Scientific Papers. Series B, Horticulture. Vol. LXI, 2017
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

# SOIL MICROBIAL ACTIVITY IN AN ORGANIC EDIBLE ROSE CROP

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***Abstract***

*The paper presents the evolution of the microbial activity analyzed through the evolution of the soil respiration, bacteria and fungi density between March 2015 and November 2016, in an organic edible rose culture under the influence of three ameliorative species and two mulching systems. Beginning with the spring of 2015, with the goal of planting an edible rose culture, in the experimental field of USAMV Bucharest, a special soil preparation was applied using three ameliorative plants, Sinapis alba L., Tagetes patula L. and Phacelia tanacetifolia L. They have a special role to control pathogens in soil and were used in seven different combinations (V1-V7) and a control plot was kept without seeding (V8). After flowering and seed formation, the mature plants were trimmed and incorporated into the soil. They were seeded in the organic roses culture also, same variants between the rose rows in the spring of 2016. In the summer of 2016, two mulching variants were applied for each initial variant (Vn), on the rose's rows: Vn.1. wood chips and Vn.2. wool, while the control Vn.3. was represented by unmulched soil. Microbial activity was stimulated especially in variants with two plant species. The highest potential of soil respiration was characteristic for combinations including Tagetes but also in the variant with Sinapis alone that stimulated the bacterial activity in microbial communities. Generally, the bacteria and fungi density and species number was higher in V1-V7 variants than in the V8 control variant. Microbial species identified included ubiquitous bacteria and fungi with high metabolic capabilities to degrade various substrates such as cellulose from vegetal wastes or keratine from sheep wool added, due to efficient production of cellulase and keratinolytic protease enzymes (bacteria from genera Bacillus, Xanthomonas, Actinomycetes and fungi from genera Trichoderma, Aspergillus, Penicillium, Cladosporium, Paecilomyces, Myrothecium), many of them contributing to biological control of potential plant pathogens and nematodes in rose cultures.*

***Key words**: bacteria, fungi, soil respiration, wool mulch, wood chips mulch.*

## INTRODUCTION

One of the most important activities in organic agriculture is maintaining and enhancing the soil health respectively the soil organic matter (IFOAM, 2010; Berca 2011; Reeve, 2007).
In the same time, as it is stated by the principle of ecology (IFOAM, 2010), the organic agriculture should be based on living ecological systems and cycles, work with them, emulate them and help sustain them.
An important component for increasing the soil fertility and health can be green manure, cover crops, living mulch (Crossland et al., 2015).
Different kind of organic matter can bring additional positive effects on yield through amelioration of soil life, water retention, humus content (van Opheusden et al., 2012; Butcaru et al., 2016).
Maintaining diversity is another important aspect for perennial cultures in organic agriculture is. Intercropping can be a way of increasing crop diversity (Andersen, 2005; Butcaru et al., 2016).
The present paper presents the results of the microbiological activity in the soil after using an alternative and innovative method for improving the soil activity by using three ameliorative species: *Sinapis alba* L., *Tagetes patula* L. Sparky Mix and *Phacelia tanacetifolia* L., before and after the plantation of an organic edible rose culture.
In addition to the three ameliorative species, from the first year of plantation, two kind of mulch was used: wood chips and wool.
The research analyses the evolution of bacteria, fungi population and respiration coefficient, measured before the establishment of the edible rose culture and after one year and reflect the potential of the ameliorative plants and mulch to develop and maintain the soil activity.

## MATERIALS AND METHODS

The research was conducted in the experimental plot at the University of Agronomic Sciences and Veterinary Medicine of Bucharest of a total area of 1,350 $m^2$ with the purpose of planting three edible rose varieties using an organic technology.

Beginning with spring of 2015 a special soil preparation was applied using three ameliorative plants, *Sinapis alba* L., *Tagetes patula* L. and *Phacelia tanacetifolia* L., with role in soil disinfection (Butcaru et al., 2015; Butcaru et al., 2016).

Crops were sown in late March, by combining the three species in 7 variants: V1 *Sinapis*, V2 *Sinapis* + *Phacelia*, V3 *Phacelia*, V4 *Sinapis* + *Tagetes*, V5 *Sinapis* + *Tagetes* + *Phacelia*, V6 *Tagetes* + *Phacelia*, V7 *Tagetes* and a control parcel V8, was kept as black field, without sowing.

The same variants were seeded between the rose rows in the spring of 2016, after the organic roses planting.

After flowering and seed formation, the mature plants were trimmed and incorporated into the soil, all three species in the same time in June 2016.

Beginning with July 2016, the roses, planted on three rows on each variant (V1-V8), were supported by wire trellis and a drip system was installed and operational.

In the summer of 2016, two mulching variants were applied for each initial variant (Vn), on the roses rows: Vn.1. wood chips and Vn.2. wool, while the control Vn.3., was represented by un-mulched soil. Both mulched rows had the same 1 m width with the specific material.

The inter-row was kept grassy through repeated mowing.

In each variant (Vn) was applied the same scheme of treatment, including: fertilizing with manure in autumn 2015 at planting and organic products in 2016; bio stimulatory and caw milk for increasing the immunity system and plant protection with different organic.

For the analysis of the soil microbiological activity, samples were collected before and after planting the organic rose culture (from the total area in March 2015, from each variant Vn in July and October 2015, from each sub-variant Vn.1., Vn.2., Vn.3. in November 2016).

Microbiological analysis studied the number of heterotrophic bacteria determined using dilution plate method - by dispersing soil suspensions on the nutrient agar medium; number of microscopic fungi determined by dispersing soil suspension on PDA medium and soil respiration determined through the substrate induced respiration method according to RS-ISI-14240-1-(2012).

The taxonomical identification were carried out on the basis of the cultural, morphological and / or physiological characteristics in accordance with bacteria Identification Manual (Bergey, 1994) and fungi in agricultural soils (Domsch & Gams, 1972).

It has been used circular chromatograms of soil extracts, with diffusion through absorption on paper Whatmann no. 1, argentic coloring, which generates information on biological quality of the soil due to analytical separation and formation of images whose model of consistency, shape, size, color, texture may indicate the degree of soil health, vitality, fertility, the intensity of biotic activity, soil conditions, the complexity of organic matter and the presence of stable humus.

## RESULTS AND DISCUSSIONS

The microbiological analyses proved an increased activity of the soil under the influence of alternative and innovative methods applied.

The bacteria population significantly increased in the March 2015 - Novembre 2016, with a relative stabilisation in the last period.

Soil samples collected in 2016 (phase IV) showed a high density of heterotrophic aerobic bacteria relative to gram of dry soil, which ranged from a minimum of 32 x $10^6$ viable cells/g dry soil to V8.2. - Control with wool sub-variant to a maximum of 88 x $10^6$ viable cells / g dry soil to the V7.2. - *Tagetes* with wood chips sub-variant (Figure 1).

The application of organic technology by using organic materials (wool, wood chips) for mulching and ameliorative crops alone or combined caused significant increases in the number of heterotrophic aerobic bacteria relative to controls plots in general.

In the fourth phase it is visible the stimulatory effect of *Sinapis* and *Tagetes* on the proliferation of bacteria. The most important values of bacterial density registered under the effect of wool mulch were obtained at variant with *Sinapis*, followed by that of *Tagetes* as ameliorative plants, but generally using wool mulch has led to less numerous bacterial populations than in the rest of variants, especially when were used combinations of two species of ameliorative plants.

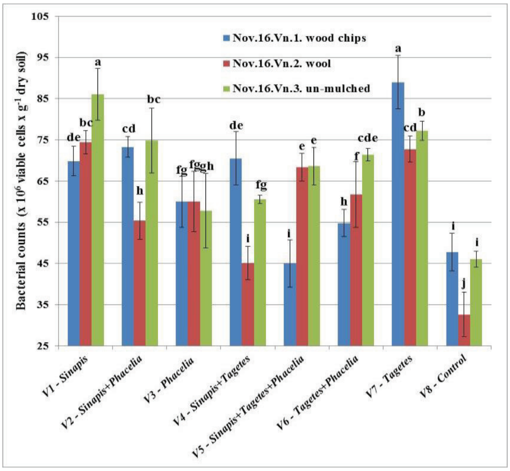


Figure 1. Influence of organic technology on bacterial microflora

Fungal microflora presented moderate values in this stage, below 100 x $10^3$ cfu / g of dry soil at control plots and to all variants with wool mulch and values considered high to a number of variants, from which the sub- variants with wood chips V3.1. *Phacelia*, V7.1. *Tagetes* or V1.1. *Sinapis* and the sub-variants un-mulched V7.3. *Tagetes*, V2.3. *Sinapis* + *Phacelia* and V6.3. *Tagetes* + *Phacelia* (Figure 2).

In terms of taxonomy, bacteria and fungi from analyzed soils include ubiquitorius species with high adaptive capacity and species equipped with enzymatic complex equipments, which enable efficient exploitation of a wide variety of substrates with very different origins. There is a considerable number of species capable to degrade and to metabolize organic substrates as wool, wood chips or debris of organic matter due to enzymes such as proteases (keratinases), cellulase and include bacteria belonging to the genera *Bacillus*, *Xanthomonas*, actinomycetes or fungal species from genera *Penicillium*, *Aspergillus*, *Paecilomyces*, *Myrothecium*, *Cladosporium*, *Trichoderma*.

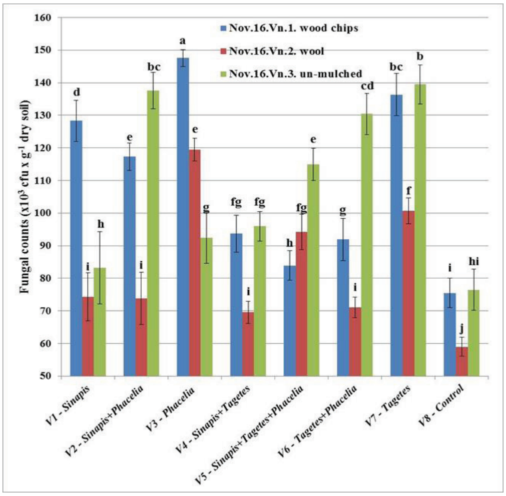


Figure 2. Influence of organic technology on fungal microflora

Many of these microorganisms, such as *Pseudomonas fluorescens*, actinomycetes, *Trichoderma viride*, *Trichoderma hazianum*, *Paecilomyces marquandii* stimulated by the presence of ameliorative plants and organic mulch represented by wool and wood chips act as antagonists against soil borne pathogens of genus *Fusarium*, *Phytophthora* and *Alternaria*, producing a beneficial effect on the health of edible rose culture (Figures 3 and 4).

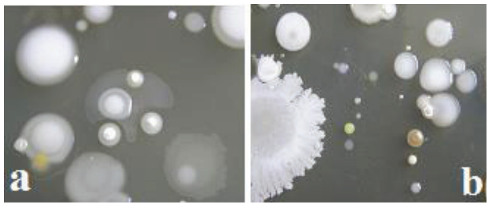


Figure 3. Bacterial microflora to V1.2. *Sinapis* with wool (a) and V7.1. *Tagetes* with wood chips (b)


Figure 4. Fungal microflora to V3.2. *Phacelia* with wool (a) and V7.1. *Tagetes* with wood chips (b)

Using ameliorative plants and different types of mulch in the organic technology for edible roses led to more dynamic global physiological activities of the soil microorganisms as reflected by a significant increase in soil respiration compared to controls, where recorded moderate values of released $CO_2$/100g dry soil (Figure 5).

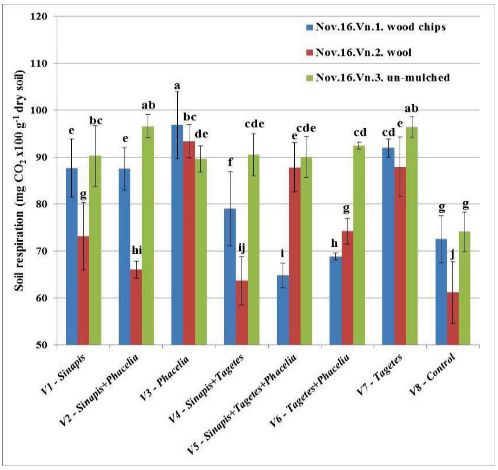


Figure 5. Influence of organic technology on soil respiration

The most intense metabolic activities were recorded at V3.1. *Phacelia* with wood chips sub-variant, mainly due to the activity of cellulolytic fungi, at V7.1. *Tagetes* with wood chips sub-variant mainly on account of bacterial activities (actinomycetes in particular) and cellulolytic fungi, followed by variants with *Tagetes* as ameliorative plant (based on fungal microflora) or combinations of two species of ameliorative plants.

In many of the variants with one or two species of ameliorative plants, soil respiration was more intense when it was used wood chips mulch compared to wool mulch but, in most cases, weaker or similar to the version un-mulched.

Figure 6 presents sectors of Pfeiffer chromate-grams for illustrating changes in soil quality under the influence of ameliorating plants (*Tagetes* and *Phacelia*) and mulch (wool and wood chips) compared to the control.

The analysis of chromatograms reveals an increasing silica organization due to biological activity though embattled shape of the outer edge of the central area in mulched variants, especially those with wood chips.

Clay shows most well-organized at the V3.1. *Phacelia* with wood chips sub-variant, the remaining variants presenting organization trends in different degrees of evolution, the organization level being correlate, in general, with the high level of chemical complexity.

The content of minerals increased significantly in V7.1. *Tagetes* with wood chips sub-variant, compared with the other experimental variants. Biological activity (bacterial and fungal), mineral diversity and enzyme activity is reflected in the organization of external and middle areas of the chromatograms, organizing corresponding to the increases of protein content, of the nutritional potential, of diversity of sources of carbon, of humic acids formation. Increases integration, connections between particles, due to increased dynamics of some processes compared with the control, but still insufficient to achieve a maximum level in the soil, possibly due to short action time of microbiota on the organic supplements. In the external area appear highlighted varied nutrient sources with an increase degree of stability, colloidal nature in the mulched variants and particularly better highlighted in the *Phacelia* variants. Formation of endings from the external area reflects the favorable evolution of organization of organic matter at variants with ameliorative plants and is more clearly evidenced at the variants mulched with wood chips.

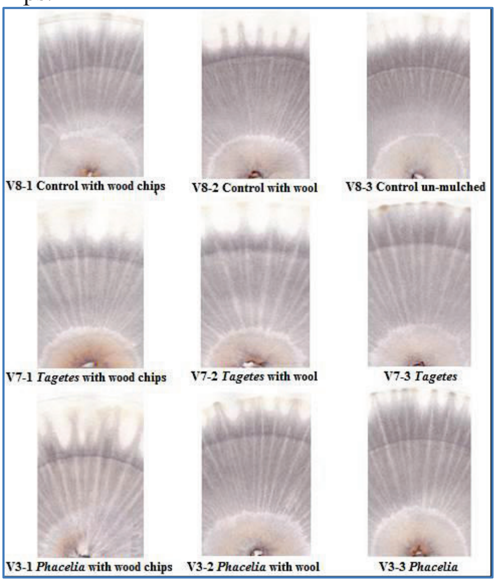


Figure 6. Soils chromatograms at the variants with ameliorative plants and mulch

## CONCLUSIONS

Positive results through organic technology applied to edible rose cultures were obtained regarding stimulating the development of fungal and bacterial microflora and increasing the global physiological activities of edaphical microorganisms compared to controls and with the initial phases.

It is remarkable the beneficial effect of *Tagetes* alone or in combination with *Phacelia* on the development of bacterial and fungal microflora.

Using wood chips mulch determined a large numbers of bacteria developing on V7. *Tagetes* variant and fungi on V7. *Tagetes*, V3. *Phacelia* variants, were was recorded the most intense soil respiration also.

Using wool as mulch induced a weaker stimulation of soil microbial populations compared with wood chips, the best results being those related to soil respiration increase on V3. *Phacelia* or V7. *Tagetes* variants, or by the stimulation of bacterial increase in V1. *Sinapis* variant.

Analysis of chromatograms revealed favorable effect on soil quality evolution in mulched variants with ameliorative plants of *Tagetes* or *Phacelia*.

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