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CONIOTHYRIUM MINITANS – BIOCONTROL AGENT AGAINST SCLEROTIUM-FORMING PLANT PATHOGENS

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Abstract: The mycoparasitic fungus *Coniothyrium minitans,* recorded for the first time in the world from California – USA (Campbell, 1947) and in Europe from the Great Britain (Tribe, 1957), is one of the most interesting subject of the biological and agricultural research due to the hyperparasitic action against sclerotium-forming plant pathogens, among them *Sclerotinia* spp., very dangerous and difficult to be controlled for many cultivated plants.

In Romania, this fungus has been recorded by Aurelia Crişan (1986). Then the bio-ecology of the fungus (Şesan, Crişan, 1988, 1998; Şesan, Baicu, 1993a) and the ways of using it as a biocontrol agent for plant protection (Şesan, Csép, 1991-1996; Şesan, Baicu, 1993b, Şesan and colab., 1997) have been studied.

It has been reviewed the results concerning the efficacy of bioproducts based on C. *minitans* under different climatic conditions in Romania in order to protect industrial and medicinal plants as well as pulses against white rot (*Sclerotinia sclerotiorum*), researches with practical importance in sustainable development of agroecosystems, results connected at the same time with the similar ones performed in the world.

Introduction

An increased interest in the study of biological control agents (BCAs) used in plant protection, among them with a particular place being *Coniothyrium minitans* Campbell, a hyperparasitic fungus specific to sclerotial parasites, mainly against *Sclerotinia sclerotiorum* (Lib.) de Bary, conducted to important investigations in this field (Şesan, 1989; Whipps, Gerlagh, 1992, 1995).

In this paper the most important data on the taxonomy and *in vitro* and *in vivo* biology of *C. minitans* have been reviewed:

a) Taxonomy and phylogeny of *C. minitans*;
b) Growth and sporulation on natural and artificial culture media; description of *C. minitans* pycnidia and pycnospores on different culture media; *C. minitans* mode of action against host pathogens;
c) Growth and sporulation on the potato-dextrose-agar medium (PDA) with different initial pH values;
d) *In vitro* relationships between *C. minitans* and sclerotium-forming fungi;
e) Testing of the efficacy of *C. minitans* in plant protection under the different climatic and field conditions in Romania;
f) The *in vitro* influence of pesticides on the growth and sporulation of the fungus, and evaluating of the chemicals' selectivity of *C. minitans* biocontrol agent.

Material and Methods

The biological material used was a *C. minitans* strain, isolated by A. Crişan in 1986 [28] and strains of sclerotium-forming fungi (*Sclerotinia sclerotiorum*, *S. minor* Jagger, *Macrophomina phaseolina* (Tassi) Goid., syn. *Sclerotium bataticola* Taubenh., *Botrytis cinerea* Pers. from the Mycology Laboratory of the Research-Development Institute for Plant Protection Bucharest, isolated by Şesan).

For obtaining the isolates of *C. minitans* several methods have been used: the classical method of isolation fungi from soil in dilution plates, the method of plant tissue segments and the method of infected sclerotia (Whipps, 1987; Whipps, Gerlagh, 1992; Gerlagh et al., 1994, 1996; Şesan, 1993).

A number of 17 culture media were tested, and among these the PDA medium with 10 different initial values, ranging between 4.0 and 13.00, aiming a determining the culture parameters of this fungus for its mass multiplication. The evaluation of culture media and pH values has done by measuring the diameter of the colonies until they completely covered the surface of the culture medium in Petri dishes in the most favourable variant. The results have been compared with the check (water-agar medium). Sporulation has been appreciated after observations under the microscope.

In order to reveal *in vitro* relationships between *C. minitans* and the test-sclerotial plant pathogenic fungi, the method of double culture has been used (Jouan et al., 1964). Scoring was done by calculating the ratio **x** between the inner (i) and outer (e) radius of the test-fungus (A) and the hyperparasite *C. minitans* (B), with the formula: x = iA/iB x eB/eA. Evaluation of activity has been done based of the x values: x <1 – antagonism; x = 1 – no influence; x >1 - no antagonism.

For testing the efficacy of *C. minitans* treatments in protecting sunflower, soybean and bean, randomized field trials have been performed during 1993-1998 in different experimental research units: Agricultural Experimental Research Station (AERS) Oradea-Bihor District, Research Institute for Cereals and Industrial Plants (RICIP) Fundulea-Călăraşi District, Research Station of Irrigated Crops (RSIC) Valul lui Traian-Constanţa District.

Cultivars and hybrids used in field experiments were: Decor cvar., Florom 328 hybrid and genotype L.C. of sunflower; soybean Diamant and Hodgson cvars. and bean Avans cvar.

The experimental variables consisted of treatments with *C. minitans* (C.m.) grown on PDA solid medium applied at a rate of 250 g/m^2 to soil, in planting pits at sowing (April).

Efficacy of biological treatment was compared to that of chemical fungicides Sumilex 50 WP (1 g/kg seed), Galben M (4 g/kg seed), Fundazol 50WP (2 g/kg seed), Tiramet 60PTS (2 g/kg seed), used as standards, as well as to untreated check.

Frequency (F%) of diseases plants and yield (kg/ha) were recorded and data were analyzed using Abbot's formula. Frequency of healthy plants and diseased plants and seed yield were examined by ANOVA programme.

The *in vitro* action of 36 pesticides, 12 fungicidal mixtures, 4 insecticidal mixtures, 8 insecto-fungicidal mixtures, 12 herbicides from various chemical groups (Tables 4-7) has been tested against *C. minitans*. The method was inclusion of the products in the nutritive PDA medium at the rate recommended by the *Codex of plant protection products registered for use in Romania* (2004) in three succesive halved dilutions. Observations consisted in measuring the diameter of the test fungal colonies until they completely covered the surface of the culture medium of Petri dishes in the check variant (without pesticides).

Rating has been performed by calculation of inhibition (I%), lethal concentrations (LC 50 mg/l active ingredient and LC 90 mg/l a.i.), regression line equations (RLE) and correlation coefficients (Ccf) by the method of dose-logarithm, mortality-probit.

The used abbreviations for the biological activity of chemicals were: strongly inhibitory or toxic (T), moderately inhibitory (MT) and slightly inhibitory (ST) or non-toxic.

All *in vitro* tests have been organized in variants with 4 replications each, data being treated by analysis of variance.

Results and Discussion

a) Taxonomy and phylogeny of *C. minitans*.

The first description of the fungus belonging to Campbell (1947) was included by Domsch et al. (1980) and by Punithalingam (1982).

This fungus has been classified in the ***Deuteromycetes*** group (*Fungi Imperfecti*), subclass *Coelomycetes*, ord. *Sphaeropsidales*, *Sphaeropsidaceae* family.

After „*The Dictionary of fungi*", 9th edition (Kirk et al., 2001), *C. minitans* is included in the family *Leptosphaeriaceae*, ord. *Pleosporales*, subclass *Dothideomycetidae*, class *Ascomycetes*, phyllum *Ascomycota*, kingdom *Fungi.*

In Romania, *C. minitans* was isolated by Aurelia Crişan in 1986 from the *S. sclerotiorum* sclerotia on carrots in Sic - Cluj District, and published by Şesan, Crişan (1988). This paper was cited in the most recent edition of the Dictionary of Fungi, 9th edition (Kirk et al., 2001, p. 126).

Coniothyrium Corda (1840) nom. cons., anamorphic *Leptosphaeria*, Cpd.0-1eP.19. 25, widespread. See Biga *et al.* (*Sydowia* **12**: 258, 1959), Sutton (*Mycol. Pap.* **123**, 1971; relationship to *Microsphaeropsis*). *C. wernsdorffiae* (brand canker of rose; Westcott, *Mem. Cornell agric. Exp. Stn* **153**, 1934). *C. minitans* (myco-parasite of sclerotia; Sesan, *Probl. Prot. Plant.* **17**: 29, 1989; Sesan & Crisan, *Stud. Cercet. Biol.* **40**: 71, 1988; Sandys-Winsch *et al.*, *MR* **97**: 1175, 1993; world dis-trib.).

Recently, Verkley et al. (2004) have presented their taxonomical investigations and considerations on *Coniothyrium*-like coelomycetes, with their formal descriptions based on anamorphic characters and with unknown teleomorphs up to the present. Their parsimony analysis of ITS (internal transcribed spacer) region of nuclear ribozomal DNA and partial SSU of the nr DNA sequences (nuclear ribosomal DNA sequences) confirmed that they belong in the order *Pleosporales* (subclass *Dothideomycetidae*, class ***Ascomycetes***) and group in a clade including *Paraphaeosphaeria s.str.*, the biocontrol agent *C. minitans*, and the ubiquitous soil fungus *C. sporulosum*.

C. minitans and *C. sporulosum* are therefore combined into the genus *Paraconiothyrium.* See the description below:

Paraconiothyrium minitans (W.A. Campb.) Verkley, **comb. nov.** MycoBank MB500085. *Basionym*: *Coniothyrium minitans* W.A. Campb., Mycologia 39: 191. 1947.
In *Paraconiothyrium minitans* conidiomata are thin-walled pycnidia, the conidiogenous cells are discrete or integrated (small protruding mass of cells), enteroblastic, phialidic with a minute periclinal thickening, but often also percurrently proliferating once or twice over a small distance, to form inconspicous annellations (OA = oatmeal medium, CBS 861.71).

The anamorphs of *Paraphaeosphaeri michotii* (Westend.) O.E. Erikks. and *P. pilleata* Kohlm. Volkm.-Kohlm. & O.E. Erikss. are regarded representative of ***Paraconiothyrium*** Verkley, **anam.gen.nov.** MycoBank MB 500080, but remain formally unnamed. *Paraconiothyrium* species are phylogenetically distant from typical members of the other coelomycete genera mentioned above.

b) Growth and sporulation on some natural and artificial culture media. In our tests (Fig. 1), *C. minitans* grew very well on the following media: Sabouraud, Leonian, PDA, malt extract-agar, Weindling, malt extract + carrot extract, PDA + carrot extract, carrot extract-agar, molasses, Hansen, soybean meal (Şesan, Crişan, 1988; Şesan, Baicu, 1993a). The poorest growth was noted on Czapek, Bilai and agarized water medium (Şesan, Crişan, 1988; Şesan, Baicu, 1993a).

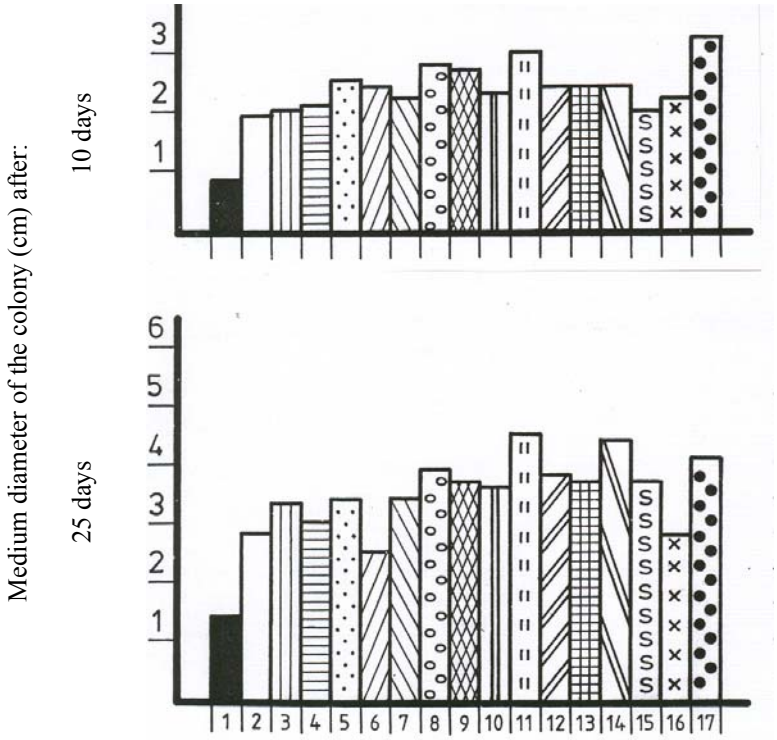


Fig. 1: Influence of solid culture media on *Coniothyrium minitans* growth (Şesan, Baicu, 1993a)

These results agree with those obtained by Iakubova & Chaban (1986), when culturing on liquid Leonian, PDA and potato extract media, as well as those reviewed by Whipps & Gerlagh (1992).

Description of *C. minitans* pycnidia and pycnospores. Pycnidia and pycnospores' sizes are presented in the Table 1, built after the literature by Whipps & Gerlagh (1992), table which includs our personal researches, too .

Table 1: Pycnidia and pycnospores' size of *Coniothyrium minitans* (after Whipps, Gerlagh, 1992)

Culture medium	Pycnidia Diameter (µm)	Pycnospores Lenght x width (µm)	Authors
Potato Dextrose Agar (PDA)	200.0-700.0 430.0-590.0	4.0-6.0 x 3.5-4.0 3.9-7.7 x 3.3-4.4	Campbell (1947) Phillips (1985)
Malt extract-agar	95.0-350.0	3.1-7.7 x 1.9-5.8	Schmidt (1970)
Oat (meal)-agar	150.0-600.0	4.0-7.0 x 3.0-4.0	Punithalingam (1982)
S. sclerotiorum sclerotia	150.0-700.0	4.0-7.0 x 2.5-3.5	Punithalingam (1982)
14 agarized culture media	116.4-795.4	3.8 x 7.5-8.75	Şesan & Crişan (1988)

***C. minitans* mode of action against host pathogens**. The hyperparasitic fungus *C. minitans* parasites the host by direct penetration of *S. sclerotiorum* mycelial filaments by their hyphal tips without formation of appressoria as it could be seen in SEM micrographs (Huang, Hoes, 1976; Huang, Kokko, 1987, 1988; Trutmann et al., 1982) (Fig. 2).

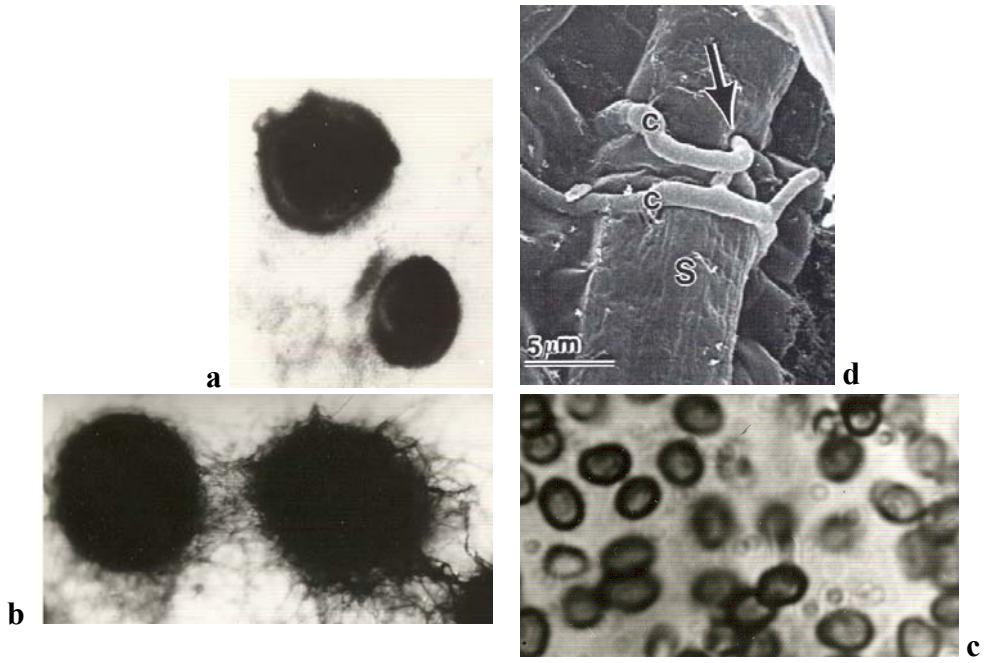


Fig. 2: ***Coniothyrium minitans***

a, b – pycnidia in Petri dish on PDA medium (10 x, Amplival microscope); c – pycnospores (40 x) (Şesan, Crişan, 1988); d – SEM micrograph with low magnification showing direct penetration of the host mycelial filament (S) by hyphal tips of *C. minitans* (c) without formation of appressoria (arrow) (Huang, Kokko, 1988, J. Phytopathology, **123**: 133-139, with authors' permission).

c) Growth and sporulation on the potato-dextrose-agar medium (PDA) with different initial pH values. Among the pH values of the PDA medium, the most favourable for *C. minitans* growth and sporulation proved to be with a slightly acid reaction (pH 4.0-6.0) up to the neutral (pH 7.0), as it results from the Fig. 3.

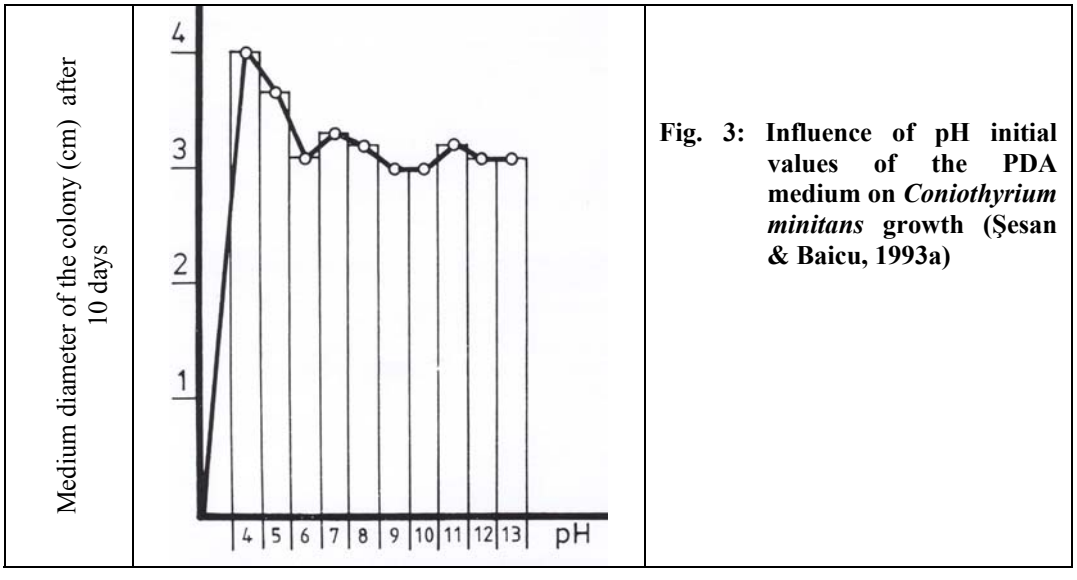


Fig. 3: Influence of pH initial values of the PDA medium on *Coniothyrium minitans* growth (Şesan & Baicu, 1993a)

Based on the results of our experiments (Şesan, Crişan, 1988; Şesan, 1989; Şesan, Baicu, 1993a), the optimal biological development parameters of *C. minitans* have been established: **culture media** - Sabouraud, Leonian, PDA, malt extract-agar, Weindling, malt extract + carrot extract, PDA + carrot extract, carrot extract, molasses, Hansen, soybean meal; **pH initial values:** 4.0 - 7.0; **temperature:** 20ºC. These parameters are important for the mass multiplication of the fungus used in practice as a biocontrol agent.

d) *In vitro* relationships between *C. minitans* and several sclerotium-forming fungi: *Sclerotinia sclerotiorum*, *S. minor*, *Macrophomina phaseolina,* syn. *Sclerotium bataticola*, *Botrytis cinerea*. It is presented in the Table 2.

Table 2: *In vitro* relationships between *Coniothyrium minitans* and sclerotial fungi

Test-fungi	Provenience - host-plants	**x** (average)
Sclerotinia sclerotiorum – Scl.	sunflower	1.465^{000}
Scl. s.	soybean	0.761^{**}
Scl. m.	carrot	0.889
Scl. p_1	parsnip	0.732^{**}
Scl. p_2	parsnip	0.978
Scl. p_3	parsnip	0.595^{***}
Sclerotinia minor – S.m.	lettuce	0.739^{**}
$S.m._1$	sunflower	0.997
Sclerotium bataticola – Scl. bat.	sunflower	0.811^{*}
Botrytis cinerea – B.c.	sunflower	0.468^{***}
$B.c._3$	parsnip	0.719^{**}

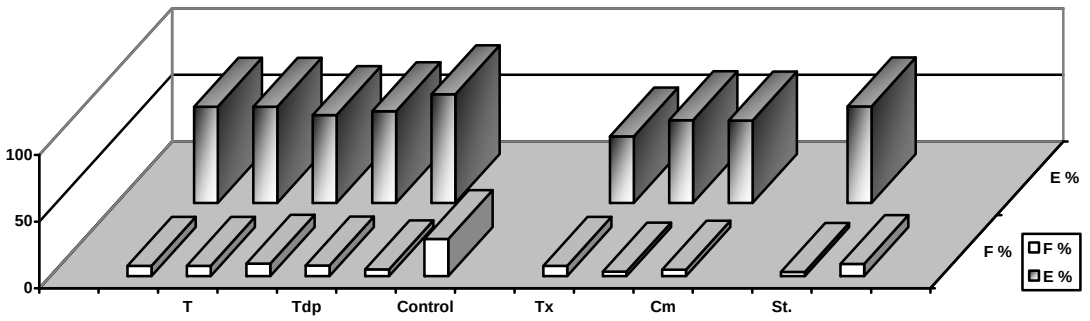
LD 5% 0.158; LD 1% 0.212; LD 0.1% 0.280

C. minitans proved a variable inhibitory action against the tested pathogenic fungi. It has been the most active against the isolates Scl. p_3 of *S. sclerotiorum* and B.c. of *B. cinerea* (x = 0.468-0.595), followed by a good inhibitory action against the isolates Scl.s., Scl. p_1, S.m. and $B.c._3$ (x = 0.719-0.761). A lower action of *C. minitans* has been revealed against isolates Scl.m., Scl. p_2, $S.m._1$, Scl.bat. (x = 0.811-0.997). In case of the isolate Scl., from sunflower, *C. minitans* has been non-antagonistic (x = 1.465).

e) Testing of the efficacy of *C. minitans* in plant protection under the different field conditions in Romania.

All the field experiments conducted during 1993-1998, in different zones (AERS Oradea, RICIP Fundulea, RSIC Valul lui Traian), under irrigation or without irrigation, showed that *C. minitans* treatment had a good efficacy in protecting sunflower, soybean and bean against white rot, but always under the efficacy obtained in the variants with chemical treatments with specific fungicides (standards) (Figs. 3-5).

Fig. 3 - Efficacy of biological treatments with *Coniothyrium minitans* in prevention of sunflower white rot (*Sclerotinia sclerotiorum*) (1993-1997)



Oradea – Not irrigated Average (1993-1997)

Constanța – Irrigated Average (1993-1997)

Fig. 4 - Efficacy of biological treatments with *Coniothyrium minitans* in prevention of soybean white rot (*Sclerotinia sclerotiorum*) 1993 - 1996 - Oradea

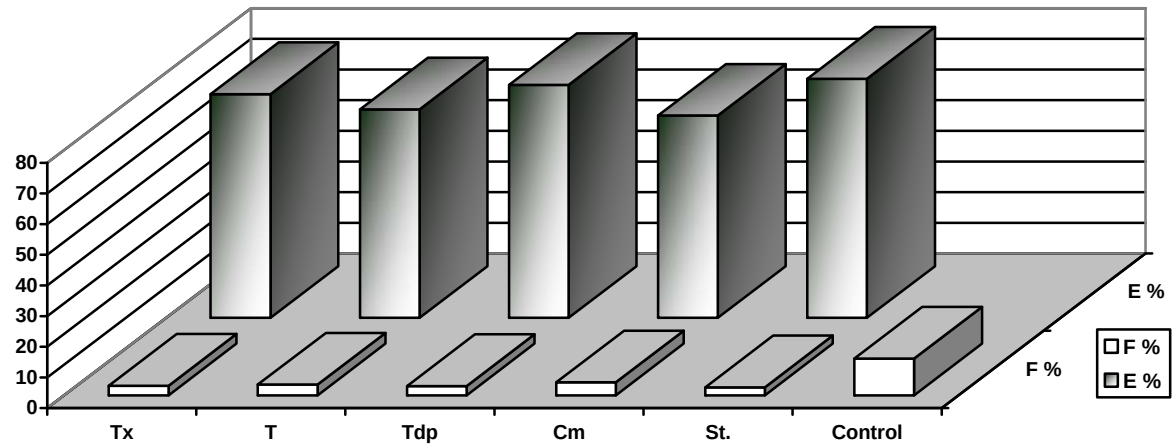
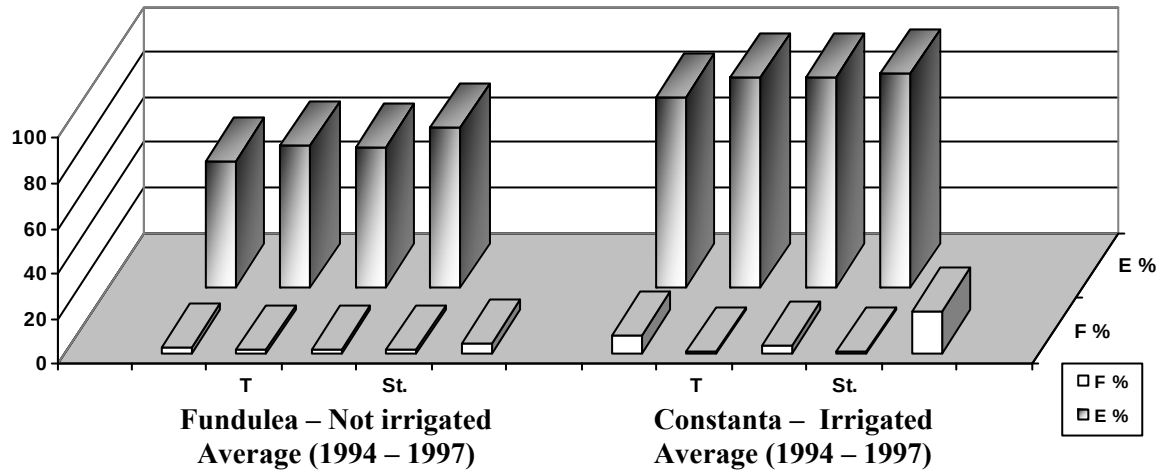


Fig. 5 - Efficacy of biological treatments with *Coniothyrium minitans* in prevention of bean white rot (*Sclerotinia sclerotiorum*)

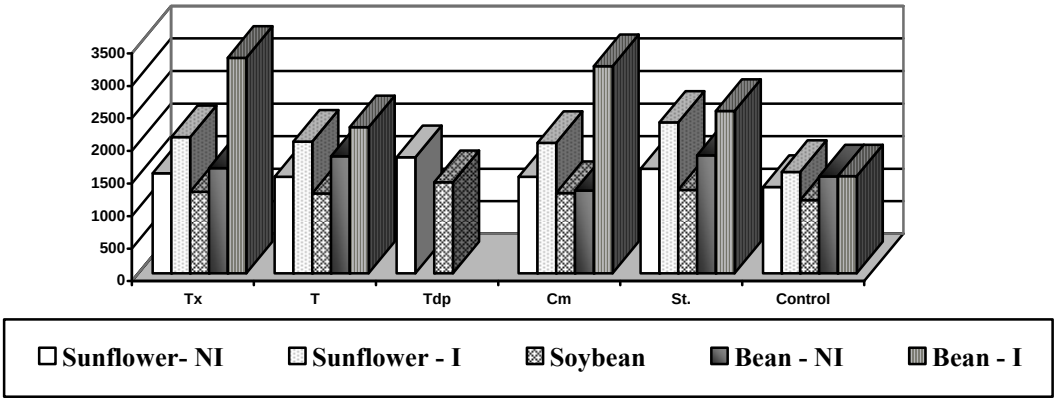


Many scientists have been interested in *C. minitans* as a plant protection mean, mainly against white rot of vegetables, industrial and medicinal crops, the most strong research group being the British-Dutch-German one (Whipps, 1987; Whipps, Gerlagh, 1992, 1995; Gerlagh et al., 1993, 1994, 1995a, b, c, 1996, 2003, 2004; Evenhuis et al., 1995; McQuilken, Whipps, 1995 a.s.o.). Also, There are known the bioproducts Contans (Prophyta, Germany) and Koni (Hungary) (Şesan, 2002a).

In Romania, some promising results have been performed for sunflower, soybean and bean crops (Şesan, Csép, 1991-1995; Şesan et al., 1997), all these results being connected with the international ones.

Seed quantities harvested in biological treatments trials were higher in comparison with seed yield from untreated check (Fig. 6).

Fig. 6 - Seed yield obtained by biological treatments in prevention of sunflower, soybean and bean white rot (*Sclerotinia sclerotiorum*) 1993 - 1997



Although the efficacy of biological treatments is lower than chemical effectiveness, the first ones have the advantages of protecting the agroecosystem of trialled crops and the environment as well, being a non-polluting alternative in the sustainable agriculture (Şesan, Baicu, 1993b; Şesan, 2002a).

f) The *in vitro* influence of pesticides on the growth and sporulation of *C. minitans*, and evaluating of the chemicals' selectivity of this biocontrol agents.

The most tested pesticides (Tables 3-6) proved a strong toxicity to the hyperparasitic fungus *C. minitans*, this fact warning their caution use in practice, as these inducing negative effects at the level of teluric beneficial mycobiota. In this proup are framed all 12 fungicides trialled (Table 3), 5 insecto-fungicides (Tirametox 90PTS, Gammavit 80PSU, Supercarb T 80PSU, Trialin MT, table 5) and 8 herbicides (Butizin 40SC, Butizin 600SC, Butiran 1:1, Diburom 800CE, Diizocab 80CE, Mecloran 35CE, Mecloran 48CE, Sanolt Combi, Table 6).

Table 3: The *in vitro* biological action of fungicides on *Coniothyrium minitans*

Fungicides	Conc. %	I %	RLE	LC 50mg/l	CL 90 mg/l	Ccf	Activ.
Metoben 70 PU	0.10	100.00	y = 1.85 + 2.30 x	23.0900	118.84	0.990	T
Metozir	0.40	100.00	y = 1.34. + 2.46 x	30.6000	141.92	0.990	T
Fundazol 50WP	0.10	100.00	y = 2.32 + 2.20 x	16.3400	90.50	0.990	T
Cuzin 15 SC	1.00	100.00	y = 1.47 + 2.23 x	37.5200	202.89	0.970	T
Tecto 450 Fl	0.10	100.00	y = 2.56 + 2.28 x	11.5700	60.31	0.990	T
Tilt 250 EC	0.10	100.00	y = 3.37 + 2.16 x	5.6200	32.07	0.980	T
IAMN-SN-210	0.25	100.00	y = 1.69 + 2.28 x	27.8000	144.69	0.990	T
Anvile 5 SC	0.05	100.00	y = 6.23 + 2.45 x	0.3100	1.46	0.970	T
Tiracarb 60 PTS	0.2-0.025	98.70	y = 9.48 + 1.98 x	0.0054	0.0243	0.813	T
Tiracarb 600 SC	0.2-0.025	77.5-98.7	y = 7.99 + 1.19 x	0.0031	0.0367	0.896	T
Tiramet 60 PTS	0.2-0.025	77.5-98.7	y = 9.19 + 1.75 x	0.0400	0.0215	0.808	T
Tiramet 600 SC	0.2-0.025	77.5-98.7	y = 9.19 + 1.75 x	0.0400	0.0215	0.808	T

Table 4: The *in vitro* biological action of insecticides on *Coniothyrium minitans*

Insecticides	Conc. %	I %	RLE	LC50 mg/l	CL90 mg/l	Ccf	Activ.
Oleoekalux + Quinalphos 3%	0.2-0.025	30.9-58,9	y = 5.84 + 0.76 x	0.0774	3.66367	0.921	MT
US 1 - RV	0.2-0.025	42.0-51.1	y = 5.18 + 0.26 x	0.1899	1538.10	0.944	MT
Lindane 400 SC	0.2-0.025	25.0-58.3	y = 5.92 + 0.79 x	0.0706	2.8182	0.991	MT
Lindane 666 SC	0.2-0.025	33.3-58.3	y = 5.72 + 0.49 x	0.0353	12.9840	0.999	MT

Table 5: The *in vitro* biological action of insecto-fungicides on *Coniothyrium minitans*

Insecto-fungicides	Conc. %	I %	RLE	LC 50mg/l	LC 90mg/l	Ccf	Activ.
Diniconazole 10g/l + Lindane 500g/l	0.2-0.025	62.9-98.1	y = 11.6 + 4.56 x	0.035	0.0668	0.870	MT
Tebuconazole 150g/l + Lindane 500g/l	0.2-0.025	10.0-97.5	y = 9.73 + 2.67 x	0.016	0.0508	0.862	MT
Tirametox 90 PTS	0.2-0.025	99.3-99.5	y = 7.66 + 0.14 x	< 0.01	< 0.1000	0.900	T
Gammavit 85 PSU	0.2-0.025	99.1-99.5	y = 7.76 + 0.24 x	< 0.01	< 0.1000	0.940	T
Supercarb T 80 PSU	0.2-0.025	99.5-99.7	y = 6.75 + 0.63 x	601.9	5.6050	0.696	T
Procarb L	0.2-0.025	99.4-99.7	y = 6.66 + 0.68 x	256.6	3.5715	0.699	T
Trialin	0.2-0.025	44.0-98.8	y = 11.0 + 0.52 x	0.024	0.0544	0.908	T
Trialin MT	0.2-0.025	92.0-96.8	y = 7.28 + 0.52 x	< 0.10	0.0100	0.954	T

Table 6: The *in vitro* biological action of herbicides on *Coniothyrium minitans*

Herbicides	Conc. %	I %.	RLE	LC50mg/l	LC90 mg/l	Ccf	Activ.
Butizin 40 SC	0.2-0.025	56.3-96.0	y = 16.1 + 6.98 x	0.025	0.039	0.969	T
Butizin 600 SC	0.2-0.025	98.9-99.1	y = 7.42 + 0.08 x	< 0.01	< 0.01	0.974	T
Butiran 1:1	0.2-0.025	79.2-96.0	y = 13.9 + 5.56 x	0.024	0.041	0.958	T
Icedin Super RV	0.2-0.025	25.6-55.3	y = 5.82 + 0.98 x	0.142	2.876	0.952	ST
Diburom 800 CE	0.2-0.025	99.3-99.7	y = 7.91 + 0.28 x	< 0.01	< 0.01	0.927	T
Diizocab 80 CE	0.2-0.025	99.3-99.7	y = 10.1 + 2.73 x	0.013	0.038	0.888	T
Mecloran 35 CE	0.2-0.025	75.0-99.2	y = 10.2 + 2.80 x	0.013	0.039	0.895	T
Mecloran 48 CE	0.2-0.025	99.3-99.7	y = 7.91 + 0.28 x	< 0.01	< 0.10	0. 927	T
Oltisan M	0.2-0.025	14.0-99.3	y = 15.6 + 6.59 x	0.024	0.038	0.967	ST
Oltisan Extra	0.2-0.025	65.7-99.3	y = 8.30 + 2.89 x	0.072	0.199	0.828	MT
Sanolt Combi 400 SE	0.2-0.025	65.7-100.00	y = 15.6 + 6.59 x	0.024	0.038	0.967	T
2.4 SDMA 600 RV	0.2-0.025	11.1-99.6	y = 11.5 + 5.06 x	0.050	0.091	0.927	MT - ST

An other group of products usable without disturbing so much the agroecosystems of various agricultural crops, is represented by moderately toxic products (8) among which: 4 insecticides (Oleoekalux + Quinalphos 3%, US 1-RV, Lindane 400SC, Lindane 666SC), 2 insecto-fungicides (Diniconazole + Lindane, Trialin) and 2 herbicides (Oltisan Extra, 2.4 SDMA – 600RV).

A very low number (4) of the tested chemicals had non-toxic action on the test-fungus, and namely: the insecticidal mixture Tebuconazole + Lindane and the herbicides: Icedin Super RV, Oltisan M and 2.4 SDMA – 600RV.

These results are a new contribution in addition with of our previous ones referring to the other biocontrol agents, like *Trichoderma viride* Pers. ex S.F. Gray, *Trichothecium roseum* Link, *Gliocladium roseum* Bainier, *Epicoccum purpurascens* (Şesan, Oprea, 1998, 1999, 2000, 2001, 2002/2003; Şesan et al., 1998; Şesan, 2002b).

For integrated plant protection systems (IPPS) it is obligatory to evaluate the biological activity of pesticides on the beneficial antagonistic fungi used as BCAs in order to apply in the protecting technologies only the efficient chemicals with low toxicity against BCAs (Table 7).

Table 7: Evaluation *in vitro* of pesticides' biological activity against *Coniothyrium minitans*

Activity	Pesticides
1	2
TOXIC (T)	***Fungicides*: Tiracarb 60 PTS, Tiracarb 600 SC, Tiramet 60 PTS, Tiramet 600 SC, Metoben 70 PU, Metozir, Fundazol, Cuzin 15, Tilt 250, Tecto 450 Fl, IAMN-SN 210, Anvile;** ***Insecticides*: -** ***Insecto-fungicides*: Tirametox 90 PTS, Gammavit 85 PSU, Supercarb T 80 PSU, Trialin MT;** ***Herbicides*: Butizin 600 SC, Butizin 40 SC, Butiran 1:1, Diizocab 80 EC, Diburon 800 EC, Mecloran 35 EC, Mecloran 48 EC, Sanolt Combi 400 SE;**
MODERATELY-TOXIC (MT)	***Fungicides*: -** ***Insecticides*: Oleoekalux+Quinalfos 3%, US 1-RV, Lindane 400 SC, Lindane 666 SC;** ***Insecto-fungicides*: Diniconazole+Lindane, Trialin, Tirametox 625 SC, Supercarb T 585 SC;** ***Herbicides*: Oltisan Extra, 2.4 SDMA 600 RV;**
SLIGHTLY TOXIC (ST)	***Fungicides*: -** ***Insecticides*: Tebuconazole+Lindane;** ***Insecto-fungicides*: -** ***Herbicides*: Oltisan M, Icedin Super RV, 2.4 SDMA 600 RV**

Conclusion

a) Taxonomical considerations on *Coniothyrium*-like coelomycetes based on the parsimony analysis of ITS region of nuclear ribozomal DNA and partial SSU of the nr DNA sequences showed that they belong in the order *Pleosporales* (subclass *Dothideomycetidae*, class *Ascomycetes*) and group in a clade including *Paraphaeosphaeria s.str.*, the biocontrol agent *C. minitans*, and the ubiquitous soil fungus *C. sporulosum*. Verkeley et al. (2004) combined *C. minitans* and *C. sporulosum* into the new genus *Paraconiothyrium*, anamorph of *Paraphaeosphaeria*. *Paraconiothyrium* species are phylogenetically distant from typical members of the other coelomycete genera.

b – c) The optimal biological development parameters of *C. minitans* have been established: the culture media: Sabouraud; Leonian, PDA, malt extract, Weindling, malt extract + carrot extract, PDA + carrot extract, carrot extract, molasses, Hansen, soybean meal; pH initial values: 4.0-7.0 ; temperature: 20ºC.

d) C. minitans proved in vitro an inhibitory action against the test-fungi depending on the species, from strong antagonism until no antagonism. This variability of the antagonistic ability of the hyperparasitic fungus C. minitans explains why it is so important to do these tests for establishing the level of antagonism, in order to select for practice the most antagonistic isolates against the target plant pathogens.

e) Efficacy of *C. minitans* as a biocontrol agent under the field conditions has been demonstrated for sunflower, soybean and bean, in irrigated and non-irrigated fields, the seed treatment, at a rate of 2 g/kg, proved a good efficacy in protecting these crops against white rot (*S. sclerotiorum*), almost similar with the chemical treatment with specific fungicides.

f) It has been evaluated *in vitro* the biological activity of 36 pesticides against the hyperparasitic fungus *C. minitans*, with a view to setting up their selectivity (Table 7).

- ✓ The most tested chemicals proved **a strongly toxic influence** to hyperparasitic fungus *C. minitans*, namely all 12 fungicides trialled, 5 insecto-fungicides (Tirametox 90PTS, Gammavit 80PSU, Supercarb T 80PSU, Trialin MT) and 8 herbicides (Butizin 40SC,

Butizin 600SC, Butiran 1:1, Diburom 800CE, Diizocab 80CE, Mecloran 35CE, Mecloran 48CE, Sanolt Combi);

- ✓ Only 8 chemicals, among them: 4 insecticides (Oleoekalux + Quinalphos 3%, US 1-RV, Lindane 400SC, Lindane 666SC), 2 insecto-fungicides (Diniconazole+ Lindane, Trialin) and 2 herbicides (Oltisan Extra, 2.4 SDMA – 600RV), revealed **a moderately inhibitory action** to the beneficial antagonistic fungus *Coniothyrium minitans*, used as a biocontrol agent, showing an increased practical importance, being efficient in crop protection and not inducing imbalances to the agroecosystem where applied;
- ✓ A very low number (4) of the tested chemicals had **non-toxic action** on the test-fungus, and namely: the insecticidal mixture Tebuconazole + Lindane and the herbicides: Icedin Super RV, Oltisan M and 2.4 SDMA – 600RV. This group of products is highly important for practice, being usable in systems of crop integrated protection without causing harm to agroecosystems.

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CONIOTHYRIUM MINITANS – AGENT BIOLOGIC DE COMBATERE A PATOGENILOR SCLEROȚIALI AI PLANTELOR

(Rezumat)

Coniothyrium minitans, ciupercă micoparazită semnalată pentru prima dată în lume din California (Campbell, 1947), iar în Europa din Anglia, în 1957 (Tribe, 1957), este un subiect dintre cele mai interesante pentru cercetarea biologică şi agricolă prin acțiunea de hiperparazitare a patogenilor scleroțiali, foarte periculoşi şi dificil de combătut la numeroase plante de cultură, între aceştia fiind pe primul loc reprezentanții genului *Sclerotinia*.

În România, ciuperca a fost izolată prima dată de Aurelia Crişan (1986). Ulterior, au fost studiate bio-ecologia acestei ciuperci (Şesan, Crişan, 1988, 1998; Şesan, Baicu,1993a) şi modalitățile de utilizare ca agent biologic de protecție a plantelor (Şesan, Csép, 1991-1996; Şesan şi colab., 1997) .

Sunt trecute în revistă rezultatele experimentale referitoare la eficacitatea biopreparatelor pe bază de *C. minitans*, în diferite condiții pedoclimatice din România, pentru protejarea plantelor industriale, medicinale şi a leguminoaselor anuale față de putregaiul alb (*Sclerotinia sclerotiorum*), rezultate cu aplicații în dezvoltarea durabilă a agroecosistemelor, racordate, în acelaşi timp, la cele obținute pe plan mondial.