THE COMPARATIVE STRUCTURE OF FUNGAL COMMUNITIES IN RHIZOSPHERE OF SOYBEAN TREATED WITH CHEMICAL AND BIOLOGICAL AGENTS FOR PATHOGENS CONTROL

Gabi-Mirela MATEI, S. MATEI

National RD Institute for Soil Science, Agrochemistry and Environmental Protection
Bd. Marasti 61, sect.1, cod.011464 Bucuresti,
Corresponding author: so_matei602003@yahoo.com

Abstract: *In agriculture, control of plant pathogens can be done by chemical or by biological methods. Both of them act on pathogen population as well as on rhizospheric microflora. Research have been carried out in order to study the structure of rhizospheric fungal community of two soybean cultivars under the influence of chemical and biological agents for the control of gray mould. A green house experiment was designed to compare the structure of fungal rhizospheric microflora of soybean cv. PR91M10 sensible to gray mould and PR92B62 resistant, treated with systemic and contact fungicides, as well as with four fungal preparations from genera Botrytis, Trichoderma and Penicillium administrated on plants and in soil. The taxonomic composition of fungal community was assessed by plating soil fragments on water agar and identifying the developed structures. A total number of 21 genera were identified for sensible soybean cultivar PR91M10 and 18 genera for resistant cultivar PR92B62. The frequency registration allowed the genera to be classified as constant accessory and accidental. Thus, the status of each taxon was the same or modified as a function of the nature of treatment (with biological or chemical control agents) or method of administration (on leaves or in soil). Apart of significant influence of fungal extracts on improving plant health, the ecological analysis of the rhizospheric fungal communities assessed the influence of biological control agents provided by selected microbial strains, on natural microflora composition and number. The use of water agar substrate allowed as to isolate and identify species of nematophagous genera Arthrobotrys, Dactylaria, Nematoctonus and Harposporium, some of them rarely isolated on other usual media. The development of ring form fungal traps or adhesive knobs and haustoria-like hyphae was possible to be monitored as well as trapped nemathods digestion by predaceous fungi, demonstrating the dynamic relationships between microflora and fauna in the rhizosphere of cultivated plants such as soybean under the influence of control agents for gray mould.*

Key words**:** *fungi, rhizosphere, resistance, soybean, biological control*

INTRODUCTION

The need for healthy food, as well as avoiding soil pollution by excessive use of pesticides in agriculture resulted in development of alternative methods for protecting cultivated plants against pathogens. Various studies have indicated that the susceptibility of plants to certain diseases is not synonymous with the absence of the genetic potential for resistance mechanisms to these diseases. The resistance to the phytopathogens can be induced by various biological control agents, especially those derived from fungal strains selected for the property of eliciting immunity reactions in plants (EDLICH et. Al 1989, DE MARCO et al.2003, HANSON & HOWELL, 2004, MATEI&MATEI, 2009, FERNANDEZ-ACERO et. al., 2007).

The defence reactions that are activated in plants cover a broad spectrum of chemical, biochemical and mechanical defense (WEST, 1984, NAMDEO, 2007).

Because less knowledge are available concerning the natural microflora behaviour under the impact of biological control agents, a study has been carried out to assess the composition and structure of rhizospheric fungal microflora of two soybean cultivars treated with chemical and biological agents for the control of gray mold.

MATERIAL AND METHODS

A greenhouse experiment was designed with soybean plants. Seeds belonging to soybean cultivars PR91M10 susceptible to *B. cinerea* attack and PR92B62 resistant have been seeded in vegetation pots with 5kg soil in 3 replications per variant.

Experimental variants included: untreated control, chemical contact pesticides Captan, Teldor and Batron administrated on plant; chemical systemic pesticides Topsin, Rovral and Topsin M administrated in soil; biological control agent E1 (monostrain extract of selected *Botrytis* isolate administrated on leaves and in soil); biological control agent E2 (multistrain extract of selected *Botrytis* isolates administrated on leaves and in soil); biological control agent E3 (multistrain extract of selected *Trichoderma* and *Penicillium* isolates administrated on leaves and in soil); biological control agent E4 (multistrain extract of selected *Botrytis*, *Trichoderma* and *Penicillium* isolates administrated on leaves and in soil).

Soybean plants were experimentally infected with inoculum of *B. cinerea*.

Four treatments with chemical or biological agents of intervals of two days have been done. Three days after, rhizospheric soil samples were collected from pots. Soil crumbs were plated on water-agar in Petri dishes 10cm diameter and incubated at 25°C for 5 days. The developed fungal structures were taxonomically identified under optic microscope according to DOMSCH & GAMS (1970) and SAMSON & HOEKSTRA (1988).

The average number of colonies / variant and the frequency of each taxa registered allowed their grouping in 3 classes of constancy, respectively in constant, accessory and accidental taxa.

RESULTS AND DISCUSSIONS

A total number of 18 fungal genera have been identified in samples collected from rhizosphere of resistant soybean cultivar PR92B62 and 21 from the cultivar PR91M10 susceptible to gray mold (Table 1 and 2).

At cultivar PR91M10 the average number of colonies developed from a soil crumb ranged between 9 and 16 as a function of treatment nature and method of administration and at cultivar PR92B62 this number ranged between 6 and 13.

In soybean cultivar PR91M10 the number of colonies was lower at both chemical treatments, biological preparations E2 and E3 both application methods as compared with control. At treatments with E1 and E4, especially when applied on plant, the number of fungal colonies was higher than in control rhizosphere.

Generally, the communities are formed by 5 to 8 genera, slowly varying from the 7 taxa identified to control. The greatest diversity of microflora was recorded for E4 administrated on plants.

In soybean cultivar PR92B62 the number of colonies isolated from rhizosphere of plants treated with E2 applied to soil exceeded the others including the control. Systemic and contact fungicides induced reductions of number and diversity in fungal communities more than biological control agents E1-E4.

As a general characteristic, at this cultivar, the treatment with biological preparations administrated in soil preserved better the number and diversity of rhizosphere fungal communities.

With few exceptions, *Actinomycetes* for PR91M10 and *Humicola* for PR92N62 had the status of constant genera in all experimental variants, their very high frequency being connected with general and stable conditions in rhizosphere. They were less influenced by experimental factors than other fungal taxa.

A number of 1 to 3 genera are common with those from control rhizosphere, but 1 to 4 differential genera occur in rhizosphere of plants under the influence of chemical or biological control agents for each soybean cultivar.

Botrytis cinerea was present in the rhizosphere of control at cultivar PR92B62 and in the communities from plants treated with E1 and E3 at cultivar PR91M10, with status of accidental genus, at frequencies below 25%. A special group developed on water-agar was that of nematophagous (predaceous) fungi, which feed with soil nematodes.

In cultivar PR91M10, *Harposporium anguillulae* (Fig.1) was constant species in control rhizosphere. It was also present in the rhizosphere of plants treated with E1 administrated in soil (as accidental species) and with E4 administrated on plants (as accessory species). *Acaulopage* appeared as a constant species in rhizosphere of plant treated with systemic fungicides and as accessory species under the influence of E2 administrated on plants. The same preparation administrated in soil, as well as both E3 variants were favorable to *Nematoctonus* isolated as accidental species. In the rhizosphere of plants treated with E4, *Arthrobotrys* was identified as accessory genus when control agent was administrated on plants and as accidental genus when it was administrated in the soil.

At cultivar PR92B62, *Nematoctonus* had accessory status in control and accidental in variant E3 administrated in soil.

Table 1

The structure of fungal communities in rhizosphere of soybean cultivar PR91M10 treated with various pathogen control agents

No	Fungal genera	Experimental variants										
		Control	Chemical treatment		Extract 1		Extract 2		Extract 3		Extract 4	
			contact	systemic	plant	soil	plant	soil	plant	soil	plant	soil
1	*Actinomycetes*	constant	accessory	constant	constant	constant	constant	constant	constant	accessory	constant	constant
2	*Harposporium*	constant				accidental					accessory	
3	*Mortierella*	accessory	constant		accessory	constant					accessory	constant
4	*Alternaria*	accidental						accidental				
5	*Fusarium*	accidental		accidental	accessory	accidental	accessory			accidental	accidental	accessory
6	*Papulaspora*	accidental	accidental									
7	*Humicola*	accidental	accidental	accidental	accessory			constant	constant	constant		
8	*Curvularia*		accidental				accessory			accidental		
9	Sterile mycelia		accidental									constant
10	*Acaulopage*			constant			accessory					
11	*Aspergillus*			accidental								
12	*Acremonium*			accidental			accessory		accidental	accidental	accessory	
13	*Cunninghamella*				constant		accessory					
14	*Myrothecium*				accessory							
15	*Botrytis*				accidental				accidental			
16	*Actinomucor*					accidental						
17	*Nematoctonus*							accidental	accidental	accidental		
18	*Rhinocladiella*							accidental				
19	*Arthrobotrys*										accessory	accidental
20	*Trichoderma*										accidental	
21	*Geotrichum*										accidental	accidental
	No. colonies	**12**	**9**	**10**	**15**	**12**	**10**	**9**	**9**	**9**	**16**	**13**

constant genera (red)
accessory genera (yellow)
accidental genera (green)

Arthrobotrys (Fig. 2) which developed adhesive three-dimensional network traps, immobilized nematodes and consumed their bodies by enzymatic lysis, has the status of constant species in E1 administrated in soil, accessory species in variant with contact pesticides and accidental species in variant with systemic fungicides.

Dactylaria had constant status in variant with E1 on plant and *Harposporium* developed as accessory species in rhizosphere of soybean treated with E4 in soil.

Table 2

The structure of fungal communities in rhizosphere of soybean cultivar PR92B62 treated with various pathogen control agents

No	Fungal genera	Experimental variants										
		Control	Chemical treatment		Extract 1		Extract 2		Extract 3		Extract 4	
			contact	systemic	plant	soil	plant	soil	plant	soil	plant	soil
1	*Humicola*											
2	*Nematoctonus*											
3	*Mortierella*											
4	*Actinomicete*											
5	*Botrytis*											
6	*Cylindrocarpon*											
7	*Rhinocladiella*											
8	*Arthrobotrys*											
9	Sterile mycelia											
10	*Alternaria*											
11	*Cunninghamella*											
12	*Acremonium*											
13	*Dactylaria*											
14	*Gliocladium*											
15	*Fusarium*											
16	*Rhizopus*											
17	*Aspergillus*											
18	*Harposporium*											
	No. colonies	11	8	6	8	9	10	13	9	9	9	10

constant genera (red)
accessory genera (yellow)
accidental genera (green)

Figure 1: Nematode parasitized by *Harposporium anquillulae* Soybean PR91M10 rhizosphere (x300)

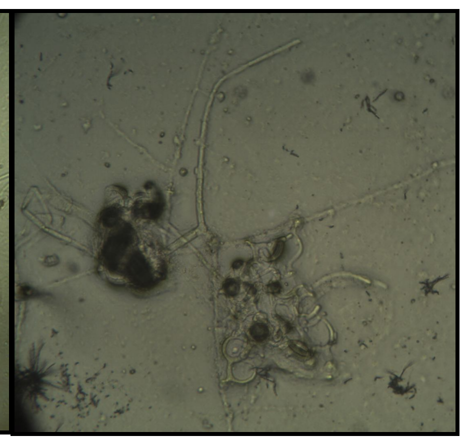

Figure 2: *Arthrobotrys oligospora* adhesive three-dimensional network traps Soybean PR92B62 rhizosphere (x300)

CONCLUSIONS

The structure of fungal communities in rhizosphere of soybean varied as a function of cultivar, the nature of control agent and the method of administration

18 fungal genera were identified from variants of soybean susceptible to pathogens PR91M10 and 21 genera from soybean cultivar PR92B62 resistant to pathogens

In resistant cultivar PR92B62, the treatments with biological control agents E1 and E4 administrated to soil supported numerical increments of fungal structures and E4 administrated on plants stimulated the fungal diversity.

In susceptible cultivar PR91M10, the biological control agents administrated to soil preserved better the number and diversity of fungal communities in rhizosphere.

A number of 1 to 3 genera were common with control, but 1 to 4 differential genera occurred in rhizosphere of plants under the influence of chemical or biological control for each soybean cultivar.

In susceptible cultivar PR91M10, genus *Botrytis* was present accidentally in variants with E1 and E3 administrated on plants and in resistant cultivar PR92B62 only in control, the other treatments with biological or chemical control agents being efficient in limiting pathogen proliferation.

The presence of four fungal nematophagous species recorded with status of constant, accessory or accidentally taxa in experimental variants and control, too.

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