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MICROBIOLOGICAL ASPECTS IN RHIZOSPHERE OF HORTICULTURAL PLANTS CULTIVATED ON VARIOUS GROWTH SUBSTRATES IN GREENHOUSE

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Abstract: *The goal of the present paper was to present microbial aspects in rhizosphere of four horticultural plants grown on various substrates based on use of perlite and its combinations with peat, as easily biodegradable material and to reveal the interrelations between natural antagonists and potential plant pathogenic species. A better control of pathogens developed in greenhouse, due to the ease of perlite substrate disinfection and its recycling was signaled as beneficial for yields and its quality, with cost benefits and important advantages for the health of consumers. Samples from rhizosphere of tomato, pepper, cucumber and lettuce plants cultivated in soilless systems on substrates from perlite or perlite-peat mixture were analyzed from a microbiological point of view comparatively with non-cultivated substrates for assessing the bacterial and fungal density and taxonomic composition of community. Peat-perlite substrate was more favorable to bacterial microflora than perlite alone, with highest values in the rhizosphere of pepper plants. Cucumber roots found good aeration conditions in the perlite from nutritive mattresses and its exudates stimulated the increase of microbial community number and diversity more than in pepper or tomato rhizosphere. Microbial counts and global physiological activity were determined in lettuce rhizosphere cultivated on perlite-peat mixt substrate and showed doubled values comparatively with samples from extra-radicular substrate in mattress. Dominant rhizosphere fungal species belonged to genera Penicillium, Trichoderma, Fusarium, Aspergillus and bacteria to genera Psedomonas, Bacillus, Arthrobacter, both accompanied sometimes by actinomycetes. The existence of antagonistic species (e.g. Trichoderma viride strains D2, D6 and D11 with proved in vitro effect on the pathogenic isolates of Fusarium and Penicillium) indicated a good relationship antagonist-pathogen in rhizosphere of horticultural plants. The possibilities of biological control of pathogens by natural antagonists, beneficial influence of plant root exudates and rhizosphere effect similar to soil conditions recommend perlite-based substrates as environmental friendly for using in greenhouse soilless systems for horticultural plants.*

Key words: *perlite, rhizosphere, antagonism, Trichoderma, growth substrate.*

INTRODUCTION

For the majority of horticultural species of plants, prior to greenhouse cultivation is needed to be produced important number of seedlings, with rise production costs, especially for the first cycle of culture (January-June).

If classic seedlings were produced in Jiffy seven and Jiffy pots from peat, Grodan pots for mineral wool or alveolar blades, new substrate based on perlite alone or mixt with other easy biodegradable materials have been proposed in the last years.

New combinations can be used for soilless culture systems due to their advantages that do not interact with nutritive solutions for plant growth and are recyclable (ASADUZZAMAN et al. 2013).

The microbial communities in rhizosphere of horticultural plants were a less studied aspect when used soilless cultivation systems.

Very important in rhizosphere communities are the interactions between plant pathogenic and antagonistic species. Literature cites numerous natural biological antagonistic bacteria such as lactic acid bacteria (DALIE ET AL., 2010; MATEI AND CORNEA, 2014; SICUIA ET AL., 2014), various stains of genera *Pseudomonas* and *Bacillus* (TRIAS el al., 2008) or fungi from genera *Trichoderma* (HASSAN et al., 2013), *Fusarium oxysporum, Gliocladium virens* or actinomycetes.

The aim of the present paper was to present the results of the research carried out for assessing microbial aspects in rhizosphere of various horticultural species cultivated in soilless systems on substrates using perlite and peat-perlite mixture and to reveal the interrelation between antagonistic and pathogenic species with implication on biological control.

MATERIAL AND METHODS

Samples from various substrate compositions from greenhouse experiments carried out in the period 2014-2015 were analyzed from the microbiological point of view in order to assess the effect of such compositions based on perlite alone or mixed with peat on the development of rhizosphere microflora of horticultural plants (tomato, pepper, cucumber and lettuce).

Qualitative and quantitative analyses of bacterial and fungal microflora

Substrate decimal dilutions were plated on potato-dextrose-agar (PDA) culture medium in Petri plates (10cm diameter) and Topping medium in Petri plates (12cm diameter) for isolation of fungal, respectively bacterial microflora.

The plates were incubated in the dark at 25^0C.

Bacterial colonies were counted after 48 hours and fungal colonies after seven days.

Bacteria were taxonomically identified according with Bergey's manual of Systematic Bacteriology (1994) and fungi according to Domsch and Gams (1970) and Watanabe (2002).

The level of CO2 released by microbial community in rhizosphere of lettuce and in perlite-peat mix from the mattress was determined by respiration method according to standard ISO 14240-1:1997.

Pure cultures of *Trichoderma viride, Fusarium verticillioides* and *Penicillium expansum* were isolated from colonies developed on Petri plates and maintained on PDA slants in test tubes at 4^0C.

In vitro assay of antagonistic activity

In vitro antagonism between potential pathogens and *Trichoderma* isolates D2, D6 and D11 was performed by dual culture technique (Phuoc, 1988). Fungal spore suspensions were placed on opposite halves of Petri plates on PDA and incubated at 25^0C. Colonies growth has been periodically monitored.

RESULTS AND DISCUSSION

Microbial density of heterotrophic bacteria (viable cells x 10^6g^{-1} dry substrate) and fungi (colony forming units-cfu x 10^3g^{-1} dry substrate) as well as taxonomic composition of microflora in rhizosphere of pepper and tomato were determined when cultivated on perlite or on peat-perlite mix comparatively with microbial parameters of variants without plants.

Data from Table1 show low bacterial and fungal densities in variants with perlite alone, but when it cultivates with either tomato or pepper, the fungal counts increase and bacterial communities are three times more numerous (Fig.1).

Table 1

Quantitative analysis of rhizosphere and substrates microflora

No. sample	**Variant**	**Fungal counts x 10^3 cfus xg^{-1}ds.**	**Bacterial counts x 10^6 viable cells xg^{-1}ds.**
1	Perlite	21	2.6
2	Perlite (tomato rhizosphere)	34.2	7.3
3	Perlite (pepper rhizosphere)	32.6	7.4
4	Peat + Perlite (1/1)	29.5	15.8
5	Peat + Perlite (1/1) (tomato rhizosphere)	30.7	14.3
6	Peat + Perlite (1/1) (pepper rhizosphere)	1.5	42.9
7	Peat	57,1	100,2

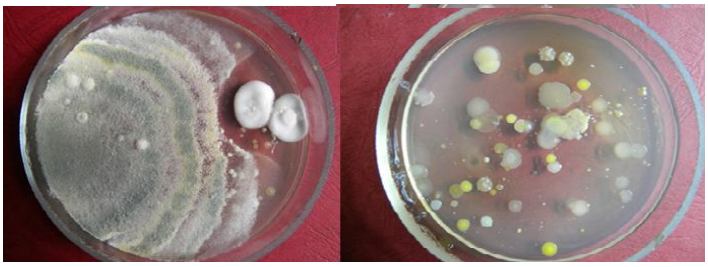

Figure 1 Antagonistic fungal microflora in pepper rhizosphere cultivated on perlite (left) and bacterial microflora in rhizosphere of tomato cultivated on perlite (right)

Peat microflora was rich in fungi (Fig.2) and especially in bacteria.

When it was mixed with perlite stimulated bacterial (Fig.2) but not fungal microflora in the substrate and in the rhizosphere of tomatoes.

Pepper root exudates stimulated the bacteria (Fig.2) to increase its activities three times than in the variant without peat, the balance between fungi and bacteria being significantly in the favor of the last.

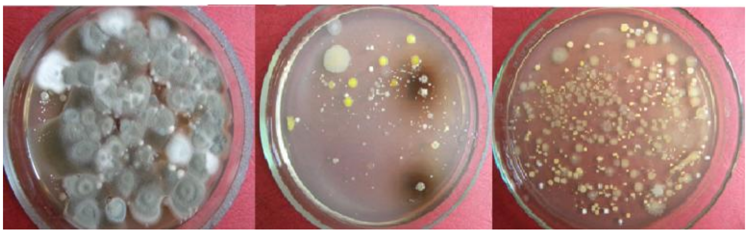

Figure 2 Fungal microflora of peat (left) and bacterial microflora in rhizosphere of tomato (center) and pepper (right) cultivated on peat-perlite substrate

Taxonomic composition of fungal microflora (Table 2) included cosmopolite species of genera *Penicillium* that accompany the antagonist *Trichoderma viride* in rhizosphere of both tomato and pepper. Figure 1 illustrates large inhibition zone of the contact limit between *Trichoderma viride* and other fungal colonies in rhizosphere of pepper cultivated on perlite.

Another well represented genus was *Fusarium*, with the antagonist *Fusarium oxysporum* in the mix peat-perlite and the potential plant pathogen *Fusarium verticillioides* sin. *moniliforme* dominant in the simple perlite.

Table 2

Taxonomic composition of fungal microflora

No. sample	**Variant**	**Fungal species**
1	Perlite	*Fusarium verticillioides, Penicillium funiculosum, Penicillium griseofulvum*
2	Perlite (tomato rhizosphere)	*Trichoderma viride, Penicillium sp.*
3	Perlite (pepper rhizosphere)	*Trichoderma viride, Verticillium leccani, Mortierella sp.*
4	Peat + Perlite (1/1)	*Acremonium strictum, Penicillium sp., Fusarium oxysporum, Trichoderma viride*
5	Peat + Perlite (1/1) (tomato rhizosphere)	*Trichoderma viride, Penicillium sp., Penicillium glabrum*
6	Peat + Perlite (1/1) (pepper rhizosphere)	*Trichoderma viride*
7	Peat	*Penicillium glabrum, Penicillium janthinellum, Stachybotris chartarum,* Actinomycetes Series Albus

Actinomycetes from Series Albus were present on PDA in peat samples and on Topping medium in samples from pepper rhizosphere (Fig.2). Actinomycetes from Series Griseus and Fuscus (producing brown pigment) were detected in rhizosphere of tomatoes cultivated on perlite mixed with peat (Fig. 2). Generally, taxonomic composition of bacterial microflora (Table 3) was represented in perlite by beneficial species belonging to genus *Pseudomonas* (with fluorescent and non-fluorescent species) that were found in peat samples, too. They were accompanied by species of genus *Bacillus* (*B. megaterium, B. subtilis, B. circulans, B. sphaericus*), *Arthrobacter* (*A. globformis, A. citreus, A. oxydans*), *Sarcina* or *Micrococcus*. They contributed to improvement of rhizosphere conditions by dynamics of physiological processes they developed and determined the character “environmental friendly” for substrates utilized in the greenhouse experiments.

Table 3

Taxonomic composition of bacterial microflora

No. sample	**Variant**	**Bacterial species**
1	Perlite	*Bacillus cereus, Pseudomonas fluorescens, Bacillus circulans, Arthrobacter oxydans, Bacillus megaterium,*
2	Perlite (tomato rhizosphere)	*Pseudomonas fluorescens, Pseudomonas sp., Pseudomonas pseudogleyi, Arthrobacter citreus, Pseudomonas aurantiaca, Bacillus circulans, Bacillus cereus* Actinomycetes Series Albus, Fuscus
3	Perlite (pepper rhizosphere)	*Bacillus circulans, Pseudomonas fluorescens, Pseudomonas sp., Arthrobacter citreus, Arthrobacter oxydans, Micrococcus sp.*
4	Peat + Perlite (1/1)	*Arthrobacter citreus, Bacillus sphaericus, Pseudomonas fluorescens, Pseudomonas sp., Bacillus circulans, Bacillus subtilis, Micrococcus sp.*

No. sample	**Variant**	**Bacterial species**
5	Peat + Perlite (1/1) (tomato rhizosphere)	*Arthrobacter citreus, Pseudomonas fluorescens, Micrococcus sp.* Actinomycetes Series Fuscus, Griseus
6	Peat + Perlite (1/1) (pepper rhizosphere)	*Bacillus subtilis, Pseudomonas fluorescens, Bacillus sphaericus* Actinomycetes Series Albus
7	Peat	*Pseudomonas fluorescens,* Actinomycetes Series Albus, Griseus, Luteus, Ruber

Microbiological analysis of composition and numerical effectives in the rhizosphere of tomatoes, pepper and cucumbers cultivated in nutritive mattresses with perlite revealed the presence of a low level of fungal structure (below 50x 10^3cfus g^{-1} dry substrate), doubled under cucumbers as compared with pepper and tomatoes rhizosphere. Bacterial communities were similar in number and with values considered of medium level (Table 4).

Fungal microflora was represented by 5-6 species from genera *Fusarium, Penicillium* and *Aspergillus* (Table 5).

Table 4

Quantitative analysis of tomato, pepper and cucumber rhizosphere microflora

No. sample	**Variant**	**Fungal counts x 10^3 cfus xg^{-1}ds.**	**Bacterial counts x 10^6 viable cells xg^{-1}ds.**
1	Tomato rhizosphere (mattresses with perlite)	9.4	13.04
2	Pepper rhizosphere (mattresses with perlite)	10.3	15.06
3	Cucumber rhizosphere (mattresses with perlite)	18.7	16.3

Table 5

Taxonomic composition of bacterial microflora of tomato, pepper and cucumber rhizosphere

No. sample	**Variant**	**Fungal species**
1	Tomato rhizosphere (mattresses with perlite)	***Fusarium verticillioides, Verticillium tenerum, Fusarium culmorum var. roseum, Cladosporium cladosporioides, Penicillium glabrum***
2	Pepper rhizosphere (mattresses with perlite)	***Fusarium culmorum var. roseum, Penicillium janthinellum, Fusarium oxysporum, Aspergillus niger, Penicillium aurantiogriseum, Fusarium verticillioides***
3	Cucumber rhizosphere (mattresses with perlite)	***Fusarium culmorum var. roseum, Aspergillus fumigatus, Fusarium oxysporum, Alternaria alternata, Aspergillus niger, Trichoderma viride***

The presence of potential pathogenic representatives of genus *Fusarium* under healthy plants was explained by biological control represented by antagonistic activity of *Fusarium oxysporum, Penicillium* and fluorescent bacteria from genus *Pseudomonas*. They dominate bacterial communities of 3-5 species, being accompanied by typical rhizosphere species from genera *Bacillus* and *Arthrobacter* (Table 6).

Table 6

Taxonomic composition of bacterial microflora of tomato, pepper and cucumber rhizosphere

No. sample	**Variant**	**Bacterial species**
1	Tomato rhizosphere (mattresses with perlite)	***Pseudomonas sp., Arthrobacter globiformis, Bacillus megaterium***
2	Pepper rhizosphere (mattresses with perlite)	***Pseudomonas fluorescens, Pseudomonas sp., Bacillus megaterium Arthrobacter globiformis***
3	Cucumber rhizosphere (mattresses with perlite)	***Pseudomonas sp., Pseudomonas fluorescens, Bacillus megaterium, Arthrobacter citreus, Bacillus circulans***

Results concerning the microbial aspects in rhizosphere of lettuce comparatively with those from the mass of perlite-peat mix in nutritive mattresses (Fig.3) revealed two fold higher numbers of microorganisms in rhizosphere than in the perlite with peat from the mattresses (Table 7). The level of global physiological activities of microflora was also twice higher in lettuce rhizosphere (72.814 mg CO_2x100 g^{-1} dry substrate) than in the mattresses (38.720 mg CO_2x100 g^{-1} dry substrate).

Figure 3 Lettuce plants cultivated on nutritive mattresses with perlite substrate

Table 7

Microbial counts and physiological activities in perlite from mattresses and rhizosphere of lettuce

Microbiological characteristics	**UM**	**Sample**	
		1. Rhizosphere	**2. Perlite (in mattresses)**
Bacterial counts	x 10^6 viable cells xg^{-1}ds.	169.021	35.027
Fungal counts	x 10^3 cfus xg^{-1}ds.	65.429	29.689
Substrate induced respiration	mg CO_2x100 g^{-1} ds.	72.814	38.720

Bacterial species identified belong especially to genera *Pseudomonas, Bacillus* and *Arthrobacter*. Fungal representatives of genera *Aspergillus*, *Penicillium*, *Cladosporium* were present in lettuce rhizosphere and *Aspergillum* and *Fusarium* in the mix peat-perlite from mattresses (Table 8).

Table 8

Taxonomic composition of bacterial and fungal microflora in perlite from mattresses and rhizosphere of lettuce

Taxonomic composition	**Sample**	
	1. Rhizosphere	**2. Perlite (in mattresses)**
Bacterial species	*Pseudomonas fluorescens,* *Escherichia freundii,* *Bacillus megaterium,* *Bacillus cereus,* *Pseudomonas* sp.	*Pseudomonas fluorescens,* *Pseudomonas* sp. Actinomycetes Series *Albus**
Species number	5	3
Fungal species	*Aspergillus niger,* *Cladosporium herbarum,* *Penicillium glabrum* *Penicillium* sp., *Aspergillus fumigatus*	*Aspergillus niger,* *Fusarium culmorum var. roseum*
Species number	5	2

Results are in concordance with previous research when fungal and bacterial effectives were twice higher in substrates mulched with peat or manure than in non-mulched control due to the richness of organic matter. Similarly significant stimulation of *Trichoderma, Penicillium* and *Aspergillus* was reported in substrates cultivates with aubergines under metalized plastic mulch (MATEI et al., 2001) or beneficial effects on microflora from rhizosphere of lettuce cultivated in the field.

Greenhouse experiments showed that the root exudates of bean, soybean and maize plants stimulated the development of a bacterial microflora doubled as numeric values comparatively with non-rhizosphere soil and the global physiological activities were more dynamic, too (MATEI AND MATEI, 2003).

In vitro antagonism was assessed between *Trichoderma viride* D2 (Fig. 4) isolated from cucumber rhizosphere in mattresses with peat-perlite mix, *Trichoderma viride* D6 (Fig. 4) isolated from tomato rhizosphere cultivated in pots with perlite and *Trichoderma viride* D11 (Fig. 4) isolated from mattresses with peat and potential plant pathogenic *Fusarium verticillioides* (from lettuce roots) and *Penicillium expansum* (from apples).

Figure 4 *Trichoderma viride* isolates D2 (left), D6 (center) and D11 (right)

Table 9 synthesizes data illustrated in figures 5 and show particular way of antagonistic action of each interaction assayed. Thus, *Trichoderma viride* D6 strongly influenced fungal growth of *Fusarium verticillioides* colony with over 50% growth inhibition.

The presence for six days of clear zone between the two colonies proved the existence of chemical interactions.

However, the growth of both colonies stops at the contact zone.

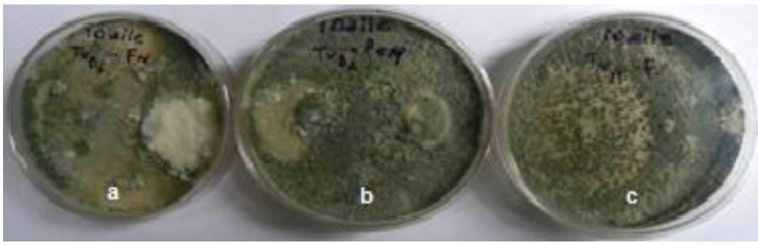

Figure 5 Inhibitory potential of 3 *Trichoderma* isolates on mycelial growth of pathogenic species (10 days). a) *T.viride* D6 vs *F.verticillioides*, b) *T.viride* D2 vs *P. expansum*, c) *T.viride* D11 vs *F.verticillioides*

Table 9

Interaction between plant pathogenic and Trichoderma species with antagonistic properties

Potential pathogenic species	***Trichoderma viride* strains**	**Interaction**
Fusarium verticillioides	*Trichoderma viride D6*	++
Fusarium verticillioides	*Trichoderma viride D11*	+++
Penicillium expansum	*Trichoderma harzianum D2*	++/+++

\- *the antagonist doesn't inhibit pathogen and the pathogen overgrows it*
\+ *the antagonist inhibits the pathogen growth but the pathogen overgrows it*
++ *the antagonist inhibits the pathogen growth but stops to inhibition limit*
+++ *the antagonist inhibits the pathogen and overgrows it*

Trichoderma viride D2 presented inhibition of fungal growth of *Penicillium expansum* but the absence of clear zone indicated that no chemical interaction produced between the two fungal species. The antagonist strain *Trichoderma viride* D2 had the tendency to overgrow the pathogen due to its ability to compete with it for space and nutrients.

Trichoderma viride D11 from peat was the most competitive antagonistic strain that overgrew *Fusarium verticillioides* from lettuce roots and had the strongest inhibitory effect on pathogen growth (Fig.11). Previous research (MATEI et al., 2014) showed antagonistic effect of the *Trichoderma* isolates of four Fusarium species. When *T. viride* L11, *T. viride* L5 and *T. harzianum* 57 were tested for antagonistic activity, *Fusarium verticillioides* was the less susceptible and *Fusarium culmorum* var. *roseum* the most susceptible pathogenic species to exposure to a mixed culture of the three compatible strains of *Trichoderma*, with competition for space and nutrients as main antagonistic mechanism of action. *Fusarium verticillioides* was inhibited by *Myrothecium verrucaria* and a bacterial strain of *Pseudomonas fluorescens via* chemical antagonism.

Recent studies (RAUT et al., 2014) of antagonism in dual cultures showed the inhibitory effect of *Trichoderma asperellum* isolates and culture filtrates on pathogenic *Fusarium*, *Rhizoctonia* and *Pytium* by releasing non-volatile compounds.

Cornea et al., (2010) reported important growth inhibition of *Rhizoctonia solani* and *Pytium* sp. by isolate *T.viride* TV2, a mutant strain producing volatile compounds. Other isolates (*T. viride* SP456 and Thz P8) presented growth inhibition rates over 80% against two *Botrytis cinerea* isolates and *Phytophtora* sp. P1. The antifungal action of antagonistic strains ranged from direct contact with mycelia and appresoria formation to vacuolization of pathogen hyphae followed by cell disintegration. In our study, mycoparasitism was also involved in antifungal activity of *T. viride* D11 agains *F. verticillioides*, when the antagonist overgrew the pathogen and its mycelia hiperparasitized pathogen's hyphae. Concerning the biochemical interactions between antagonist and pathogen, our previous research (MATEI et al., 2011) showed that antagonistic isolates from genus *Trichoderma* were able to produce antifungal enzymes such as: chitinase, phenyl-alanin-ammonium liase, β-1,3 -glucanase and peroxidase, in variable amounts depending on genetic structure of each fungal isolate. The presence of various species known as antagonists (e.g. *T. viride*, *F. oxysporum*, *Penicillium* sp., *Actinomycetes*, *Pseudomonas fluorescens*) in the rhizosphere or mass of various mixtures as substrates in soilless systems of cultures of horticultural plants showed that equilibrium could be maintained between potential pathogenic species and natural antagonists. Root exudates can help the development of antagonists and also a rhizosphere effect was observed similar with the case of culture on soil substrates.

CONCLUSIONS

Specific charge in microbial counts and taxonomic composition were determined as a function of substrate composition, the presence of plant roots, and the species of horticultural plant.

The presence of plant roots significantly stimulated bacterial but not fungal development comparatively with non-cultivated substrate.

Mixt substrate with peat and perlite increase the bacterial proliferation especially in pepper rhizosphere.

Rhizosphere effect was detected for lettuce, with doubled microbial counts and physiological activities comparatively with the substrate mix of peat-perlite from nutritive mattresses mass.

Fungal antagonists isolated from rhizosphere belonging to genus *Trichoderma* were able to control the development of fungal pathogens, proving the environmental friendly character of proposed soilless culture substrates and their beneficial influence on plant health conditions.

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