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EVALUATION OF THE MICROFUNGAL COMMUNITY FROM SOIL TO ONION CROP IN AN INTEGRATED PROTECTION SYSTEM

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Abstract

Soil is a reservoir of microorganisms including microfungi who play a key role as saprotrophs, plant mutualists, symbionts, decomposers, pathogens and excellent bioindicators of soil quality. The diversity of soil fungi communities is influenced by crop protection products. This study aimed to evaluate the diversity of soil fungal community in onion crop. Two plant protection methods were applied - i) diatomite in three different doses: 52.5 kg ha^{-1} (T1), 105 kg ha^{-1} (T2), and 210 kg ha^{-1} (T3) and ii) biological control agent Trichoderma asperellum Td85 strain (T4). Of 58 operational taxonomic units isolated from all treatments (including control) only 7 operational taxonomic units were found in common. The highest value of colonization frequency was observed in T2 (167%), followed by control (125%), T3 (58%), T4 (50%) and T1 (42%). Results indicate that the degree of soil colonization with Trichoderma is related with the dose of diatomite.

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) and 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 and Bouma, 1996; Girvan et al., 2004; Mader et al., 2002) even for onion crop (Knerr et al., 2020). It is important to reveal and understand the interactions of fungal diversity in soil when different organic amendments are applied to select the best option for ecology (Swer et al., 2011).
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. has 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 (Elena et al., 2015; 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). In Romania, onion is one of the most cultivated vegetable crop with 30.000 ha in 2018 (https://www.madr.ro/horticultura/fructe-si-legume.html). Many studies of onion crop targeted the arbuscular mycorrhizal fungi (AMF) (Bolandnazar, 2009; Charron et al., 2001; Galván et al., 2009) but soil fungal communities were less addressed. Onion crop may also enhance soil microorganisms communities of other plants when cultivated in intercropping system (Li et al., 2020). We expected *Trichoderma* strain will supress some

soil fungi and a less genera will be found in the plot treated with it. The aim of this study was to determine i) the diversity of the soil fungal communities in onion crop and ii) if relative abundance of *Trichoderma asperellum* is influenced by treatments.

MATERIALS AND METHODS

Field experiment. The experiment was carried out in 2020 at the Research and Development Station in Vegetables Buzau, Romania. In the trial onion variety 'De Buzau' was seeded at 4th March in soil with pH 8.2, 2.57% organic matter content and 4.3% $CaCO_3$. Previous crop was dwarf bean. Onion crop was fertilized with 366 kg ha^{-1} of a complete fertilizer mixture of 20-20-0-13 N-P_2O_5-K_2O-S. The herbicide Cerlit (333 g/L fluroxypyr) was applied at 0.3 L ha^{-1}. Treatments employed were i) diatomite 52.5 kg ha^{-1} (T1), ii) diatomite 105 kg ha^{-1} (T2), iii) diatomite 210 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). Plots were established at 7 m^2 (1.4 m x 5 m), with four repetitions per treatment with a total of 20 experimental units in a complete randomized block design.

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

Isolation of fungi. Soil samples per treatment (each with 4 repetitions) were manually blended and 3 g of each soil sample repetition was divided into three replicates, each with 1 g, finally having 12 samples per treatment or 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 corresponds 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 subcultured. Eventually, 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 index, Shannon index and Simpson's dominance index were used. Margalef index (Cosoveanu et al., 2018) 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 dominance of Simpson (Cosoveanu et al., 2018) 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.

Effective number of OTUs were calculated according to Jost (2006) for Shannon diversity

index and Simpson index. The number of equally-common species required to give a particular value of an index is called the "effective number of species". This is the true diversity of the community in question. 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

The first morphological inspection resulted in 484 colonies clustered in 58 OTUs.
Only six OTUs were found with values of colonization frequency per one gram of soil in all treatments, higher than 50% while 15 OTUs registered values of 10% and 50%. The majority of soil fungi OTUs were found with CF < 10% per gram of soil (Figure 1).

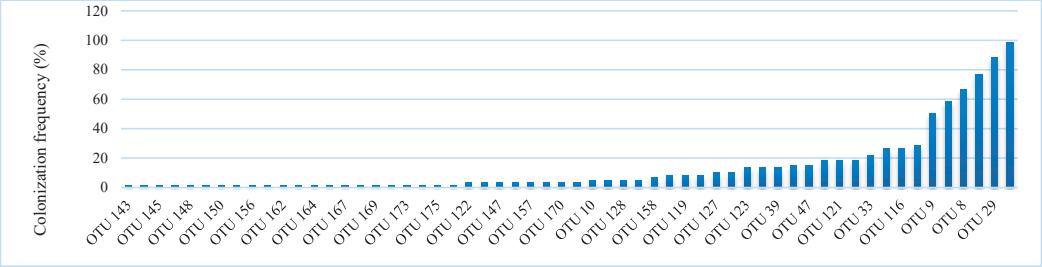

Figure 1. Mean values of colonization frequency of each OTU per 1 gram of soil in all plots (all five treatments including control, each with four repetitions and three replicates)

Although the bioinoculant *T. asperellum* strain T85 was applied in T4 plots, the highest value of colonization frequency was observed in T2 (167%), followed by not treated plots - control (125%), T3 (58%), T4 (50%) and T1 (42%) (Figure 2). Present identification does not rely on molecular data, therefore OTU *Trichoderma* might gather different strains and species of *Trichoderma* naturally present in the soil. Experimental design also might have played a role in the registered values as not-treated plots (control) was set up in two repetitions next to T4 plots. Yet, plots of T2 were not placed nearby control.

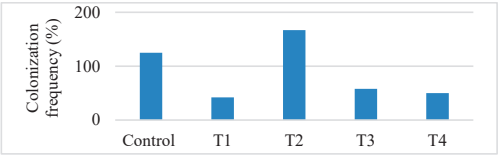

Figure 2. Colonization frequency % of *Trichoderma* strains per gram of soil across treatments: T1-diatomite 52 kg ha^{-1} ; T2- diatomite 105 kg ha^{-1} ; T3- diatomite 210 kg ha^{-1} ; T4- *T. asperellum* Td85 strain (1×10^{7} ml^{-1})

OTUs number per treatment slightly varied with the lowest value registered for T4 (25 OTUs) and the highest value registered for T1 (30 OTUs). Of 58 OTUs isolated in all treatments, including not-treated plot (control), only 12% were found in common. Single OTUs per treatment varied from five in T4 to eight in T2 and control. Generally, only one OTU was found common for at least two treatments. The highest number of common OTUs between at least two treatments was four (Figure 3). Different dosages of organic compost (low versus high), nitrogen fertilizer and untreated control were found to shift the selection of bacterial species and their abundance (Enebe et al., 2020). Therefore, in this study it comes easy to speculate that singleton OTUs were isolated due to treatments applications which restricted their habitat.
Control plots registered highest values individual counted 115 colonies, followed by T1 (106 colonies) and T4 (93 colonies). 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 the highest value in T3 (H = 2.99) and the lowest in T2 (H = 2.76). Thus, it comes easy to speculate that diatomite has an effect on richness of fungal soil community, as T3 was amended with the highest dose of diatomite (210 kg ha^{-1}).

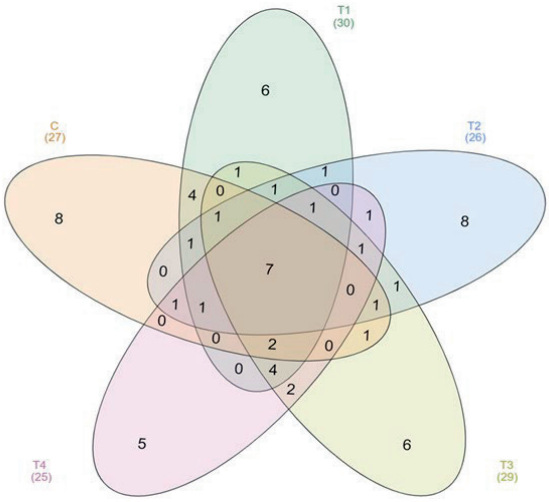

Figure 3. Venn diagram showing common OTUs among treatments: C - control; T1 - diatomite 52 kg ha^{-1} ; T2 - diatomite 105 kg ha^{-1}; T3 - diatomite 210 kg ha^{-1}; T4 - *T. asperellum* Td85 strain (1×10^{7} ml^{-1})

Application of large amounts of vermicompost and mushroom residues enhanced the biodiversity of soil bacterial communities in *Leymus chinensis* grasslands (Shang et al., 2020). Converting to effective number of species, which is the true diversity of species, it can be observed that the differences between the values of this index are not that high (T2 = 16 OTUs versus T3 = 20 OTUs). It is noteworthy to underline that T3 resulted in the highest diversity of fungal soil community (H = 2.99), also having high value of evenness (Simpson 1-D = 0.93) and highest species richness index (Margalef = 6.32). Relative abundances of several bacterial species were positively correlated with increasing organic fertilizer in the rhizosphere soil of grapes (Wu et al., 2020). Lowest values for species richness, diversity and increased dominance were found in not-treated plots (Table 1).

Table 1. Diversity indices per treatment: C-control; T1-diatomite 52 kg ha^{-1}; T2-diatomite 105 kg ha^{-1}; T3-diatomite 210 kg ha^{-1}; T4-*T. asperellum* Td85 strain (1×10^{7} ml^{-1})

	C		T1		T2		T3		T4	
	Diversity indices	Effective species	Diversity indices	Effective species	Diversity indices	Effective species	Diversity indices	Effective species	Diversity indices	Effective species
Taxa_S	27		29		26		29		25	
Individuals	115		106		82		84		93	
Simpson_1-D	0.90	10.13	0.93	14.27	0.90	10.10	0.93	15.08	0.94	15.65
Shannon_H	2.79	16.22	2.94	18.93	2.76	15.83	2.99	19.81	2.94	18.90
Margalef	5.48		6.00		5.67		6.32		5.30	

CONCLUSIONS

This study suggests that application of diatomite in high dose is positively correlated with higher diversity and evenness of soil fungal communities. Further analysis is to be considered to identify the isolated strains of *Trichoderma* and to evaluate the scarce colonization of soil of the bionoculant T. *viridae* T85.

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