BIOLOGICAL CONTROL OF BROWN COLLAR ROT (*RHIZOCTONIA SOLANI* KÜHN) IN ANNUAL PULSES

Tatiana Eugenia Șesan[1)], Tudorel Baicu and Constantin Gogoașă[2)]

ABSTRACT

Rhizoctonia solani **induces brown collar rot in many cropped plants among which annual pulses are often affected. Several *Trichoderma viride* isolates were tested against *R. solani* in order to find some non-chemical means to control *Rhizoctonia*-disease in bean. A number of 16 isolates proved to be highly antagonistic to this pathogen. At Research Institute for Plant Protection Bucharest (RIPP), application of the fungus *T. viride* (isolate Td 50) to soil (10-15 g/kg soil) and seed (2-4 g/kg dry biomass) and of 7 mutants originating from the isolate Td 5 (Td C, Td E, Td I, Td K, Td M, Td N) as seed dressing (2-4 g/kg dry biomass) provided protection of bean seedling Progres cv. from incidence and evolution of disease in greenhouse. Percentages of healthy plants recorded in the entries with biological treatments were comparable to those in standard entries (Methylthyophanate 3 g/kg, AC 8-2 g/kg). The best efficacy was shown by the mutant Td K, followed by Td N, Td M, Td J, Td I under greenhouse conditions (RIPP Bucharest) but also in field (Uzlina - Tulcea). Seed dressing of beans with a product based on *T. viride* has been included as biological method to develop an Integrated Management System.**

Key words. beans, biological treatments, *Rhizoctonia solani*, seed dressing, soil treatment, *Trichoderma viride*.

INTRODUCTION

Rhizoctonia solani Kühn, a cosmopolitan polyvorous parasite, induces root and collar rot in annual pulses. It was first recorded in Romania in greenhouse beans (Stan and Raicu, 1973) and later the disease was frequently identified on bean (Șesan and Dumitraș, 1979; Șesan, 1981/1983, 1982; Șesan and Ciurdărescu, 1982), peas (Șesan and Ioniță, 1983) (Figure 1), chickpea (Șesan and Procopovici, 1995).

Figure 1. Spreading of *Rhizoctonia solani* on bean and pea seeds in Romania

Starting from progress known in the world (Boosalis, 1956; Papavizas, 1986 and others), several studies on the antagonistic capacity of some saprophytic fungi, particularly *Trichoderma viride* Pers. ex S. F. Gray, in order to select biological agents for bean protection from *R. solani* have been performed in Romania since 1980 (Dumitraș and Șesan, 1980; Șesan, 1981/1983, 1985 a, b). The purpose of these investigations was to select biological agents for bean protection against *R. solani* and to include the biological control means (antagonistic fungi) in an integrated protection system of pulse crops, in general, and bean crop, in particular (Baicu et al., 1988, 1989).

MATERIALS AND METHODS

The material used consisted of test-fungus *R. solani* isolated from bean seeds and cultivated on PDA for 7-10 days. A number of 16 isolates of saprophytic fungi, belonging to 7 species were collected from various substrata and grown on PDA for 7-10 days (Table 1). *T. viride* mutants (Td A - Td O), resistant to methlythyophanate and TMTD were previously obtained from standard isolate Td 5 (Șesan and Baicu, 1989). Tests were performed on two bean varieties, Progres and Magna. The fungicide AC-8 (Prosemin) (methlythyophanate 25% + thiuram 50% + chloramphenicol 0.6%), developed at RIPP-Bucharest, applied at a rate of 2 g/kg seed was used as standard.

In vitro tests were performed using the method of double culture on PDA medium to establish the relationship of the saprophytic fungi and *T. viride* mutants with *R. solani*. Scores were expressed by the "index x", calculated after Jouan et al. (1964).

In vivo tests were performed in ICPP

[1)] Research Institute for Plant Protection - Bucharest, Romania
[2)] Research Institute "Delta Dunării" - Tulcea, Romania

greenhouse in 1988, and in experimental field at Uzlina-Tulcea in randomized complete block design with 4 replications and plots of 10 m^2, in 1987-1988.

Seed treatments consisted of a mixture of dry biomass from *T. viride* isolates and mutants at rates of 2-4 g/kg and carboxymethyl cellulose 0.5% as adhesive.

Soil treatment was made by mixing homogeneously a glass vial with *T. viride* culture of 7-10 days with one kg of soil (about 10-15 g culture/kg of soil).

Efficacy assessment included plant emergence (percentages of healthy and attacked plants), plant height in cm (greenhouse) and grain yield (field), compared to data obtained in untreated check and chemical standard AC-8 (Prosemin) - 0.2%.

ANOVA was computed to estimate the effect of biological treatments and significance of differences.

RESULTS AND DISCUSSIONS

Among the 16 isolates of the saprophytic fungi tested for antagonism to *R. solani* isolated from beans (Table 1), only *T. viride* isolates exhibited antagonism to various extents (Td 50, Td, 5, Td 23, Td 49).

Table 1. In vitro relationship between some saprophytic fungi and *Rhizoctonia solani* from bean estimated by "x" coefficients (after Jouan et al., 1964)

Saprophytic fungi	Isolates	"x" coefficient	Relationship[1]
Trichoderma viride	Td 5	0.520	A
	Td 23	0.550	A
	Td 49	0.700	A
	Td 50	0.380	HA
Gliocladium roseum	Gl 1	1.200	NA
	Gl 2	1.300	NA
Epicoccum purpurascens	Ep 1	1.300	NA
	Ep 2	1.500	NA
	Ep 3	1.700	NA
Aspergillus clavatus	Asp.	1.800	NA
Coniothyrium minitans	C.m.	0.880	SA
	C 15*	0.900	SA
	C 18*	1.140	NA
	IVT 1*	1.220	NA
Ulocladium chartarum	Ulocl.	0.910	SA
Sordaria fimicola	Sord.	1.890	NA
CHECK	-	1.000	-

1) = A - antagonism; HA = heavy antagonism;
SA = slight antagonism; NA = no antagonism;
* = isolates from dr. M. Gherlagh (IPO-DLO Wageningen - The Netherlands)

Two *Conyothyrium minitants* Campbel isolates (C.m. and C 15) and one *Ulocladium chartarum* (Preus) Simmons (Ulocl.) isolate were slightly antagonistic. Other fungi (*Gliocladium roseum* Bainier, *Epicoccum purpurascens* Ehrenb. ex Schlecht., *Aspergillus clavatus* Desmazieres, *Sordaria fimicola* (Rib.) Ces. et De Not.), and other two *C. minitans* isolates (C 18, IVT 1) did not prove to be antagonistic to *R. solani.*

Among the 15 *T. viride* mutants obtained from the isolate Td 5, resistant to methylthyophanate and TMTD, a number of five (Td N, Td M, Td K, Td J, and Td I) were more antagonistic to *R. solani* than the isolate of origin Td 5, while the other were less active (Table 2).

Table 2. In vitro antagonism of *Trichoderma viride* mutants versus *Rhizoctonia solani*

Trichoderma viride mutants	"x" coefficient
Td A	0.740
Td B	0.780
Td C	0.740
Td D	0.720
Td E	0.580
Td F	0.760
Td G	0.720
Td H	0.620
Td I	0.440
Td J	0.420
Td K	0.360
Td L	0.720
Td M	0.320
Td N	0.290
Td O	0.540
Td 5 (standard)	0.520

In greenhouse tests (Table 3) good results were obtained by soil treatment with Td 50, the percentage of healthy plants after 26 days being 25 fold higher than in the infected, untreated check, and 7 fold lower than the same check, when Td 50 was applied to seed. In both entries with biological treatments, fair plant development was noted.

Seed treatment with Td 50 resulted in high percentages of healthy plants only in the first 10 days after sowing, the effect of biological treatment decreasing subsequently.

The biological treatment with Td 50 to soil proved to be more efficient, its effect increasing, as compared to untreated check, from 116 % after 11 days to 125% when scored after 25 days. The higher amount of

inoculum required by soil treatment created more suitable conditions for inoculation with the antagonists and led to a better effect in controlling *R. solani*, than seed application which, seemingly, provided an insufficient inoculation with saprophytic fungi.

Table 3. Prevention of *Rhizoctonia solani* in bean (Progres cv.) by biological treatments with *Trichoderma viride* under greenhouse conditions

Entry	% healthy plants after			Plant height (cm)
	11 days	15 days	25 days	
Methylthyophanat 3 g/kg seed	71***	80***	87***	15.5
Td 50 2 g/kg seed	70***	73	60	22.0
Td 50 10-15 g/kg soil	58**	76**	79***	21.0
CHECK (soil artificially inoculated)	50	71	63	8.5

,* - significant for P<0.01 and P<0.001, respectively

Greenhouse trials with *T. viride* mutants (Table 4) demonstrated good emergence of seedling in the entries with mutants Td N and Td M (74-85%), and with the isolate Td 5 (72%), close to the standard product AC-8 (Prosemin), and respectively a lower frequency of plants attacked (15-28%). Efficacy of both mutants Td N (76%) and Td M (58%) was higher than that of the standard isolate Td 5 (55%). The other mutants yielded lower efficacy (45-49%) than the isolate Td 5 (55%). In a single circumstance the mutant Td N gave a superior efficacy to the chemical standard AC-8 (Prosemin) (65%).

Table 4. Efficacy of seed treatments with *Trichoderma viride* mutants under prevention of *Rhizoctonia solani* in bean (Magna cv.) under greenhouse conditions – 1988

Entry	Rate (g/kg seed)	Healthy plants emerged (%)	Frequency of attacked plants (%)	Efficacy (%)	Plants height (cm)
Td C	4	66	34	45	9.5
Td I	4	68	32	48	14.2
Td J	4	69	31	49	19.1
Td K	4	71	29	48	17.2
Td M	4	74	26	58	19.6
Td N	4	85	15	76	18.9
Td 5 standard	4	72	28	55	18.9
AC-8 standard	2	78	22	65	20.7
CHECK	-	38	62	-	9.7

In field (Tables 5 and 6) good seedling emergence was recorded in mutant isolates, frequently higher or equal to the isolate Td 5, and in all cases superior to the untreated check; the attack level before harvest was similar in all entries with *T. viride* mutants, as reflected by a relatively good efficacy (between 54 and 67% in 1987 and 50-61% in 1988).

Grain yield obtained in the entries with *T. viride* mutants was in all cases higher than untreated check, in 1988 being statistically significant.

The higher yield of standard AC-8 (Prosemin) was probably due not only to its fungicide action, but also to its bactericidal effect.

Good results from greenhouse and field suggest the possibility of the utilization of

Table 5. Efficacy of seed treatments with *Trichoderma viride* mutants in prevention of seed-borne pathogens (including *Rhizoctonia solani)* in bean (Magna cv.) under field conditions at Uzlina-Tulcea, 1987

Entry	Rate (g/kg seed)	Healthy plants emerged (%)	Frequency of attacked plants (%)	Attack degree before harvest (%)	Efficacy %	Yield (t/ha)
Td E	4	89	11	23	56	1.76
Td K	4	88	9	17	67	1.87*
Td I	4	89	9	18	65	1.79
Td M	4	90	12	24	54	1.66
Td N	4	87	11	21	60	1.65
Td 5 standard	4	88	7	17	67	1.98**
AC-8 standard	2	89	6	13	75	2.07**
CHECK	-	86	17	52	-	1.56

*, ** - significant for P<0.05 and P<0.01, respectively

Table 6. Efficacy of seed treatments with *Trichoderma viride* mutants in prevention of seed-borne pathogens (including *Rhizoctonia solani*) in bean (Magna cv.) under field conditions at Uzlina- Tulcea district, 1988

Entry	Rate (g/kg seed)	Healthy plants emerged (%)	Frequency of attacked plants (%)	Attack degree before harvest (%)	Efficacy %	Yield (t/ha)
Td E	4	92	13	21	52	2.01***
Td K	4	85	10	18	59	2.55***
Td I	4	92	8	17	61	2.51***
Td M	4	84	14	22	50	1.80***
Td N	4	86	13	22	50	2.05***
Td 5 standard	4	88	15	20	55	2.27***
AC-8 standard	2	90	8	15	66	2.68***
CHECK	-	59	25	44	-	1.32

*** - significant for P<0.001

T. viride mutant isolates as a technological element in a system of integrated protection of bean crop, particularly in the protected zone of the Danube Delta (Baicu et al., 1988, 1989).

CONCLUSIONS

T. viride isolates showed antagonism to *R. solani* on bean; two *C. minitans* isolates (C.m., C 15), and the isolate of *U. chartarum* were merely slightly antagonistic to the pathogen under study. The other saprophytic fungi tested (*G. roseum, E. purpurascens, A. clavatus, S. fimicola*) had not biological inhibitory activity against *R. solani.*

Td N, Td M, Td K, Td J and Td I mutant isolates of *T. viride* were significantly more antagonistic to *R. solani*, as compared to the isolate Td 5, which they originated from.

Efficacy of biological treatment with *T. viride* (Td 50) applied to seed and soil in greenhouse was in favour of soil application, which provided protection to bean plants against *R. solani* throughout the whole vegetation period, whereas the seed treatment was efficient only during the first 10 days after application.

Bean seed treatment with *T. viride* mutants (4 g/kg) demonstrated efficacy, the most active mutants in greenhouse being Td N and Td M, as well as the parental isolate Td 5.

Bean seed treatment with *T. viride* mutants provided good protection from seed pathogens, including *R. solani* in field as reflected by a low attack level before harvest and grain yield gains, statistically significant.

Efficacy of biological applications with *T. viride* to seed and soil did not generally overpass the chemical treatments, but the advantage of being non-polluting recommended them for crop protection. The biological treatment with *T. viride* could constitute an efficient measure to be included in a system of integrated disease control of bean crops, ensuring protection particularly in the early phases of plant development emergence and seedling. This type of treatment is a non-polluting alternative, highly advocated for agricultural crops in protected areas, such as the Danube Delta.

REFERENCES

Baicu, T., Gogoașă, C., Șesan T., 1988.Complex de măsuri pentru combaterea bolilor și dăunătorilor fasolei și soiei. Probl. Prot. Plant., XVI(3): 195-209.

Baicu, T., Șesan T., Gogoașă, C., 1989. Eficacitatea unor izolate și mutante de *Trichoderma viride,* rezistente la fungicide, în combaterea biologică a unor ciuperci fitopatogene. Probl. Prot. Plant., XVII(3): 205-218.

Boosalis, M.G., 1956. Effect of soil temperature and green manure amendment of unsterilized soil on parasitism of *Rhizoctonia solani* by *Penicillium vermiculatum* and *Trichoderma* sp. Phytopathology, 46(9): 473-478.

Dumitraș L., Șesan T., 1980. Aspecte privind antagonismul ciupercii *Trichoderma viride* Pers. ex Fr. față de *Rhizoctonia solani* Kühn. St. Cerc. Biol., Biol. Veget., 32(1): 87-92.

Jouan, B., Lemaire, J.M., Arnoux, L., 1964. Éléments d'appréciation des interactions entre champignons cultivés *in vitro*. Phytiatrie-Phytopharmacie, 13: 185-195.

Papavizaz, G.C., 1985. *Trichoderma* and *Gliocladium*: biology, ecology and potential for biocontrol. Annu. Rev. Phytopathol., 23: 23-54.

Stan, G., Raicu, C., 1973. *Rhizoctonia solani* Kühn la fasole. Rev. Hort. Vitic., 22(10): 80-86.

Șesan, T., 1981, 1983. Tratarea semințelor - element de integrare în combaterea bolilor fasolei. II. Folosirea ciupercii *Trichoderma viride* Pers. ex Fr. în combaterea unor ciuperci ce se transmit prin sămânță și sol. St. Com. Șt., Inst. Inv. Sup. Bacău: 239-254.

Şesan, T., 1982. Ciuperci parazite pe sămânţă de fasole şi combaterea lor. Red. Prop. Tehn. Agr. Buc., 38 pg., 15 figs.

Şesan, T., 1985 a. Studiul biologic al principalelor specii de ciuperci antagoniste faţă de unii patogeni ce produc micoze la plante. Ph. D. Thesis, Buc., 189 pg., 46 figs.

Şesan, T., 1985 b. Cercetări privind prevenirea agenţilor patogeni ai seminţelor şi plantulelor leguminoase pentru boabe prin mijloace biologice. Lucr. celei de a IX-a Conf. Naţ. Prot. Plant., Buc., 5-6 Sept. 1985: 213-224.

Şesan, T., Baicu, T., 1989. Selectarea de mutante de *Trichoderma viride* Pers. ex.S.F. Gray, rezistente la fungicide, antagoniste faţă de diferite ciuperci fitopagene. St. Cerc. Biol., Biol. Veget., 41(2): 155-164.

Şesan, T., Ciurdărescu, G., 1982. Răspândirea unor agenţi patogeni care au atacat seminţele de fasole în anii 1978-1980. Analele ICCPT-Fundulea, XLIX: 205-212.

Şesan, T., Dumitraş, L., 1979. Efectul unor fungicide asupra unor ciuperci ale seminţei şi asupra formării nodozităţilor la fasole. Analele ICPP Buc., XV: 95-101.

Şesan, T., Ioniţă, A., 1983. Micoflora seminţelor de mazăre cultivată în R.S.România. Analele ICPP Buc., XVII: 57-63.

Şesan, T., Procopovic,i E., 1996. Micoflora seminţelor de năut (*Cicer arietinum* L.) din România. Analele ICPP Buc., XXVII: 45-57.

Table 1. *In vitro* relationship between some saprophytic fungi and *Rhizoctonia solani* from bean estimated by "x" coefficients (after Jouan et al., 1964).

Saprophytic fungi	Isolates	"x" coefficient	Relationship[1]
Trichoderma viride	Td 5	0.520	A
	Td 23	0.550	A
	Td 49	0.700	A
	Td 50	0.380	HA
Gliocladium roseum	Gl 1	1.200	NA
	Gl 2	1.300	NA
Epicoccum purpurascens	Ep 1	1.300	NA
	Ep 2	1.500	NA
	Ep 3	1.700	NA
Aspergillus clavatus	Asp.	1.800	NA
Coniothyrium minitans	C.m.	0.880	SA
	C 15*	0.900	SA
	C 18*	1.140	NA
	IVT 1*	1.220	NA
Ulocladium chartarum	Ulocl.	0.910	SA
Sordaria fimicola	Sord.	1.890	NA
CHECK	-	1.000	-

1) A - antagonism; HA = heavy antagonism;

SA = slight antagonism; NA = no antagonism

* = isolates from dr. M. Gherlagh (IPO-DLO Wageningen - The Netherlands)

Table 2. *In vitro* antagonism of *Trichoderme viride* mutants versus *Rhizoctonia solani*

Trichoderma viride mutants	''x'' coefficient
Td A	0 740
Td B	0 780
Td C	0 740
Td D	0 720
Td E	0 580
Td F	0 760
Td G	0 720
Td H	0 620
Td I	0 440
Td J	0 420
Td K	0 360
Td L	0 720
Td M	0 320
Td N	0 290
Td O	0 540
Td 5 (standard)	0 520

Table 3. Prevention of *Rhizoctonia solani* in bean (Progres cv.) by biological treatments with *Trichoderma viride* in greenhouse conditions.

Entry	% healthy plants after			Plant height (cm)
	11 days	15 days	25 days	
Thiophanate methyl 3 g/kg seed	71***	80***	87***	15.5
Td 50 2 g/kg seed	70***	73	60	22.0
Td 50 10-15 g/kg soil	58**	76**	79***	21.0
CHECK (soil artificially inoculated)	50	71	63	8.5

,* - significant for P<0.01 and P<0.001, respectively

Table 4. Efficacy of seed treatments with *Trichoderma viride* mutants in prevention of *Rhizoctonia solani* in bean (Magna cv.) in greenhouse conditions - 1988.

Entry	Rate (g/kg seed)	Healthy plants emerged (%)	Frequency of attacked plants (%)	Efficacy (%)	Plants height (cm)
Td C	4	66	34	45	9.5
Td I	4	68	32	48	14.2
Td J	4	69	31	49	19.1
Td K	4	71	29	48	17.2
Td M	4	74	26	58	19.6
Td N	4	85	15	76	18.9
Td 5 standard	4	72	28	55	18.9
AC-8 standard	2	78	22	65	20.7
CHECK	-	38	62	-	9.7

Table 5. Efficacy of seed treatments with *Trichoderma viride* mutants in prevention of seed-born pathogens (including *Rhizoctonia solani)* in bean (Magna cv.) in field conditions at Uzlina-Tulcea, 1987.

Entry	Rate (g/kg seed)	Healthy plants emerged (%)	Frequency of attacked plants (%)	Attack degree before harvest	Efficacy %	Yield (t/ha)
Td E	4	89	11	23	56	1.76
Td K	4	88	9	17	67	1.87*
Td I	4	89	9	18	65	1.79
Td M	4	90	12	24	54	1.66
Td N	4	87	11	21	60	1.65
Td 5 standard	4	88	7	17	67	1.98**

AC-8 standard	2	89	6	13	75	2.07**
CHECK	-	86	17	52	-	1.56

*, ** - significant for P<0.05 and P<0.01, respectively.

Table 6. Efficacy of seed treatments with *Trichoderma viride* mutants in prevention of seed-born pathogens (including *Rhizoctonia solani*) in bean (Magna cv.) in field conditions at Uzlina- Tulcea district, 1988.

Entry	Rate (g/kg seed)	Healthy plants emerged (%)	Frequency of attacked plants (%)	Attack degree before harvest	Efficacy %	Yield (t/ha)
Td E	4	92	13	21	52	2.01***
Td K	4	85	10	18	59	2.55***
Td I	4	92	8	17	61	2.51***
Td M	4	84	14	22	50	1.80***
Td N	4	86	13	22	50	2.05***
Td 5 standard	4	88	15	20	55	2.27***
AC-8 standard	2	90	8	15	66	2.68***
CHECK	-	59	25	44	-	1.32

*** - significant for P<0.001