THE MODIFICATIONS OF SOIL MICROBIAL DIVERSITY AND STRAWBERRY YIELDS IN RESPONSE TO VARIOUS METHODS OF CONTROL OF GREY MOULD IN FIELD EXPERIMENTS

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Abstract: *The intense use of pesticides in strawberry to control various diseases, induced pathogens increased resistance and environment pollution. The main purpose of the present study was to assess the efficacy of alternative methods for the control of grey mould, based on microbial products as well as their impact on structure and diversity of soil fungal communities. Previous research showed that microbial strains of bacteria or fungi were successfully utilized for the bio-control of plant pathogens in cultivated plants of horticultural or agricultural interest. The paper presents the results of the experiment designed in the field of Biophan S.A. in Arges county to assess the influence of two biological preparations of microbial origin, administered alone or along with chemical fungicides on the control of grey mould attack frequency, as well as on yields. A comparative study of fungal diversity was carried out in soil cultivated with strawberry plants cv. Senga Sengana. The new bio-control methods tested involve the increase of plant resistance under the influence of fungal preparations. The results confirmed that both bio-control agents administered alone or along with chemical fungicides were able to protect strawberry plants against the attack of grey mould. This has practical implications on yields quantity and quality, increased as compared with untreated control and healthy due to reduced pesticides consume in variants treated with biological and chemical control agents, or total absence when treated only with fungal preparations. An original aspect treated in the paper refers to the implications of bio-control agents of microbial origin on soil fungal coenoses. Species composition, diversity index, homogeneity and species status in soil fungal communities were studied. Results showed that preparations of fungal origin were able to stimulate immune reactions in strawberry plants, to reduce grey mould attack, thus manifesting a high biological efficacy and to increase yields. The paper reveals the benefits of alternative control methods proposed, based on reducing pesticides consume and activating natural defence mechanisms against pathogens for both healthy food and environment protection. Research was supported by PNCDI II Research Program, Projects nos. 31-078/2007 and 52-112/2008.*

Key words**:** *fungi, diversity, plant resistance, strawberry, biological control*

INTRODUCTION

The development of resistance to chemical pesticides and their negative impact on the environment has prompted researches to evaluate alternative methods for the control of important agricultural pests (CORNEA et al., 2009)

A variety of biological control agents can be utilized as either single or combined living strains of bacteria and fungi or their metabolites that stimulate plant response to pathogens attack (WALTERS and BOYLE, 2005; COGALNICEANU et al., 2010; MATEI et al., 2010).

They act through plant-based mechanisms rather than on direct attack on the pathogenic agents, thus avoiding direct undesired effect on non-pathogenic organisms.

These methods confer protection for the whole plant, with effects extending post harvest and might also, be beneficial in thwarting other stress in the field conditions.

The paper presents the results of the experiment designed in the field of BIOPHAN S.A. in Arges county to assess the influence of two biological preparations of microbial origin, administrated alone and along with chemical fungicides on the control of grey mould attack, as well as on yields and diversity of soil fungal microflora.

MATERIAL AND METHODS

An experimental field was organized to Biophan S.A. in Arges county and cultivated with strawberry plants (*Fragaria* x *ananassa* Duch.) cv. Senga Sengana.

Experimental variants with three replicates each included: untreated control, chemical contact fungicides Captan, Teldor and Batron administered on plant; chemical systemic fungicides Topsin, Rovral and Topsin M administered in soil; biological control agent E1 (multistrain extract of selected *Botrytis* isolates administered on leaves and in soil); biological control agent E2 (multistrain extract of selected *Trichoderma* and *Penicillium* isolates administered on leaves and in soil); combined treatment with E1 and fungicides administered on leaves and in soil; combined treatment with E2 and fungicides administered on leaves and in soil.

Four treatments with chemical or biological agents at intervals of two days have been done. Climatic parameters, such as precipitations, average air temperature and relative humidity have been registered daily during the experimentation. Soil samples were collected from field, for each variant. Soil dilutions were plated on culture medium potato-dextrose-agar in Petri dishes 10cm diameter and incubated at 25^{o}C for 5 days. The developed fungal structures were counted and taxonomically identified under optic microscope according to Domsch & GAMS (1970) and SAMSON & HOEKSTRA (1988).

The fungal density expressed as average number of colonies forming units (cfu) x g^{-1} dry soil, relative abundance, diversity index of Shannon-Wiener and the homogeneity represented the ecological indicators for the effect of pathogen control agents on soil fungal coenoses (SHEN,1997).

Experimental data were statistically interpreted by means of Student test.

RESULTS AND DISCUSSIONS

According to measurements carried out during the period of experimentation, climatic conditions were favorable to absorption of chemical and biological fungicides to test, at both soil and leaves level (Fig.1).

In variants of administration on leaves, the frequency of pathogen attack on strawberry fruits was 50,69% (Fig. 2) in untreated control, but the biological and chemical control agents significantly reduced it.

The most efficient were contact fungicides and mixed biological product E2 when administrated on plant leaves along with contact fungicides (over 82% biological efficacy).

Strawberries biomass was double in treated plants when compared with untreated control (estimated yield of over 20t/ha comparatively with 10t/ha) (Fig. 3).

When applied to soil, pathogen control agents were less efficient with biological efficacy of 55-60% for biological agents alone or combined with fungicides and of 62% for systemic fungicides (Fig. 4).

Strawberries biomass was slightly decreased than in variants treated by spraying on leaves, but it was also double for treated plants comparatively with untreated control (Fig. 5).

The results indicate that fungal extracts E1 and E2 induced resistance mechanisms in treated plants, evidenced at histological and biochemical level, with effects on plant health and consequently on yields quality and quantity, as also pointed out other authors for similar microbial preparations (KOMBRICK & SOMSSICH, 1995; CAMERA et al., 2004).

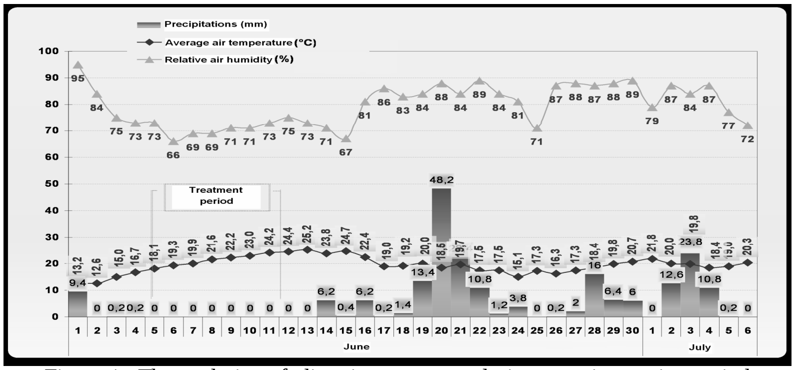

Figure 1: The evolution of climatic parameters during experimentation period

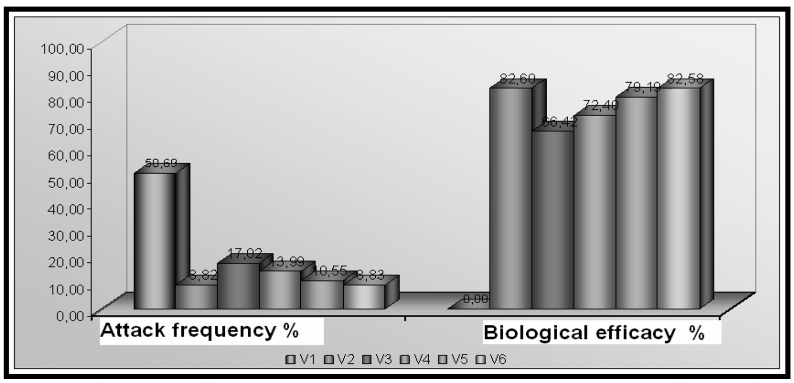

Figure 2: The frequency of pathogen attack on strawberry and the biological efficacy of control treatments applied on plant leaves

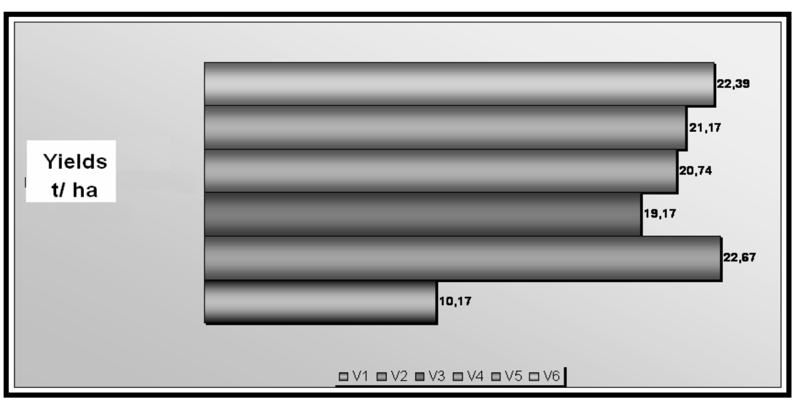

Figure 3: Yields to Senga Sengana strawberry cultivar at variants treated on leaves with biological and chemical fungicides

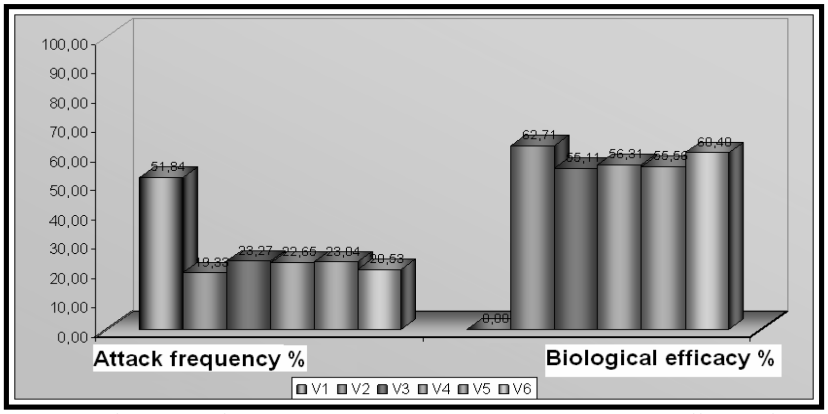

Figure 4: The frequency of pathogen attack on strawberry and the biological efficacy of control treatments applied to soil

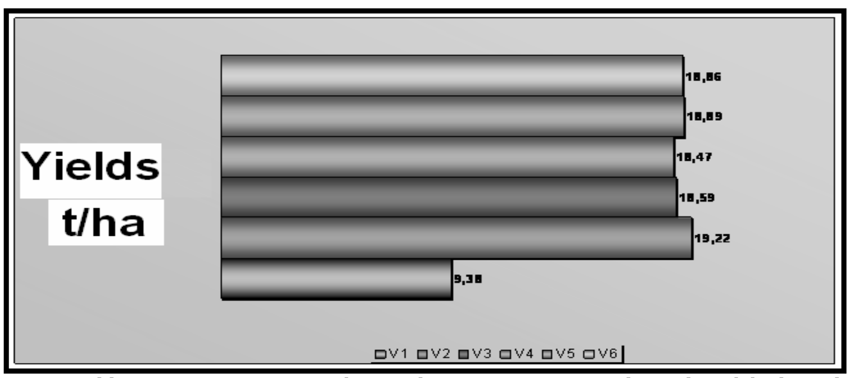

Figure 5: Yields to Senga Sengana strawberry cultivar at variants treated to soil with biological and chemical fungicides

As an additional observation, strawberries harvested from variants treated with fungal extracts kept in refrigerator rested fresh and healthy 3-4 days longer than strawberries harvested from variants with chemical fungicides or from untreated control.

Concerning the presence of the pathogen *Botrytis cinerea,* it was found into the soil from untreated control, but not in variants with biological or chemical control agents (Table 1).

When administrated alone or along with chemical fungicides on either leaves or soil, E1 kept the diversity index H' of soil fungal communities similar to control and contact fungicides variants.

Fungal diversity was not significantly influenced by E2 when administrated along with chemical fungicides, but the number of species was lower in variants treated with E2 and in variants with systemic fungicides comparatively to control.

Generally, fungal communities consisted of 6 to 10 species, with more homogeneity in control and the tendency of increasing heterogeneity in treated variants, especially with E2 administered along with chemical fungicides by spraying on plant leaves.

Species belonging to cosmopolitan genera *Fusarium*, *Penicillium* and *Aspergillus* were the most abundant in all fungal communities.

Table 1

The structure of soil fungal communities and ecological indices in field experiment with strawberry cv. Senga Sengana treated with chemical and biological control agents

Nr.	Fungal species	Experimental variant										
		Control	Chemical		Extract 1		Extract 2		Extract 1+chemical fungicide		Extract 2+chemical fungicide	
			contact	systemic	plant	soil	plant	soil	plant	soil	plant	soil
1	*Penicillium glabrum*	▒										▒
2	*Penicillium janthinellum*	▒										
3	*Aspergillus fumigatus*	▒	▒			▒					▒	
4	*Fusarium culmorum*	▒	▒		▒	▒			▒	▒		▒
5	*Aspergillus niger*	▒					▒					
6	*Rhizopus stolonifer*	▒	▒			▒	▒			▒	▒	▒
7	*Botrytis cinerea*	▒										
8	*Aspergillus glaucus*	▒										
9	*Epicoccum nigrum*	▒	▒									
10	*Fusarium oxysporum*		▒	▒	▒	▒		■	▒	▒	▒	▒
11	*Fusarium sp.*		▒					▒				
12	*Penicillium sp.*		▒		▒	▒		▒				
13	*Trichoderma viride*		▒	▒	▒	▒	▒				▒	
14	*Verticillium leccani*		▒		▒			▒	▒	▒		
15	*Trichoderma harzianum*			▒		▒		▒	▒			▒
16	*Penicillium steckii*			▒								
17	*Cladosporium cladosporioides*		▒	▒	▒				▒			
18	*Aspergillus terreus*			▒	▒						▒	
19	*Penicillium*				▒	▒	▒		▒	▒		▒
21	*Fusarium pallidoroseum*				▒						■	
22	*Aspergillus versicolor*				▒		▒					
23	*Fusarium culmorum var. roseum*						▒				▒	
24	*Cladosporium herbarum*					▒	▒			▒		
25	*Acremonium strictum*							▒	▒			
26	*Penicillium aurantiogriseum*								▒			▒
27	*Penicillium verrucosum*									▒		
28	*Fusarium verticillioides*										▒	
29	*Verticillium tenerum*											▒
	No fungi (x10^3 cfu/g dry soil)	180,132	141,586	133,021	173,892	149,275	126,871	163,976	167,994	119,690	158,739	147,926
	Diversity Index H'	2,370	2,206	1,627	2,069	2,107	1,820	1,602	1,927	1,894	1,859	1,970
	Diversity Index H'_1	2,120	1,884	1,479	1,833	1,821	1,570	1,446	1,709	1,621	1,626	1,720
	Homogeneity ε	0,920	0,818	0,825	0,834	0,828	0,806	0,806	0,821	0,833	0,782	0,827
	Var (H')	0,027	0,037	0,031	0,028	0,033	0,040	0,031	0,031	0,033	0,045	0,032
	Semnif.Student test	Mt	ns	*	ns	ns	*	*	ns	ns	ns	ns
	H max	2,303	2,303	1,792	2,197	2,197	1,946	1,792	2,079	1,946	2,079	2,079

A=67-100%
■ A=34- 66%
▒ A=0 -33%

Antagonistic species *Trichoderma viride* and *Trichoderma harzianum* developed well in soils treated with fungicides or biological control agents, as well as in variants with both treatments, but not in control.

The same status of differential species owned *Cladosporium cladosporioides* and *Cladosporium herbarum*.

Previous research (Matei et al., 2010) on soybean showed a number of 1 to 4 differential species in experiments with similar biological control agents, but generally, the fungal communities were not as rich in species as in strawberry.

Similarity was higher in soybean, with 1-3 species common with control as compared with strawberry.

Both in soybean and strawberry experiments, control agents of microbial origin exerted a beneficial influence on soil fungal communities, stimulating the development of antagonistic species and limiting grey mould attack.

CONCLUSIONS

Both chemical and biological control agents reduced the frequency of grey mould attack on strawberry and increased yields irrespective of application method when compared with untreated control.

Pathogen control was more efficient when chemical and biological fungicides applied by spraying on plant leaves than into the soil.

Biological control agent E2 efficacy was similar to chemical fungicides when administrated both alone and along with he pesticide, irrespective of application method.

E1 preparation was more efficient when administrated along with chemical fungicides

Species diversity of soil fungal communities was not significantly influenced by treatment with E1 alone, E1 and E2 along with chemical fungicides and contact fungicides but the number of species decreased when administrated E2 or systemic fungicides.

Botrytis cinerea was present in fungal communities from control but no in variants treated by biological or chemical fungicides.

Alternative bio-control strategies reduced losses caused by plant pathogens and were effective and affordable with maintaining environmental quality.

BIBLIOGRAPHY

1. CAMERA, S.L., GOUZERH, G., DHONDT, S., HOFFMANN, L., FRITIG, B., LEGRAND, M., HEITZ, T., 2004 Metabolic reprogramming in plant innate immunity: the contributions of phenylpropanoid and oxylipin pathways. Immunol. Rev. 198:267-284.
2. COGALNICEANU, G., MITOI, M., MATEI, M.G., MATEI, S., 2010 Biochemical changes induced in regenerants of *Fragaria X ananassa* Duch. by the *in vitro* treatment with fungal elicitors. Rom.Biotechnol.Letts. 15(4): 5512-551970.
3. CORNEA, P., POP, A., MATEI, S., CIUCA, M., MATEI, M.G., POPA, G., VOICU, A., STEFANESCU, M. 2009 Antifungal action of new *Trichoderma* spp. Romanian isolates on different plant pathogens. Biotech. Biotechnol. Equip. SE on line. 23(2):766-770.
4. DOMSCH, K.H., GAMS, W., 1970 Fungi in agricultural soils. T&A Constable Ltd. Edinburgh, London. 290 p.
5. KOMBRINK, E., and SOMSSICH, I.E., 1995 Defense responses of plants to pathogens. Adv. Bot. Res. **21**:1-34.
6. MATEI, M.G., MATEI, S., 2010 The comparative structure of fungal communities in rhizosphere of soybean treated with chemical and biological agents for pathogens control. Res. J. Agr. Sci. 42(2):81-85.
7. MATEI, S., MATEI, G.M., CORNEA, P., POPA, G., VOAIDES, C., DRAGHICI, E.M., DOBRESCU, A., BADULESCU, L., 2010 Experimentally-induced plant defense response by “vaccination” with fungal elicitors. Ed. Sitech. Craiova. p.9-12.
8. SAMSON, A. R., HOEKSTRA, R.S.E., 1988 Introduction to food borne fungi. Ed. CBS Netherlands. p.1-209.
9. SHEN, D., 1997 Microbial diversity and application of microbial products for agricultural purposes in China. Agric. Ecosist. Environ. 62:237-245.
10. WALTERS, D.R., BOYLE, C., 2005 Induced resistance and allocation costs: what is the impact of pathogens challenge. Physiol. Mol. Plant Pathol. 68:40-44.