IN VITRO RELATIONSHIPS BETWEEN FUNGI ISOLATED FROM *RIBES NIGRUM* L. PLANTS

RELAȚIILE *IN VITRO* DINTRE UNELE SPECII DE CIUPERCI IZOLATE DE PE PLANTE DE *RIBES NIGRUM* L.

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Abstract: *Fungal isolates from Ribes nigrum L. have been screened in vitro for antagonism against the two pathogens Botrytis cinerea Pers. and Alternaria tenuissima (Kunze)Wiltshire. Among saprophytic isolates tested, strains of Trichoderma sp. were the most competitive against B. cinerea and A. tenuissima pathogenic isolates. The other fungi - Trichothecium roseum Link, Epicoccum nigrum Link and Clonostachys rosea (Link) Schroers, Samuels, Seifert & W. Gams have showed a very slight antagonistic action or lack of antagonism towards the two phytopathogens of blackcurrant.*
Key words: *Ribes nigrum*, phytopathogens, antagonistic fungi, biological control

Rezumat: *Cercetările au urmărit evaluarea capacității antagonice in vitro a unor ciuperci izolate de pe plante de Ribes nigrum față de ciupercile fitopatogene Botrytis cinerea Pers. şi Alternaria tenuissima (Kunze)Wiltshire. Dintre ciupercile testate față de cei doi patogeni ai coacăzului, tulpinile de Trichoderma sp. au manifestat cea mai puternică acțiune antagonistă față de cei doi fitopatogeni-test. Față de Botrytis cinerea, ciupercile Epicoccum nigrum Link şi Clonostachys rosea (Link) Schroers, Samuels, Seifert & W. Gams au prezentat o activitate slab antagonistă, iar Trichothecium roseum Link. nu a manifestat antagonism. Față de Alternaria tenuissima, izolatele de T. roseum şi C. rosea au dovedit un antagonism de valoare medie, în timp ce E. nigrum a fost antagonistul cel mai puțin eficient.*
Cuvinte cheie: *Ribes nigrum*, ciuperci fitopatogene, ciuperci antagoniste, control biologic

INTRODUCTION

In the last years, due to development in the world and in Romania of gemotherapy, as new field of phytotherapy, is seeking for the glycerol-hydroalcoholic extracts from fresh meristematic plant tissues. For this purpose there are used black currant buds (Raiciu, 2011). Recent studies of Romanian researchers have demonstrated antimicrobial activity of some gemoderivatives containing buds extracts of *R. nigrum* (Mihele, 2007), and the ability of some *R. nigrum* essential oils to reduce inert substrate adhesion of bacteria by inhibiting the expression of microbial adhesins (Oprea, 2008). It is considered that the plant

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organs with a therapeutic value are not only the buds, but also the leaves, fruits or seeds (Bojor, 2003).

Since the quality of these products used to obtain phytopharmaceutical products may be impaired by various pathogens, especially fungi both in vegetation period of culture, and in the storage period, the establishment of biological control measures of these pathogens it is necessary.

Our researches have been focused on the use of some fungi antagonistic to black currant crop pathogens *Alternaria tenuissima* (Kunze) Wiltshire and *Botrytis cinerea* Pers., for integration in sustainable crop protection.

MATERIAL AND METHODS

In vitro testing was performed by dual culture method (Jouan, 1964), which is the method used in the work of Romanian researchers to study the relationships of different species of fungi (Hulea, 1973; Drăgoescu, 1996; Raicu, 1970; Şesan, 1985 to present). Each variant had 3-5 repetitions. In petri plates, whereby it was assigned malt-extract-agar medium (MEA), at equal distances from the centre of the vessel, it was inoculated the test pathogenic fungus (marked A) and potential antagonistic fungus (marked B).

To assess the degree of antagonism, it was used the value of the x ratio between of the internal rays (i) and external rays (e) of the test pathogenic fungus (A) and the potential antagonistic fungus (B), by the formula x=iA/iB x eB/eA, whereby iA= internal radius of the colony of the phytopathogenic fungus test A; iB = internal radius of the colony of the antagonistic fungus B; eB = external ray of the colony of antagonistic fungus B; eA = external ray of the colony of the phytopathogenic fungus test A.

The ratio x>1 indicates no mutual interaction or antagonism between the two fungi; x<1 indicates a phenomenon of varying degrees of antagonism.

As biological material we used the test phytopathogenic fungi like *Botrytis cinerea*, isolated from the fruits of black currant, and *Alternaria tenuisssima*, isolated from the leaves of currant in experimental plots of S.C. Hofigal S.A. Bucharest.

Fungi of which antagonistic activity was evaluated were: *Trichoderma viride* Pers. – Td1 strain, *Trichotecium roseum* Link, *Epicoccum nigrum* Link, *Clonostachys rosea* (Link) Schroers, Samuels, Seifert & W. Gams, isolated from the leaves of black currant plantation S.C. Hofigal S.A. Bucharest, and the strain of *Trichoderma viride* Td2, isolated from black currant plantation SC Hofigal S.A. Bucharest, and the strain of Trichoderma viride Td 2, isolated from *Ribes nigrum* leaves from the plantation of Furculeşti, Teleorman County.

A first assessment of the relationship between the two colonies of fungi that grow in the same petri vessel was based on macroscopic analysis from contact line between the two fungal isolates tested.

RESULTS AND DISCUSSIONS

In all cases whereby the action of *Trichoderma* isolates was tested against the phytopathogens *Botrytis cinerea* and *Alternaria tenuissima*, there was initially a slight distance action on pathogenic fungi colony by the persistence of a very narrow area of mycelium weakly developed, that can be considered a zone of inhibition. Concavity of the contact line was directed

towards *T. roseum*, wich had a slower growth. (fig.1a,b; fig. 2a,b). In the case of test fungus *B. cinerea* and *Clonostachys rosea* and of the potential antagonist *Trichotecium roseum*,, the contact curve concavity was directed to *T. roseum*, which had a slower growth (fig. 1e).

In the experimental variant *B cinerea* and *Clonostachys rosea* it was noticed a slight concavity oriented towards the fungus *C. rosea*, which had a slower growth. Although the phytopathogen increased faster, the portion of colony oriented towards the antagonist presents a more rarefied aspect of growth compared to the portion of colony from the wall of Petri vessel (fig. 1d).

In the variant whereby it was tested the action of fungus isolate *E. nigrum* on the *B. cinerea*, the concavity of marking area was more pronounced and oriented to the antagonist fungus *E. nigrum*, as consequence of the faster growth rate of the colony of phytopathogenic fungus (fig. 1c). When it was tested the influence of antagonist *E. nigrum* on *A. tenuissima*, the marking curve was smoother, the concavity being forwarded to the colony of patogenic fungus (fig. 2c).

In control variants (phytophathogenic fungi placed face to face with itself), contact line was straight, sign of uniform growth of both colonies placed in the same petri dish (fig. 1f, fig. 2f).

There was a linearly correlation between the linear aspect of the contact area in control and value equal to unity of the x coefficient between external and internal rays of the colonies, both in the version that was tested fungus *B. cinerea* influence by itself and in version that was tested isolate of *A. tenuissima* (tab.1, tab. 2).

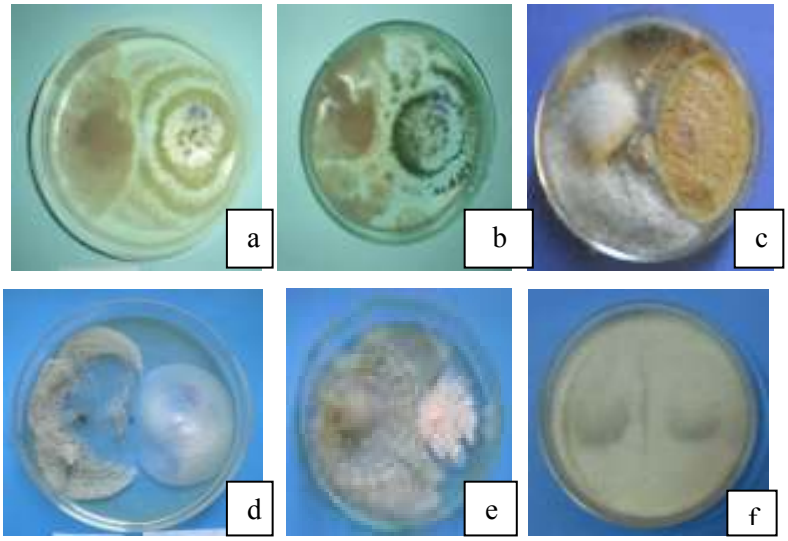

Fig. 1 – Macroscopic *in vitro* aspects of the antagonism of Td1 (a), Td2 (b), *Epicoccum nigrum* (c), *Clonostachys rosea* (d), *Trichothecium roseum* (e) şi *Botrytis cinerea* (f) isolates of the phytopathogen *Botrytis cinerea*, on MEA medium

Analysis of x coefficient value shows that strains of *Trichoderma* have expressed antagonism to the pathogen *B. cinerea*. (tab. 1). The stronger antagonistic strain was the fungus isolate from leaves from currant plantation S.C. Hofigal S.A. of Furculeşti, Teleorman County.

The fungi *E.nigrum* and *C. roseum* showed slight antagonistic activity, whereby they obtained average values of the coefficient x close to the control. In case of the confrontation of fungi *B. cinerea* and *T. roseum* was obtained a higher than one value of coefficient x, which showed that *T. roseum* strain expressed no antagonism against *B. cinerea* isolate (Tab. 1).

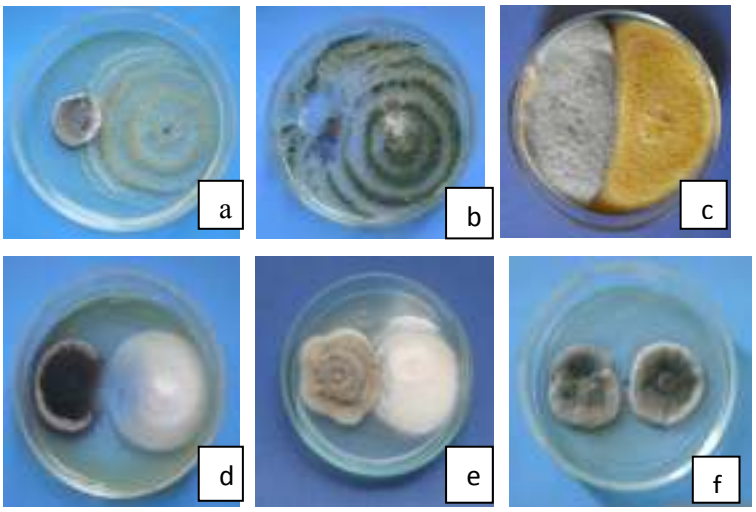

Fig. 2 – Macroscopic *in vitro* aspects of the antagonism of Td1 (a), Td2 (b), *Epicoccum nigrum* (c), *Clonostachys rosea* (d), *Trichothecium roseum* (e) şi *Botrytis cinerea* (f) isolates of the phytopathogen *Alternaria tenuissima*, on MEA medium

Table 1

The *in vitro* relationsips between *Botrytis cinerea* and some antagonistic fungi, expressed by the x coefficient, calculated at 7 days, after Jouan and colab. (1964)

Variant		x	Behavior
1.	*B. cinerea / Trichoderma* 1	0,63	A
2.	*B. cinerea / Trichoderma* 2	0,59	A
3.	*B. cinerea / Clonostachys rosea*	0,88	SA
4.	*B. cinerea / Trichothecium roseum*	1,25	N
5.	*B. cinerea / Epicoccum nigrum*	0,97	SA
martor	*B. cinerea / Botrytis cinerea*	1,00	I

Legend.: x > 1 absent antagonism, nonatagonist izolat e (N);
x < 1 antagonism (A), strong antagonist (PA), weak antagonist (SA);
x = 1 absence of mutual influences, indifferently (I).

Against pathogen *Alternaria tenuissima* (table 2), both strains of *Trichoderma* tested showed antagonism. The strain of Trichoderma Td 2, Furculeşti origin, showed the strongest antagonistic, while the strain Td,

provenance Bucharest, was less effective. Isolates of *T. roseum* and *C. rosea* showed antagonism, but of mean value. The fungus *E. nigrum* showed no antagonism against *Alternaria tenuissima*, so the average of coefficient x is close to that of the control (tab. 2).

Table 2

The *in vitro* relationsips between *Alternaria tenuissima* and some antagonistic fungi, expressed by the x coefficient, calculated at 7 days, after Jouan and colab. (1964)

Variant		x	Behavior
1.	*A. tenuissima / Trichoderma 1*	0,72	A
2.	*A. tenuissima / Trichoderma* 2	0,50	A
3.	*A. tenuissima / Clonostachys rosea*	0,81	A
4.	*A. tenuissima / Trichothecium roseum*	0,80	A
5.	*A. tenuissima / Epicoccum nigrum*	1,06	N
martor	*A. tenuissima / Alternaria tenuissima*	1,00	I

Legend: x > 1 absent antagonism, nonatagonist izolat e (N);
x < 1 antagonism (A), strong antagonist (PA), weak antagonist (SA);
x = 1 absence of mutual influences, indifferently (I).

CONCLUSIONS

1. Against pathogen *Botrytis cinerea*: 1.1. both strains of *Trichoderma* tested – Td1 and Td2- showed antagonism on malt-extract-agar medium; the strain with stronger antagonistic action was Td2, the strain isolated from the leaves of currant plantation S.C. Hofigal S.A. from Furculeşti, Teleorman County; 1.2. the isolates of *E. nigrum* and *C. rosea* were slight antagonistic activity against the fungus *Botrytis cinerea*; 1.3. *T. roseum* showed no antagonism against the pathogen *Botrytis cinerea*.

2. Against pathogen *Alternaria tenuissima*: 2.1. The two strains of *Trichoderma* isolated from *Ribes nigrum* leaves behaved differently: Td 1 Furculeşti origin, showed the strongest antagonistic action; 2.2. Isolates of *T. roseum* and *C. rosea* showed a mean antagonism to pathogen *Alternaria tenuissima*; 2.3. Fungus *E. nigrum* showed no antagonism against *A. tenuissima*;

3. From the strains with an antagonist potential tested *in vitro* on the two pathogens of the black currant culture, those of *Trichoderma* had the most powerful biological activity. There were registered differences of the two isolated antagonistic depending on their origin. Strain Td 2 Furculeşti origin was stronger antagonistic to both pathogens tested - *B. cinerea* and *A. tenuissima.*

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