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ORIGINAL RESEARCH PAPER

Impact of AMF *Claroideoglomus etunicatum* on the structure of fungal communities in the tomato rhizosphere

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Abstract
Mycorrhizal fungi influence the development and activity of communities of soil microorganisms. The purpose of this study was to estimate the effect of arbuscular mycorrhizal fungus *Claroideoglomus etunicatum* (W. N. Becker & Gerd.) C. Walker & Schüβler on the population structure of fungal colonies in the rhizosphere of tomatoes grown in a plastic tunnel. The field experiment was conducted from 2015 to 2017 at an ecological farm in Grądy, central eastern Poland. The object of study were the three tomato cultivars: 'Antalya F_1', 'Esmira F_1', and 'Pelikan F_1'. Tomato seedlings were inoculated with *C. etunicatum*; spores were introduced about 5 cm deep in the rhizosphere of the studied plants (25–30 spores of *C. etunicatum* for each plant). Each year, mycological analysis of the tomato rhizosphere was conducted using Warcup's method; structure of fungal communities of the tomato rhizosphere varied depending on the AMF applied. Saprotrophic fungi such as *Trichoderma* ssp., *Mucor* spp., and *Penicillium* spp. were often more isolated from the rhizosphere of plants inoculated with *C. etunicatum* than that of the control samples. It can be concluded that AMF directly impacted the development of fungal biodiversity in the tomato rhizosphere, particularly regarding the number of saprotrophs in the soil.

Keywords
Lycopersicon esculentum; mycorrhiza; soil-borne pathogens; antagonistic fungi

Introduction

Arbuscular mycorrhizal fungi (AMF) comprises the most common group of soil fungi that exhibits mutualistic relationships with vascular plants [1–3]. AMF form a mycorrhizal symbiosis with a wide range of cultivated plant species and play an important role in decreasing the incidence of plant disease [4,5]. By altering the composition and amount of root exudates, mycorrhizal fungi influence the development and activity of communities of soil microorganisms. The phenomenon of mycorrhizal symbiosis, therefore, does not always yield expected effects because the process is dependent on soil and environmental factors [6]. Stimulation of spore germination and contact between AMF and plants can be supported by metabolites of soil microorganisms, especially certain saprophytic bacteria and fungi [7,8]. AMF also create favorable conditions for rhizosphere microorganisms activity that positive affects plants [9]. The formation of the mycorrhizosphere modifies growth conditions for microorganisms in the root zone, protecting plants against harmful microorganisms [10,11].

This study was developed to estimate the effect of the AMF *Claroideoglomus etunicatum* (W. N. Becker & Gerd.) C. Walker & Schüβler on the diversity of fungal communities in the rhizosphere, especially saprotrophs and pathogens, of tomato plants grown in plastic tunnels.

Material and methods

Field experiment

The study was conducted in the Department of Plant Protection at the University of Life Sciences in Lublin in the years 2015–2017. A field experiment was conducted at an ecological farm in Grądy (district Lublin) (51°05′36″ N, 22°12′33″ E), central eastern Poland. The objects of examination included three tomato cultivars (*Lycopersicon esculentum* Mill.) that are important for commercial production: 'Antalya F_1', 'Esmira F_1', and 'Pelikan F_1'. Crop rotation was used in the experiment with cucumber plants as a forecrop. Tomato seedlings produced in a specialized horticulture farm were planted in a plastic tunnel in the first week of May, spaced at 0.80 × 0.50 m. Each year, the field experiment was established as a two-factor experiment in a random block design in five replications. *Claroideoglomus etunicatum* (syn. *Glomus etunicatum* W. N. Becker & Gerd) spores were used to inoculate each of the tomato seedling cultivars (AC: Antalya F_1 cv. control; ACE: Antalya F_1 cv. + *C. etunicatum*; EC: Esmira F_1 cv. control; ECE: Esmira F_1 cv. + *C. etunicatum*; PC: Pelikan F_1 cv. control; PCE: Pelikan F_1 cv. + *C. etunicatum*). Fungal spores were obtained from the the Institute of Soil Science and Plant Cultivation – National Research Institute (SSPC-NRI) collection in Puławy, Poland. Before planting, tomato seedlings were inoculated with *C. etunicatum*; spores were introduced about 5 cm deep in the rhizosphere of the studied plants. Fungal inoculum containing about 25–30 spores of *C. etunicatum* in physiological saline was applied to each plant. Control combinations consisted of plants without inoculum of mycorrhizal fungus. Black film was used in each row for mulching. Drip irrigation was used to irrigate plants depending on their needs. No chemical protection was applied during the vegetation period.

Laboratory analysis of rhizosphere

Throughout the study period (2015–2017), mycological analysis of the rhizosphere was conducted. The soil samples were taken from each experimental combination when the tomato fruits had fully ripened (first week of September – BBCH 89) by scraping material from each plant's roots and placed in sterile Petri dishes. Soil samples from five roots for each experimental combination was taken. In the laboratory, the soil samples were mixed into a single sample for each experimental combination. The mycological analysis of the rhizosphere was conducted using Martin's medium according to Warcup's method [12]. Twenty dishes for each experimental combination were prepared. The dishes were incubated at 24°C in darkness for 5–7 days. Fungal colonies grown from the rhizosphere soil were measured and then transferred to slants of potato dextrose agar (Difco and BBL). Next, fungal colonies were identified using the available mycological keys and monographs.

Statistical analysis

Soil fungi communities obtained from the mycological analysis of rhizosphere were compared using PAST 3.18 software [13]. The phylogenetic tree was constructed using Bray-Curtis cluster analysis with unweighted pair group method with arithmetic mean (UPGMA) and bootstrap test with 1,000 iterations. Principal component analysis was performed in STAMP 2.1.3 software based on the number of cultivable fungi count [14].

Results

During the 3-year mycological analysis of the tomato rhizosphere, 3,806 fungal colonies comprising 42 species were isolated, and the genera *Fusarium*, *Mucor*, *Penicillium*, and *Trichoderma* were found to be the most numerous (Tab. 1, Tab. 2).

Tab. 1 Number of fungi colonies occurring the tomato rhizosphere in 2015–2017.

Fungus species	AC	ACE	EC	ECE	PC	PCE	Total (%)
Acrostalagmus luteoalbus (Link) Zare. W. Gams & Schroers	9	6	-	-	-	-	15 (0.4)
Albifimbria verrucaria (Alb. & Schwein.) L. Lombard & Crous	14	-	-	1	-	-	15 (0.4)
Aspergillus fumigatus Fresen.	-	-	1	-	-	-	1 (0.02)
Aureobasidium pullulans (de Bary & Löwenthal) G. Arnaud	5	-	-	-	-	-	5 (0.1)
Boeremia hedericola (Durieu & Mont.) Aveskamp. Gruyter & Verkley	-	-	1	-	-	-	1 (0.02)
Cephalotrichum microsporum (Sacc.) P. M. Kirk	-	1	62	12	-	-	75 (1.9)
Chaetomium spp.	-	-	-	-	-	3	3 (0.08)
Chrysosporium merdarium (Ehrenb.) J. W. Carmich.	-	1	-	1	-	-	2 (0.04)
Cladosporium cladosporioides (Fresen.) G. A. de Vries	16	-	5	-	2	-	23 (0.6)
Clonostachys rosea (Link) Schroers. Samuels. Seifert & W. Gams	1	-	-	-	-	5	6 (0.2)
Domingoella asterinarum Petr. & Cif.	4	1	-	-	48	-	53 (1.4)
Fusarium culmorum (Wm. G. Sm.) Sacc.	-	-	-	1	1	-	2 (0.04)
Fusarium oxysporum Schltdl.	273	65	-	56	27	10	431 (11.3)
Fusarium solani (Mart.) Sacc.	2	-	58	-	-	-	60 (1.5)
Fusarium equiseti (Corda) Sacc.	19	-	-	9	8	11	47 (1.2)
Humicola fuscoatra Traaen	-	17	-	6	-	-	23 (0.6)
Mortierella subtilissima Oudem.	1	-	-	-	-	-	1 (0.02)
Mucor circinelloides Tiegh.	38	31	-	81	5	58	213 (6.06)
Mucor hiemalis Wehmer	23	52	-	43	214	82	414 (10.9)
Mucor moelleri (Vuill.) Lendn.	-	-	1	2	1	7	11 (0.3)
Paecilomyces fulvus Stolk & E. S. Salmon	1	-	-	-	-	-	1 (0.02)
Penicillium adametzii K. M. Zaleski	-	-	-	21	-	-	21 (0.7)
Penicillium albicans Bainier	-	4	-	13	6	-	23 (0.6)
Penicillium aurantiogriseum Dierckx	-	7	-	22	44	86	159 (4.1)
Penicillium chrysogenum Thom	10	10	186	13	1	-	220 (5.7)
Penicillium citrinum Thom	-	12	-	35	42	72	161 (4.2)
Penicillium decumbens Thom	-	-	1	1	2	5	9 (0.2)
Penicillium expansum Link	8	-	-	-	-	3	11 (0.3)
Penicillium glabrum (Wehmer) Westling	-	-	-	-	-	49	49 (1.3)
Penicillium purpurascens (Sopp) Biourge	-	-	-	-	2	5	7 (0.2)
Penicillium simplicissimum (Oudem.) Thom	30	12	1	-	34	2	79 (2.1)
Pestalotiopsis spp.	-	-	-	-	2	-	2 (0.04)
Thermoascus egyptiacus S. Ueda & Udagawa	4	-	2	54	61	72	193 (5.1)
Talaromyces diversus (Raper & Fennell) Samson, N. Yilmaz & Frisvad	-	-	-	-	-	19	19 (0.5)
Trichocladium asperum Harz	10	-	-	-	-	-	10 (0.2)
Trichoderma aureoviride Rifai	21	37	34	12	-	12	116 (3.0)
Trichoderma hamatum (Bonord.) Bainier	28	97	11	147	9	14	306 (8.0)
Trichoderma harzianum Rifai	51	230	300	43	127	145	896 (23.5)

Tab. 1 Continued

Fungus species	AC	ACE	EC	ECE	PC	PCE	Total (%)
Trichoderma koningii Oudem.	-	-	-	12	-	-	12 (0.3)
Trichoderma polysporum (Link) Rifai	-	-	-	25	-	37	62 (1.6)
Trichoderma viride Pers.	-	-	-	1	-	-	1 (0.02)
Truncatella spp.	2	-	-	-	-	-	2 (0.04)
Micelia sterilia	3	15	5	9	8	6	46 (1.2)
Total	573	598	668	620	644	703	3,806 (100.0)

AC – Antalya F_1 cv. control, ACE – Antalya F_1 cv. + *C. etunicatum*; EC – Esmira F_1 cv. control, ECE – Esmira F_1 cv. + *C. etunicatum*; PC – Pelikan F_1 cv. control, PCE – Pelikan F_1 cv. + *C. etunicatum*.

Tab. 2 Participation of mainly genus fungi in the communities obtained from the rhizosphere soil of tomato in 2015–2017.

	Number of isolates (% of isolates)					
Fungi	AC	ACE	EC	ECE	PC	PCE
Fusarium spp.	294 (51.3)	65 (10.8)	58 (8.7)	66 (10.6)	36 (5.6)	21 (3.0)
Mucor spp.	61 (10.6)	83 (13.9)	1 (0.2)	126 (20.3)	220 (34.2)	147 (20.9)
Penicillium spp.	48 (8.4)	45 (7.5)	188 (28.1)	105 (16.9)	131 (20.3)	222 (31.6)
Trichoderma spp.	100 (17.5)	364 (60.9)	345 (51.6)	240 (38.7)	136 (21.1)	208 (29.6)
Others	70 (12.2)	41 (6.9)	76 (11.4)	83 (13.5)	121 (18.8)	105 (14.9)
Total	573 (100.0)	598 (100.0)	668 (100.0)	620 (100.0)	644 (100.0)	703 (100.0)

AC – Antalya F_1 cv. control, ACE – Antalya F_1 cv. + *C. etunicatum*; EC – Esmira F_1 cv. control, ECE – Esmira F_1 cv. + *C. etunicatum*; PC – Pelikan F_1 cv. control, PCE – Pelikan F_1 cv. + *C. etunicatum*.

The presence of Ascomycota and Mucoromycota fungi was noted in the rhizospheric fungal communities; fungi from the subtype Pezizomycotina and species from classes Sordariomycetes and Eurotiomycetes, as well as species from the class Dothideomycetes, were most dominant. Within the Eurotiomycetes class, the genus *Penicillium* was most common, followed by the genus *Trichoderma* and *Fusarium* from the Sordariomycetes class (Tab. 2, Fig. 1).

Among saprotrophs, the most numerous were *Trichoderma* ssp., *Penicillium* spp., and *Mucor* spp. (Tab. 2). In total, 1,372 isolates of *Trichoderma* spp. (especially *T. harzianum* and *T. hamatum*), 739 colonies of *Penicillium* spp. (especially *P. aurantiogriseum*, *P. chrysogenum*, and *P. simplicissimum*), and 638 colonies of *Mucor* spp. (*M. circinelloides* and *M. hiemalis*) were obtained during the mycological analysis of the tomato rhizosphere between (Tab. 1). The saprotrophic fungi isolated from the rhizosphere of tomato inoculated with *C. etunicatum* were more numerous (from 471 to 577 colonies) than the control (from 188 to 534 colonies) (Tab. 1).

In the rhizosphere samples from the control tomato plants, numerous fungal colonies of the genus *Fusarium* (mainly *F. oxysporum* on 'Antalya F_1' and *F. solani* on 'Esmira F_1') and *Trichoderma* (*T. harzianum* – 'Antalya F_1' and 'Esmira F_1') were noted (Tab. 1, Tab. 2). In the rhizosphere of tomato plants inoculated with mycorrhizal fungus, the genus *Trichoderma* with the species *T. hamatum* and *T. harzianum* ('Pelikan F_1' – 29.6%, 'Antalya F_1' – 60.9%) and the genus *Penicillium* with the species *P. aurantiogriseum*, *P. glabrum*, and *P. citrinum* ('Pelikan F_1' – 31.6%) dominated (Tab. 2).

Therefore, the results indicate that the mycorrhizal fungus *C. etunicatum* influences the differentiation of the rhizosphere structure of the tomato, causing a reduction in the number of colonies of *F. oxysporum* (by 76% for 'Antalya F_1' and 63% for 'Pelikan F_1') and *F. solani* ('Esmira F_1') compared to the control group. In addition, colonies of *Trichoderrma* spp. and *Penicillium* spp. significantly increased after root inoculation with mycorrhizal fungus (Tab. 1, Tab. 2, Fig. 1).

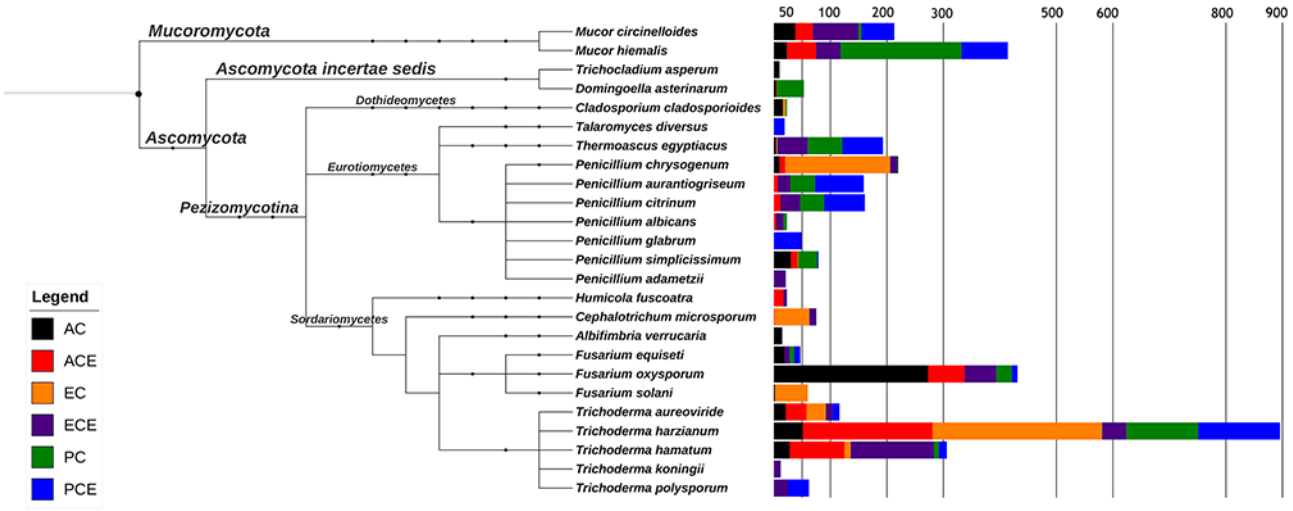

Fig. 1 Phylogenetic tree of the fungi colonizing the tomato rhizosphere, 2015–2017. AC – Antalya F_1 cv. control; ACE – Antalya F_1 cv. + *C. etunicatu*; EC – Esmira F_1 cv. control; ECE – Esmira F_1 cv. + *C. etunicatum*; PC – Pelikan F_1 cv. control; PCE – Pelikan F_1 cv. + *C. etunicatum*.

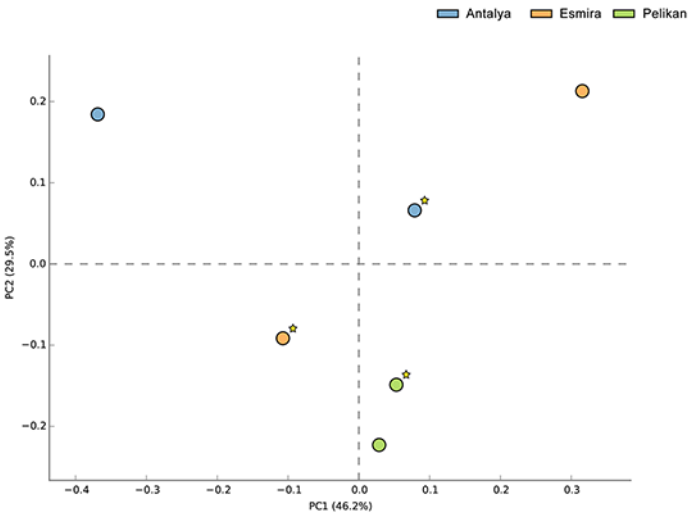

Fig. 2 Principal component analysis of cultivable fungi abundance occurring in tomato cultivars 'Antalya F_1', 'Esmira F_1', and 'Pelikan F_1' after inoculation with mycorrhizal fungus *Claroideoglomus etunicatum* (asterisk) and without inoculation (without asterisk).

Similarities between the examined fungal communities on the tested tomato cultivars are shown in Fig. 2. The communities of rhizosphere fungi of 'Antalya F_1' and 'Esmira F_1' cultivars inoculated with mycorrhizal fungus significantly differed from the noninoculated cultivars, whereas the communities of rhizosphere fungi from inoculated 'Pelikan F_1' with *C. etunicatum* did not differ significantly from the noninoculated cultivars (Fig. 2).

Discussion

AMF create favorable conditions for the biodiversity and activity of rhizosphere microorganisms that have a positive effect on plants [9,15], which was confirmed in the conducted experiment. The interactions of microorganisms are visible in soil communities that are highly complex and variable, but very important for plant development. The effect of AMF depends on a number of environmental and soil factors that can equally affect both the development and activity of AMF as well as saprophytic fungi [16].

The presence of a symbiont in plant roots also induces direct and indirect effects on rhizosphere microorganisms [17]. AMF can effectively reduce root disease caused by soil-borne pathogens [18]. AMF also exhibited a positive effect on the increase on the numbers of pathogens, especially *Fusarium* spp., on the tomato rhizosphere. Certain soil microorganisms can be bio-controlling factors of plant pathogens, frequently exhibiting synergism of protective effects on plants alongside AMF. Some amino acids, ethylene, proteins, and isoflavonoids can be mediators between the positive rhizosphere microorganisms and AMF [3]. Tolerance to *Fusarium* crown and root rot appeared in the practical decline soil. The effect of AMF species, especially *G. intraradices*, demonstrated the highest effectiveness as a biocontrol agent [19–21]. Smith and Read [1] show the phenomenon of synergetic pathogen bio-control by simultaneously using mycorrhiza and saprotrophic fungi. Martinez-Medina et al. [22] showed that coinoculation of plants with the AMF and *T. harzianum* more effectively controlled *Fusarium* wilt than AMF inoculation alone.

Stimulation of spore germination and contact between AMF and plants can also be supported by metabolites of soil microorganisms, especially certain saprophytic bacteria and fungi [8]. Our own studies have shown a significant increase in the number of *Trichoderma* spp. and *Penicillium* spp. colonies in the rhizosphere of tomatoes inoculated with AMF compared to controls for all tested cultivars. Similar results are shown by Thanoon and Jamiołkowska [12] as they present the effect of *C. etunicatum* on increasing the number of saprotrophic fungi in the tomato rhizosphere. The above-mentioned saprophytic fungi are antagonists of many plant pathogens and are used as a natural biological protection factor. *Trichoderma* has been used against wilt diseases in tomato, melon, and cotton, as has *Fusarium culmorum* on wheat. *Trichoderma harzianum* exhibited 60–83% control of *Fusarium* diseases in naturally infected field soil [16]. Similarly, *Penicillium* spp. are considered good antagonists. Patkowska et al. [21] conducted laboratory tests that demonstrated the antagonistic properties of *Panicillium* spp. against *F. oxysporum* isolated from carrot soil. Studies have shown that *P. chrysogenum*, *P. aurantiogriseum*, *P. verrucosum*, and *P. canescens* inhibits the growth of *F. oxysporum*. Communities that are rich in antagonistic fungi are able to reduce pathogen growth [23].

Conclusion

Inoculating tomato plants with *C. etunicatum* directly impacted the development of fungal biodiversity in the tomato rhizosphere, particularly by increasing the number of saprotrophs in the soil.

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