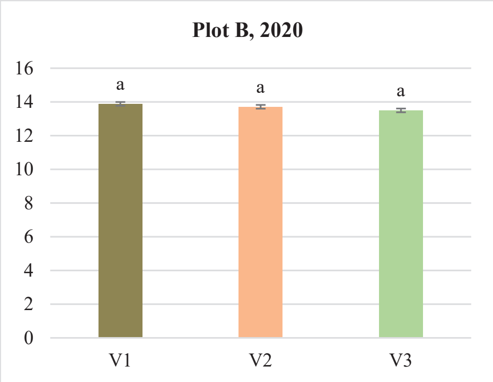

Figure 6. C:N ratio between V1, V2, V3 variants on Plot B in 2020

The rest of the variants did not relate any noticeable differences. To be mentioned that the samples from plot B, even though they were taken in the same year as the Bbmi treatment, have the C:N ratio closer to samples from plot A taken in 2020, two years after inoculation (Figure 7). This similarity may come from the fact that the samples were taken in the same month (September).

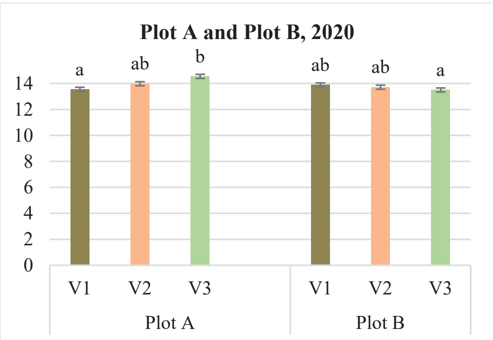

Figure 7. Difference between Plot A (treated in 2019) and Plot B (treated in 2020)

CONCLUSIONS

The treatment with microbial inoculants based on *Beauveria bassiana* fungus did not provide significant changes of C:N ratio in the same year of inoculations. As other study reported, this indicator has a rather limited variation over time and sometimes it takes years to notice a significant change (Cabral, 2012).

Anyway, the treatments may provide other beneficial effects, such as pest and disease reduction, but these aspects were not relevant for this paper, as no soil pests were impacting the tomato crop in the 2019-2020 period or in nutrient cycling, plant growth, nutrient uptake, aspects that will be considered in further studies, on medium term.

For the treatment performed in 2019, a significant increase of C:N ratio was observed in the treated variant compared with the untreated and uncultivated variant, one year after inoculation.

The modification of C:N ratio did not reach an optimal level in the studied period, but still the small increase over just one year indicates that on medium and long term the use of microbial inoculant on organic fields could improve the productive potential of agroecosystem.

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REFERENCES

Alori, E.T. & Babalola, O.O. (2018). Microbial Inoculants for Improving Crop Quality and Human Health in Africa. *Frontiers in Microbiology 9*, 2213.

Brust, G. E. (2019). Safety and Practice for Organic Food. *Academic Press, Elsevier.*

Cabral, M.R. (2012). Relation and change over time of CN-ratios throughout Swedish peatlands and in seven fertility classes. *Master's Thesis in Environmental Science, SLU, Swedish University of Agricultural Sciences* , Uppsala, Sweden

Canhilal, R. (2016). The use of entomopathogens in the controlling of insect pests of stored product. *Scientific Papers. Series A. Agronomy, LIX.* 235-240.

Edu E. M., Udrescu S., Mihalache M.&Dincă L. (2012). Research concerning the organic carbon quantity of National Park Piatra Craiului and the C/N ratio. *Scientific Papers. Series A. Agronomy,* LV:44-46.

Elson, M.K., Schisler, D.A. and Jackson, M.A. (1998). Carbon-to-Nitrogen Ratio, Carbon Concentration, and Amino Acid Composition of Growth Media Influence Conidiation of Helmintho sporium solani. *Mycologia 90*(3), 406-413.

Gao, L., Sun, M.H., Liu, X.Z. and Che, Y.S. (2007). Effects of carbon concentration and carbon to nitrogen ratio on the growth and sporulation of several biocontrol fungi. *Mycopathologia 169*, 475–481.

Jaronski, S. T. (2010). Ecological factors in the inundative use of fungal entomopathogens. *Bio Control, 55*(1), 129–145.

Jaworska, M. (1979). The role of some entomopathogenic fungi in reduction of european apple sawfly *Hoplocampa testudinea* Klug (Hymenoptera, Tenthredinidae) laboratory studies. *Bulletin de l'Academie Polonaise Des Sciences. S´erie des Sciences Biologiques 27*, 1059–1062.

Jaworska, M. (1981). Studies on the possibility of limiting populations of the apple sawfly - *Hoplocampa testudinea* Klug. (Hymenoptera, Tenthredinidae) by the use of parasitic fungi. *RocznikiNaukRolniczych, E*(9), 169–181.

Kai, H., Ahmad, Z. & Harada, T. (1969). Factors affecting immobilization and release of nitrogen in soil and chemical characteristics of the nitrogen newly immobilized, *Soil Science and Plant Nutrition, 15*(5), 207–213

Klonsky, K. (2000). Forces impacting the production of organic foods. *Agriculture and Human Values 17*, 233–243.

Micuți, M.M., Popa (Burlacu), A., Bădulescu, L. & Israel-Roming, F. (2020). Soil enzymes - bioindicators of soil health. *Scientific Papers. Series B, Horticulture. LXIV*(1), 679–684.

Miller, C. (2000). Understanding the Carbon-Nitrogen Ratio. *Acres U.S.A 30*(4)*,* 20–22.

Musat, M., Ciceoi, R., Burnichi, F., Mot, A., Dobrin, A. & Zugravu (Micuti) M. M. (2018). Characterization of soil conditions in an organic testing field for vegetable crops, Buzau county, Romania. IBIMA Conference, Education Excellence and Innovation Management through Vision 2020: From Regional Development Sustainability to Global Economic Growth (32), 2014-2022.

Nchu, F. (2020). Assessment of the Effects of *Beauveria bassiana* (Hypocreales) Inoculum on the Composting of Vegetable Wastes. Conference: 18th SOUTH AFRICA Int'l Conference on Agricultural, Chemical, Biological & Environmental Sciences (ACBES-20), 249-253.

Santos, M.S., Nogueira, M.A. & Hungria, M. (2019). Microbial inoculants: reviewing the past, discussing the present and previewing an outstanding future for the use of benefcial bacteria in agriculture. *Santos et al. AMB Expr. 9*(205), 1-22.

Sicuia, O.A., Dinu, S., Dinu, M., Fătu, C., Vălimareanu, D., Mincea, C. & Constantinescu, F. (2014). Pests and diseases management using compatible biocontrol bacteria and entomopathogenic fungal strains. *Scientific Bulletin. Series F. Biotechnologies, XVIII*, 66-72.

Soares, F.B., Monteiro, A.C., Barbosa, J.C. & Mochi, D.A. (2017). Population density of *Beauveria bassiana* in soil under the action of fungicides and native microbial populations. *Acta Scientiarum. Agronomy39* (4), 465-474.

Swiergiel, W., Meyling, N.V., Porcel, M. &Ramert, B. (2016). Soil application of *Beauveria bassiana* GHA against apple sawfly, *Hoplocampa testudinea* (Hymenoptera: Tenthredinidae): Field mortality and fungal persistence. *Insect Science 23*, 854–868.