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Dedicated to Professor Maria Ławrynowicz on the occasion of the 45th anniversary of her scientific activity

Mycobiota of rape seeds in Romania. II. Evaluation of potential antagonistic fungi isolated from rape seeds against the main pathogens of rape crop

TATIANA-EUGENIA ŞESAN

Department of Botany and Microbiology, Biology Faculty
University of Bucharest, 1-3 Portocalelor Alley, RO-060101 Bucharest 35, tatianasesan@yahoo.com

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In vitro relationships between identified seed- and soil-borne fungi from rape samples have been investigated in order to evaluate their antagonistic ability as potential biocontrol agents. The bioproduct obtained from the *Trichoderma viride* Pers. (strain Td_{50}) has been tested *in vivo* against the main phytopathogens of rape: *Sclerotinia sclerotiorum* (Lib.) de Bary, *Botrytis cinerea* Pers., *Alternaria* spp. and *Fusarium* spp. in greenhouse at the Laboratory of Mycology and Plant Pathology, Biology Faculty, University of Bucharest – Romania and in the field at the Agricultural Experimental Research-Development Station Caracal (AERDS), Olt district. The *T. viride* (strain Td_{50}) bioproduct formulated as a powder for the seed treatment has been effective in the protection of rape plantlets against the above mentioned phytopathogens.

Key words: rape, seed- and soil-borne fungi, antagonistic fungi, biocontrol, *Trichoderma*, phytopathogens, *Sclerotinia sclerotiorum*, *Botrytis cinerea*, *Alternaria* spp., *Fusarium* spp., bioproduct from *T. viride* (strain Td_{50}), Romania

INTRODUCTION

In the frame of integrated protection of rape crop, aspects concerning chemical control of pathogens, pests and weeds have been published in Romania by different authors (Baicu, Săvescu 1986; Iliescu et al. 1987; Oancea 1998; Bîlteanu 2001; Diaconu, Mateiaş 2004; Hălmăjan 2006; Mantu 2007; Popov, Raranciuc 2007).

Also, some steps for diversification of the protection means have been recorded, among them the biological non-polluting ones using antagonistic fungi (Şesan 1992, 2001, 2005; Şesan, Groza 2007; Şesan 2008).

The objective of this approach was the evaluation of potential antagonistic ability of some *Trichoderma viride* isolates against the main pathogens of rape crop: *Sclerotinia sclerotiorum* (Lib.) de Bary, *Botrytis cinerea* Pers., *Alternaria* spp., *Fusarium* spp.

MATERIAL AND METHODS

The investigations for evaluation of potential antagonistic ability of some *T. viride* isolates against the main rape phytopathogens have been conducted *in vitro* and *in vivo* (greenhouse, experimental field).

In vitro method of double cultures on the same medium in Petri plates it was used, after Jouan et al. (1964), presented in the Figure 1 the antagonistic fungal ability has been expressed by the coefficient x, calculated after the formula x = iA/iB x eB/eA. In this formula the coefficient x is the value of the quotient of inner radius (i) and outer radius (e) of the test-fungus (A) and the antagonistic fungus (B). In case of x = 1, no influence has been expressed between the two tested fungi; when x < 1, the antagonism is stronger when the coefficient x value is lower or close to 0 (zero); when x > 1, the tested isolates prove no antagonism against the checked phytopathogens.

For *in vitro* experiments, fungal isolates of *Sclerotinia sclerotiorum*, *Botrytis cinerea*, *Fusarium* spp., *Alternaria* spp. and *Trichoderma* spp. from rape seeds have been used. As antagonistic fungi, 5 strains of *Trichoderma viride* (Td_5, Td_{35}, Td_{45},Td_{49}, Td_{50}), isolated by the author, have been tested.

The bioproduct from *Trichoderma viride* - isolateTd_{50} - (Şesan, Oancea 2010; Şesan et al. 2012) has been also, tested in greenhouse at the University of Bucharest, Laboratory of Mycology and Plant Pathology for the treatment of rape seeds in comparaison with an untreated control and with a chemical standard – procymidone 50. The experiment has been performed in sterile soil, in Petri plates of 12 cm diameter, each variant having 5 replicates. A number of 10 rape seeds Hydromel cv. (from AERDS Caracal, Olt district, 2007) have been used for each replicate. The application dose for biological seed treatment was 1g powder of *T. viride* bioproduct per kg seeds.

In the field at AERDS Caracal, Olt district, the experimental variants were as follows: 1) seeds treated with Trichosemin 25 PTS – 1 g/kg seed; 2) seeds treated with a chemical standard (procymidone 50) – 1 g/kg seed and 3) untreated control. The Manitoba cv. was cultivated.

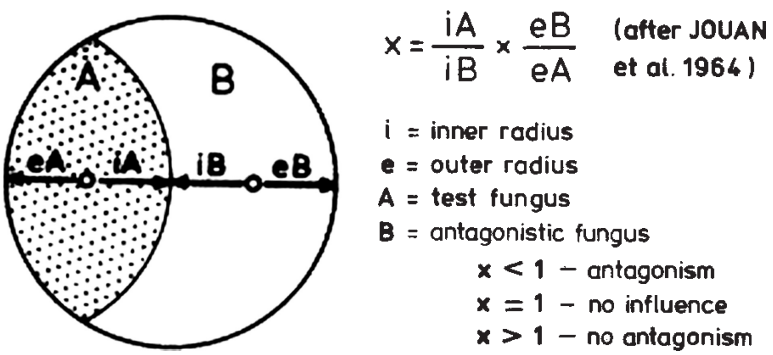

Fig. 1. Method of evaluation of the relationships between potential antagonistic fungi and fungal phytopathogens (Jouan et al. 1964).

In this experiment, evaluation of number and percent of emerging healthy plantlets in comparaison with the same parameter in the standard variant (chemical fungicide procymidone 50) and in the untreated control was performed.

RESULTS AND DISCUSSION

In vitro. Among the tested isolates (Tab. 1), the strain Td_{35} proved the strongest antagonism against tested phytopathogens, the value of x coefficient ranging between 0.22 and 0.44 (Fig. 2).

Table 1
Evaluation of the antagonistic activity of *Trichoderma viride* strains based on x coefficient (after Jouan et al. 1964)

Trichoderma viride	*Fusarium* sp.	*Alternaria alternata*	*Botrytis cinerea*	*Sclerotinia sclerotiorum*
Td_{35}	0.25	0.40	0.44	0.22
Td_{45}	0.78	0.86	0.70	0.48
Td_{49}	0.28	0.90	0.62	0.76
Td_{50}	0.30	0.42	0.35	0.54
Td_5 (control)	0.55	0.54	0.89	0.45

In the decreasing order, a good antagonistic ability have been proved for strains Td_{50} (x = 0.30-0.70) and Td_{49} (x = 0.30-0.89). The lowest antagonistic activity has been noticed for the isolates Td_{45}, with x coefficient between 0.48 and 0.86 and Td_5 (control), with x coefficient between 0.45-0. 89.

The antagonistic behaviour of the tested isolates is shown by the following decreasing order: $Td_{35} > Td_{49} > Td_{50} > Td_{45} > Td_5$.

In vivo. The experiments performed under greenhouse conditions (Tab. 2, Fig. 3) proved that the percent of emerging healthy plantlets, in comparison with the untreated control, was higher by 47%.

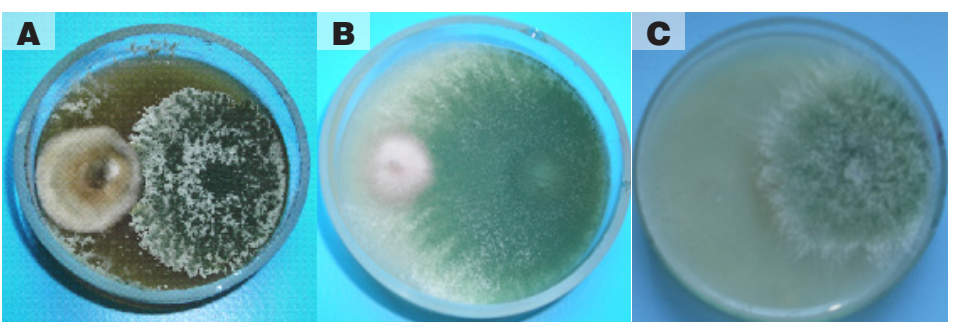

Fig. 2. Double cultures obtained by Jouan et al. (1964) method, with phytopathogens *Botrytis cinerea* (A), *Fusarium* sp. (B), *Sclerotinia sclerotiorum* (C), as test-fungi and *Trichoderma viride*, strain Td_{50}, as antagonistic fungus.

Table 2
Testing the *Trichoderma viride* (Td_{50}) bioproduct for protecting rape crop cv. Hydromel against soil- and seed-borne pathogenic fungi in greenhouse (2007 yield)

Variant	Emergence of healthy plants after 8 days		Difference in comparaison with control (%)
	No. Seedlings	% seedlings	%seedlings
1. *T. viride* (Td_{50}) bioproduct – 1 g/kg seed	100	147	+47
2. Chemical standard (procymidone 50) – 1g/kg seed	90	130	+30
3. Control (untreated)	70	100	

In the standard variant (procymidone 50), the value of the emerging and healthy rape seedlings was higher by 30% in comparaison with the untreated control.

Under the field conditions, in the variant with biological seed treatment, the percentage of emerging helthy plantlets was higher by 26% in comparaison with the untreated control and similar to the variant of the chemical standard (Tab. 3). However, after 14 days (29th August 2008), no differences have been registered between the biological and chemical treatments.

These results obtained in the rape crop were similar to other experimental results obtained by us for other industrial oilcrops (sunflower, soybean) and annual pulses (bean, soybean, cickpea) (Baicu, Săvescu 1986; Şesan et al. 1997 a, b) and to our previous results in the rape crop (Galani et al. 2008). Also, these results confirm our tests *in vivo* proving the efficacy of our bioproduct with *T. viride* (Td_{50}), patented in 2012 (Şesan et al. 2012) for protecting oilseed plants among them rape (Şesan, Oancea 2010).

In order to obtain a healthy, non-polluted and productive rape crop, the instructions for the application of biological control of rape seeds with the bioproduct based on *T. viride* were prepared. These instructions consist of: 1) seed treatment: dry; 2) dose of treatment: 1-2 g/kg seeds; 3) time of treatment application: 1-2 days before sowing; 4) storage conditions for the bioproduct: in the dry, well-aired spaces, with a good ventilation, at low temperatures, in the shadow, avoiding

Untreated control | Bioproduct from *Trichoderma viride* (Td_{50}) - 1 g/kg seed | Chemical standard (procymidone 50) - 1 g/kg seed

Fig. 3. Testing the protective activity *Trichoderma viride* bioproduct (Td_{50}).

Table 3
Testing *Trichoderma viride* (Td_{50}) bioproduct for protecting rape plants (Manitoba cv.) against seed- and soil-borne pathogens under the field conditions
AERDS Caracal – Olt District - 2008

Variant	Emergence of healthy plants after 8 days		Difference to the control (%)	
	No. plantlets	%	No.	%
1. *T. viride* bioproduct – 2 g/kg seed	86	126	+18	+26
2. Procymidone 50 (chemical standard) – 2 g/kg seed	88	129	+ 20	+ 29
3. Untreated (control)	68	100		

the direct; 5) proper conditions for bioproduct transport, protected against high temperatures and humidity. These instructions are very important for the agricultural practice.

CONCLUSIONS

1. The antagonistic ability of 5 *Trichoderma viride* strains (Td_5, Td_{35}, Td_{45},Td_{49}, Td_{50}) against 4 species of phytopathogens isolated from rape seeds (*Fusarium* sp., *Botrytis cinerea*, *Alternaria alternata*, *Sclerotinia sclerotiorum*) was evaluated *in vitro*. The antagonistic ability of strains was evaluated in decreasing order - as: $Td_{35} > Td_{49} > Td_{50} > Td_{45} > Td_{5.}$

2. Bioproduct with *Trichoderma viride* – Td_{50} strain – was efficient in the rape protection against the seed- and soil-borne pathogens (*Sclerotinia sclerotiorum*, *Botrytis cinerea*, *Fusarium* spp., *Alternaria* spp.) in the greenhouse (Hydromel cv.) and in the field (Manitoba cv.), too. Applied as seed treatment at a rate of 1-2 g/kg seeds, *T. viride* bioproduct stimulated the emergence of plantlets and their health status.

3. The instructions for the application of biological control of rape seeds with the bioproduct based on *T. viride* were proposed: 1) seed treatment: dry; 2) dose of treatment: 1-2 g/kg seeds; 3) time of treatment application: 1-2 days before sowing; 4) storage conditions for the bioproduct: in the dry, well-aired spaces, at low temperatures, in the shadow, avoiding the direct; 5) proper conditions for bioproduct transport, protected against high temperatures and humidity. These instructions are very important for the agricultural practice for obtaining a healthy, productive, non-polluted with chemicals rape crop.

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