MICROFUNGAL COMMUNITY FROM ORGANICALLY AMMENDED SOILS ASSOCIATED WITH COMMON BEAN AND BELL PEPPER CROPS

Ioan Radu[1], Lorena - Roxana Gurau[1], Emilian Mirea[2], Alexandru Cristian Toader[2]*

[1]Research - Development Institute for Plant Protection Bucharest, Romania
[2]Research and Development Station for Vegetables Growing Buzau, Buzau County, Romania

*correspondence address
Research-Development Institute for Plant Protection Bucharest
8 Ion Ionescu de la Brad, PC 013813, Bucharest, Romania
E-mail: raduioan1999@gmail.com

http://www.doi.org/10.54574/RJPP.15.04

Abstract: Soil is a reservoir of microorganisms including microfungi that play a key role as saprotrophs, plant mutualists, symbionts, decomposers, pathogens and excellent bio-indicators of soil quality. The diversity of soil fungi communities is influenced by products used in the life cycle of each crop. This study aimed to evaluate the diversity of soil fungal community in bean and bell pepper crop. For pepper crop two plant protection methods were applied - i) diatomite in three different doses: 75 kg ha^{-1} (T1), 150 kg ha^{-1} (T2), and 300 kg ha^{-1} (T3) and ii) biological control agent *Trichoderma asperellum* Td85 strain (T4). For bean crop the T4 was not applied. After soil isolation for bean crop resulted 461 colonies clustered in 49 OTUs while for pepper resulted 436 colonies clustered in 56 OTUs. Pepper crop had a higher activity of soil fungi compared to bean crop, fact evidenced by a higher index of Simpson 1-D (evenness), Shannon (richness and the evenness) and Margalef (richness) tests. Even if treatment with *Trichoderma* sp. wasn`t applied on bean crop, the fungus was present in the soil naturally and had the highest value of CF% (colonization frequency).

Key words: soil microorganisms, integrated pest management, biological control agent

INTRODUCTION

Besides bacteria, soil fungi constitute an essential component of biological characteristics in soil ecosystems playing a key role as saprotrophs, plant mutualists, symbionts, decomposers, pathogens (Peay et al., 2016; Victorino et al., 2021) being an excellent bioindicators of soil quality (Orgiazzi et al., 2012). A growing number of studies show that conventional farming leads to lower soil quality and less biological activity (i.e. microbial populations and microbial respiration rate) than organic farming to different crops (Droogers & Bouma, 1996; Girvan et al., 2004; Mader et al. 2002).

In European agriculture, the trend is to increase areas organically cultivated using biological means for plant protection or organic substances from natural sources. *Trichoderma* spp. have many roles in soil ecology such as suppress soil-borne pathogen fungi (Harman et al. 1989; Harman 2000), increase N and P nutrient contents in soil, degrade nutrients produced by photosynthesis into a state in which they can be used for plant growth, increase in the soil enzyme activity of the rhizosphere soil of seedlings, expand the contact area between the rhizosphere and soil (Halifu et al., 2019), improve the rhizosphere microbial community structure (Baldi et al., 2016; Zhang et al., 2013). Diatomaceous earth (diatomite) in agriculture mitigates plant biotic and abiotic stresses (Camargo et al., 2017; Liang et al., 2015) and increases yield acting as a fertilizer (Pati et al., 2016).

Common bean (*Phaseolus vulgaris* L.) are one of the most consumed vegetables in Romania, especially during fasting periods, but also worldwide, knowing that it has an important nutritional value (Tanase et al., 2022). The use of bioproducts for plant protection

has a positive effect on the communities of fungi in soil under the cultivation of this plant (Patkowska, 2008). Bell pepper (*Capsicum annuum* L.) is one of the most cultivated vegetable crops in the country - 17.700 ha in 2020 (https://www.madr.ro/horticultura/fructe-si-legume.html). Many studies for pepper crop targeted the arbuscular mycorrhizal fungi (Yilma, 2019; Fauziyah et al., 2017, Beltrano et al., 2013) and revealed their role for plant growth but soil fungal communities were less addressed.

The aim of this study was to determine the diversity of the soil fungal communities in common bean and bell pepper crops.

MATERIALS AND METHODS

Field experiment. The experiment was carried out in 2020 at the Research and Development Station in Vegetables Buzau, Romania. In the experiment, bean variety "Menuet" and bell pepper variety "Buzau 10" were used. The soil had a pH - 8.2, 2.57% organic matter content and 4.3% $CaCO_3$. Treatments applied for pepper were i) diatomite 75 kg ha^{-1} (T1), ii) diatomite 150 kg ha^{-1} (T2), iii) diatomite 300 kg ha^{-1} (T3), iv) bioinoculant *Trichoderma asperellum* Td85 strain, three grams of inoculated calcium alginate per plant (1×10^7 ml^{-1}) (T4) and v) not-treated plots (control). For bean, treatments were the same with the mention that T4 was not applied.

Field sampling. Soil samples were collected from each plot at 5 cm depth with a soil sampling auger at the harvest time. The samples were placed in sterile polyethylene bags, transferred to the laboratory and stored at low temperature (4°C) until analysed.

Isolation of fungi. Soil samples per treatment (each with 4 repetitions) were manually blended and 3 g of each sample repetition was divided into three replicates, each with 1 g, finally having 12 samples per treatment and control. Each sample was introduced into a sterile tube with 10 ml sterile distilled water and vortexed for 30 seconds at 2000 rpm. Samples were diluted in series (1:10 and 1:100) and the lowest one was dispensed in 9 cm-Petri dishes with potato dextrose agar (PDA) nutrient medium containing a mix of antibiotics chloramphenicol + ampicillin (0.2 mg/L) + tetracycline (0.2 mg/L). Each plate corresponded to 1 g of soil sample. Plates were incubated at +25˚C in darkness for 7 days. Fungal colonies were counted and only the fungi with visible different morphological characteristics were sub-cultured. When an endophyte was acquired in pure culture it was cultured on PDA, malt extract agar (MEA) and oatmeal agar (OA) medium for colony characterization. Fungal colonies were morphologically separated in morphotypes (Cosoveanu et al., 2018) classified according to colour and shape of mycelium, pigmentation of medium and morphological characteristics of asexual/sexual organs (Bankina et al., 2017) resulting 58 operational taxonomic units (OTUs). To separate OTUs by microscopically characters, a microscope at 40x magnification was used. For the mycological collection (long-term conservation), OTUs isolates were maintained in glycerol (20%) and mineral oil at -38°C and 5°C, respectively.

Diversity indices. Colonization frequency (CF%) was calculated as the total number of isolates of one OTU in all treatments (each with four repetitions and three samples per repetition) or per treatment divided by the total number of dispensed plates; where each plate contained 1 g of soil sample. CF% = (number of colonies of an OTU / total number of petri dishes sampled) x 100.

For the diversity of soil fungi, Margalef and Shannon indexes and Simpson's dominance index were used (Cosoveanu et al., 2018). Margalef index measures species richness while Shannon index combines richness and evenness. The Margalef index was calculated using formula d = (S-1)/ ln N, where S is the number of OTUs and N is the number of isolates in the sample. The Simpson dominance index was calculated according to the

formula D = 1 - ∑ [ni (ni-1)/N(N - 1)] , where ni is the number of isolates belonging to i OTUs and N is the total number of isolates. The Shannon diversity index was calculated according to the formula:

$$H' = -\sum_{i=1}^{S} pi \ln pi$$

Where, p is the proportion (n/N) of isolates of one particular OTU found (n) divided by the total number of isolates found (N), ln is the natural log, ∑ is the sum of the calculations and S is the number of OTUs. For the diversity indices, PAST software version 3.15 (copyright Hammer & Harper, Natural History Museum, University of Oslo, Norway) was used. Venn diagrams were performed using the web-based tool InteractiVenn (Heberle et al., 2015).

RESULTS AND DISCUSSIONS

For bean crop in the first morphological inspection resulted in 461 colonies clustered in 49 OTUs. Of these 45 OTUs were found with values of colonization frequency per one gram of soil lower than 50%. Two OTUs had CF% values between 50% and 100%. According to the study of de Oliveira et al. (2016), when bean was used as a cover crop its presence was positively associated with higher density of *Rhizoctonia solani* and *Fusarium oxysporum* and increased microbial activity (soil basal respiration, metabolic quotient and soil enzymatic activity). OTUs 29 which had the highest value of CF% were identified as *Trichoderma* sp. Although the treatment with *Trichoderma* sp. wasn`t applied on bean crop, the fungus was present in the soil naturally (Figure 1).

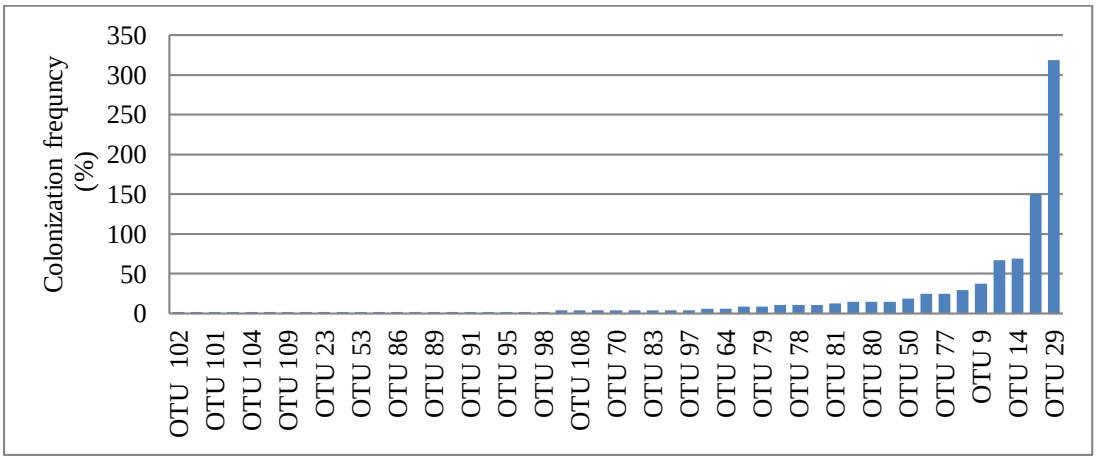

Figure 1. Mean values of colonization frequency of each OTU per 1 gram of soil in all plots (all four treatments including control, each with four repetitions) for bean crop

CF% for pepper crop reveals a higher diversity of the isolated fungi. In the first morphological inspection resulted 436 colonies clustered in 56 OTUs. The majority of soil fungi OTUs were found with CF < 20% per gram of soil. Only six OTUs were found with values of colonization frequency per one gram of soil in all treatments, higher than 50%. Here *Trichoderma* sp. had a CF of 51% (Figure 2).

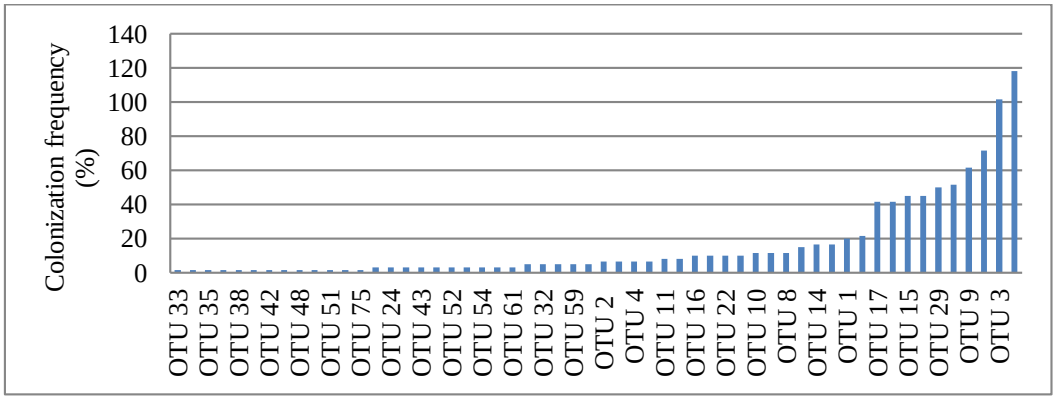

Figure 2. Mean values of colonization frequency of each OTU per 1 gram of soil in all plots (all four treatments including control, each with four repetitions) for pepper crop

OTUs number per treatment slightly varied with the lowest value registered for T3 (16 OTUs) and the highest value registered for C (27 OTUs). Of 49 OTUs isolated in all treatments, including not-treated plot, only 16% were found in common. Single OTUs per treatment varied from three in T3 to thirteen in control. Generally, only one OTU was found common for at least two treatments. The highest number of common OTUs for at least two treatments was two (Figure 3). Therefore, for bean crop it comes easy to speculate that single OTUs were isolated due to treatments applications which restricted their habitat.

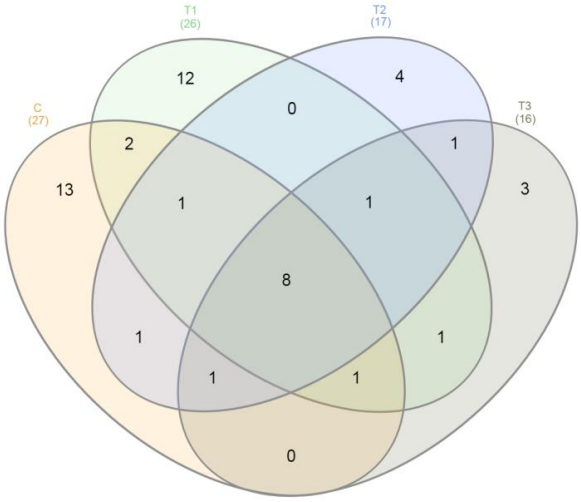

Figure 3. Venn diagram showing common OTUs among treatments for bean crop. C - control, T1 - diatomite 75 kg ha^{-1}, T2 - diatomite 150 kg ha^{-1}, T3 - diatomite 300 kg ha^{-1}

For pepper crop 6 OTUs were found in common of 56 OTUs isolated in all treatments. T1 and T2 had the same number of OTUs isolated (22) while in T3, alongside the increase of treatment rate ha^{-1}, 25 OTUs were found. Studies of Wu et al. (2020) showed that relative abundances of several bacterial species were positively correlated with increasing organic fertilizer in the rhizosphere soil of grapes. In the plot where *T. asperellum* Td85 strain (1×10^7 ml^{-1}) was applied (T4), single OTUs number isolated were the lowest (Figure 4). This can be attributed to the fungus capacity to inhibit the growth and activity of target soil pathogens (Tyskiewicz et al., 2022).

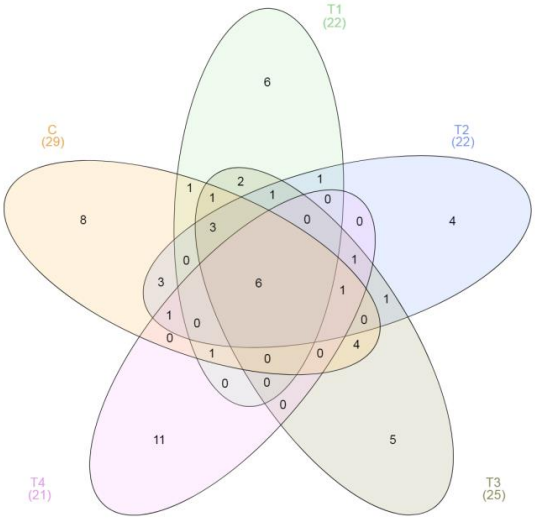

Figure 4. Venn diagram showing common OTUs among treatments for pepper crop. C - control, T1 - diatomite 75 kg ha^{-1}, T2 - diatomite 150 kg ha^{-1}, T3 - diatomite 300 kg ha^{-1}, T4 - *T. asperellum* Td85 strain (1×10^{7} ml^{-1})

Shannon diversity index was used to indicate both the richness and the evenness of the soil fungal community being sensitive to changes in rare species. Results indicate a higher value on bean (H = 3.2) than on pepper (H = 2.64). A higher value of evenness on pepper (Simpson 1-D = 0.94) and highest species richness index (Margalef = 8.12) indicate that the microfungal community differ from one crop to another in accordance with its biological needs (Table 1).

Table 1. Diversity indices for pepper and bean crops

	Bean	**Pepper**
Taxa_S	49	56
Colonies	461	536
Simpson_1-D	0,85	0,94
Shannon_H	2,64	3,20
Margalef	7,83	8,12

CONCLUSIONS

Pepper crop had a higher activity of soil fungi compared to bean crop, fact evidenced by the higher values of Simpson 1-D (evenness), Shannon_H (richness and the evenness) and Margalef tests (richness) indices.

Even if treatment with *Trichoderma* sp. wasn`t applied on bean crop, the fungus was present in the soil naturally and had the highest value of CF% (colonization frequency).

ACKNOWLEDGEMENTS

This work was supported by a grant of the Romanian Ministry of Research and Innovations, CCCDI-UEFISCDI, project number PN-III-P1-1.2-PCCDI-2017-0659 PCCDI, within PNCDI III.

REFERENCES

BALDI, E., AMADEI, P., PELLICONI, F., TOSELL, M. (2016). Use of *Trichoderma* spp. and arbuscular mycorrhizal fungi to increase soil beneficial population of bacteria in a nectarine commercial orchard: effect on root growth, nutrient acquisition and replanting disease. *Journal of Plant Nutrition*, 39, 1147-1155.

BANKINA, B., BIMSTEINE, G., NEUSA-LUCA, I., ROGA, A., FRIDMANIS, D. (2017). What influences the composition of fungi in wheat grains? *Acta Agrobotanica*, 70, 4, 1726.

BELTRANO, J., RUSCITTI, M., ARANGO, M.C., RONCO, M. (2013). Effects of arbuscular mycorrhiza inoculation on plant growth, biological and physiological parameters and mineral nutrition in pepper grown under different salinity and plevels. *Journal of soil science and plant nutrition*, 13, 1, 123-141.

CAMARGO, M.S., DE BEZERRA, B.K.L., VITTI, A.C., SILVA, M.A., OLIVEIRA, A.L. (2017). Silicon fertilization reduces the deleterious effects of water deficit in sugarcane. *Journal of Soil Science and Plant Nutrition*, 17, 99-111.

COSOVEANU, A., RODRIGUEZ SABINA, S., CABRERA, R. (2018). Fungi as endophytes in *Artemisia thuscula*: juxtaposed elements of diversity and phylogeny. *Journal of Fungi*, 4, 17.

DE OLIVEIRA, P., NASCENTE, A.S., DE BRITO FERREIRA, E.P., KLUTHCOUSKI, J., JUNIOR, M.L. (2016). Response of soil fungi and biological processes to crop residues in no-tillage system. *Pesquisa Agropecuaria Tropical*, 46, 1, 57-64.

DROOGERS, P., BOUMA, J. (1996). Biodynamic versus conventional farming effects on soil structure expressed by stimulated potential productivity. *Soil Science Society of American Journal*, 60, 1552-1558.

FAUZIYAH, N., HADISUTRISNO, B., SURYANTI, S. (2017). The roles of arbuscular mycorrhizal fungi in the intensity of the foot rot disease on pepper plant from the infected soil. *Journal of Degraded and Mining Lands Management*, 4, 4, 937.

GIRVAN, M.S., BULLIMORE, J., BALL, A.S., PRETTY, J.N., OSBORN, A.M. (2004). Responses of active bacterial and fungalcommunities in soils under winter wheat to different fertilizer and pesticide regimens. *Applied Environmental Microbiology*, 70, 2692-2701.

HALIFU, S., DENG, X., SONG, X., SONG, R. (2019). Effects of two *Trichoderma* strains on plant growth, rhizosphere soil nutrients, and fungal community of *Pinus sylvestris* var. *mongolica* annual seedlings. *Forests*, 10, 758.

HARMAN, G.E. (2000). Myths and dogmas of biocontrol: changes in perceptions derived from research on *Trichoderma harzianum* T-22. *Plant Diseases*, 84, 377-393.

HARMAN, G.E., TAYLOR, A.G., STASZ, T.E. (1989). Combining effective strains of *Trichoderma harzianum* and solid matrix priming to improve biological seed treatments. *Plant Diseases*, 73, 631-637.

HEBERLE, H., VAZ MEIRELLES, G., DA SILVA, F.R., TELLES, G.P., MINGHIM, R. (2015). InteractiVenn: a web-based tool for the analysis of sets through Venn diagrams. *BMC Bioinformatics*, 16, 169.

LIANG, Y., NIKOLIC, M., BELANGER, R., GONG, H., SONG, A. (2015). *Silicon in agriculture. From theory to practice*. Springer Publishing House, 235.

MADER, P., FLIESSBACH, A., DUBOIS, D., GUNST, L., FRIED, P., NIGGLI, U. (2002). Soil fertility and biodiversity in organic farming. *Science*, 296, 1694-1697.

MADR. Horticultura, fructe si legume. Retrieved from https://www.madr.ro/horticultura/fructe-si-legume.html.

ORGIAZZI, A., LUMINI, E., NILSSON, R.H., GIRLANDA, M., VIZZINI, A., BONFANTE, P., BIANCIOTTO, V. (2012). Unravelling soil fungal communities from different mediterranean land-use backgrounds. *PLoS ONE*, 7, 4, e34847.

PATI, S., PAL, B., BADOLE, S., HAZRA, G.C., MANDAL, B. (2016). Effect of silicon fertilization on growth, yield, and nutrient uptake of rice. *Communications in soil science and plant analysis*, 47, 284-290.

PATKOWSKA, E. (2009). Effect of bio-products on bean yield and bacterial and fungal communities in the rhizosphere and non-rhizosphere. *Polish Journal of Environment Studies*, 18, 2, 255-263.
PEAY, K.G., KENNEDY, P.G., TALBOT, J.M. (2016). Dimensions of biodiversity in the Earth mycobiome. *Nature Reviews Microbioly*, 14, 7, 434-47.
TĂNASE, B.E., ASĂNICĂ, A., VÎNĂTORU, C., BARCANU, E., AGAPIE, O.L., GHERASE, I. (2022). The organoleptic qualities of some varieties of french beans. *Scientific Papers Series B, Horticulture*, 66, 1, 564-569.
TYSKIEWICZ, R., NOWAK, A., OZIMEK, E., JAROSZUK-SCISEL, J. (2022). *Trichoderma:* the current status of its application in agriculture for the biocontrol of fungal phytopathogens and stimulation of plant growth. *International Journal of Molecular Sciences*, 23, 4, 2329.
VICTORINO, I.M.M., VOYRON, S., CASER, M., ORGIAZZI, A., DEMASI, S., BERRUTI, A., SCARIOT, V., BIANCIOTTO, V., LUMINI E. (2021). Metabarcoding of soil fungal communities associated with alpine field-grown saffron (*Crocus sativus* L.) inoculated with AM fungi. *Jornal of Fungi*, 7, 45.
WU, L., JIANG, Y., ZHAO, F., HE, X., LIU, H., YU, K. (2020). Increased organic fertilizer application and reduced chemical fertilizer application affect the soil properties and bacterial communities of grape rhizosphere soil. *Scientific Reports*, 10, 9568.
YILMA, G. (2019). The role of mycorrhizal fungi in pepper (*Capsicum annuum*) production. *International Journal of Advanced Research in Biological Sciences*, 59-65.
ZHANG, F., ZHU, Z., YANG, X., RAN, W., SHEN, Q. (2013). *Trichoderma harzianum* T-E5 significantly affects cucumber root exudates and fungal community in the cucumber rhizosphere. *Applied Soil Ecology*, 72, 41-48.