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IN VITRO EVALUATION OF THE RELATIONSHIPS BETWEEN SOME FUNGAL PATHOGENS OF BLACK CURRANT CROP AND SOME SAPROPHYTIC FUNGI

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Abstract

Blackcurrant crop is affected by fungal pathogens, such as Mycosphaerella grossulariae, Drepanopeziza ribis (Kleb.)v. Hohn., Alternaria tenuissima (Kunze) Wiltshire, Sphaerotheca mors-uvae (Schw.) Berk, Cronartium ribicola Dietr., Fusarium oxysporum E.F. Sm. Swingle, Botrytis cinerea Pers. In this study has been evaluated the in vitro antagonistic effect of some fungal saprophytic isolates towards some of these phythopathogens in order to establish which of them could be used as biological control agents of black currant crop. In all the cases has been observed the in vitro antagonistic effect of the strain of the fungus Trichoderma spp.

Key words: *blackcurrant crop, biological control agents, in vitro antagonism, phytopathogenic fungi, Trichoderma spp*

INTRODUCTION

Black currant (*Ribes nigrum* L.) is high appreciated for alimentary and therapeutic value of its fruits. Young leaves and buds are also used for obtain alimentary supplements and phytopharmaceutical products, like medicinal teas and gemoderivatives [1, 9]. The antimicrobial activity of some *Ribes nigrum* essential oils and of some gemoderivatives containing buds extracts of *R. nigrum* has been demonstrated [7].

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.

Blackcurrant crop is affected by pathogens, like viruses and fungi [8, 4]. In 2009-2012, in experimental plots of S.C. Hofigal S.A. were identified the fungal pathogens *Mycosphaerella grossulariae, Drepanopeziza ribis (Kleb.)v. Hohn., Alternaria tenuissima (Kunze) Wiltshire, Sphaerotheca mors-uvae (Schw.) Berk, Cronartium ribicola Dietr., Fusarium oxysporum E.F. Sm. Swingle*. These phytopathogens could alter the quality of the vegetable organs like leaves or fruits which are used as a raw-material for obtaining phytopharmaceutical products.

Our work aimed to screen *in vitro* activity of some saprophytic fungi against some phytopathogens isolated from experimental plots of S.C. Hofigal S.A. from Bucharest in 2010-2011 in view to establish which of them could be used as biological control agents. Biological control using such antagonistic fungi is an alternative, safety for humans and non-polluting method for control the diseases produced by phytophathogens [2].

MATERIAL AND METHOD

Biological material was represented by isolates of phytopathogenic and saprophytic fungi. The

two strains of pathogenic fungi isolated from blackcurrant crop from experimental plots of S.C. Hofigal S.A: one strain of *Botrytis cinerea* isolated from mature fruits in July 2011 and one strain of *Fusarium oxysporum* isolated from leaves in April 2010.

The saprophytic fungi of which activity against both phytopathogens was evaluated were *Trichothecium roseum* Link, *Epicoccum nigrum* Link, *Chaetomium globosum* isolated from leaves of blackcurrant in 2010 and one strain of *Trichoderma viride* isolated in 2010 from wheat grains, County Constanţa provenance.

The *in vitro* testing was performed in the laboratory at Research-Development Institute for Plant Protection Bucharest. The dual test assay [5] was used for *in vitro* screening of the relationships of pathogenic and saprophytic fungi. Each variant had 3 replicates. The control was the variant whereby in the same Petri plate, two colonies of the same pathogenic fungus were inoculated. In each variant two fungi, one pathogenic fungus and one antagonistic fungus were inoculated at equal distance one from another (3 mm distance) and from equal distance from the centre of Petri plates containing PDA (potato-dextrose-agar) medium.

Macroscopic aspect of the contact line was evaluated by the method described by Ana Hulea [3].

The type of interaction was assessed by using the x ratio between of the internal radial growth (i) and external radial growth of the pathogenic fungus (noted A) and the saprophytic fungus (noted B). This is the method used by Romanian to evaluate the degree of antagonism [11]. Formula used was x = iA/iBxeB/eA, whereby iA = internal radius of the colony of the phytopathogenic fungus, iB = nternal radius of the colony of the antagonistic fungus, eB = external radius of antagonistic fungus, eA = external radius of the colony of the phytopathogenic fungus. The ratio x>1 indicates the lack of antagonism between the two fungi, x<indicates a phenomenon of varying degrees of antagonism, the power antagonism being in variants whereby x value is close upon 0 than 1.Value of x=1 indicates no mutual interaction and an indifferently relationship.

In order to obtain more information about the antagonism it was considered only external and internal radial growth of the pathogen and inhibition percent (I %) was calculated by the formula: I % = (eA-iA/eA)x100 [6,12]. A grow category scale from 0 to 4 was used in order to establish the degree of inhibition, where 0= no growth inhibition, 1=1-25% growth inhibition; 2 = 26-50% growth inhibition; 3 = 51-75% growth inhibition, 4 = 76-100% growth inhibition, according to Živković Svetlana [12].

The observations and biometric measurements were taken at 3 and 6 days after inoculation. Photos were taken at 6 day after inoculation.

RESULTS AND DISCUSSIONS

Botrytis cinerea is known to produce grey mould on fruits [4], and flower blight in blackcurrant crop [11]. *Fusarium oxysporum* is also known to produce a vascular wilt disease on plants.

The aspect of contact area between the pathogen and antagonistic fungus which are grown in the same petri dish depends on the growing rate of the colony [3].

In control variants (the same phytophathogenic fungus placed face to face), contact line was straight, sign of uniform growth of both colonies placed in the same Petri plates (fig. 1).

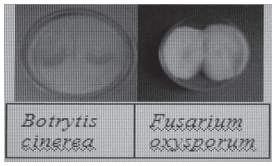

Fig. 1 Macroscopic *in vitro* aspects of the colonies of *Botrytis cinerea* and *Fusarium oxysporum* isolates on PDA medium

In the variants whereby it was tested the effect of the presence of the fungi *T.roseum*, C. *globosum* and *E. nigrum* towards fungus *B. cinerea*, the contact area was a curve, with concavity oriented towards the colony of the saprophytic fungus which had a slower growth (Fig.2).

Only in the variant whereby was tested the relationship between *B. cinerea* and *T. viride*, the aspect of the contact area was a nearly straight and higher line than the colonies of the two fungi, due to the intermingling of the

hyphae of both fungi (Fig. 2B). In portion of the colony of the strain of *B. cinerea* oriented towards those of the fungus *T. viride* an area of very weakly developed mycelium was observed. After three days to inoculation, in variant *B. cinerea*/*C. globosum*, at the contact zone between the colonies of the fungi a very narrow zone of inhibition has been observed (Fig. 2C).

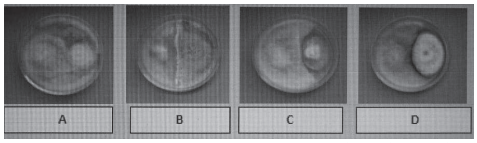

Fig. 2 Macroscopic *in vitro* aspects of the relationship between phytopathogen *Botrytis cinerea* and *Trichothecium roseum* (A), *Trichoderma viride*(B), *Chaetomium globosum* (C) and *Epicoccum nigrum* (D), on PDA medium

In variants whereby was tested the action of the saprophytic fungi towards the strain of *F. oxysporum* the contact line observed after 6 days was a line, with the exception of the variant whereby the influence of *Trichoderma viride* towards *F. oxysporum* was tested. In this case, the contact zone was a curve with concavity oriented towards the phytophatogen *F. oxysporum*, *T. viride* being the fast growth fungus (Fig. 3B). This aspect has been directly correlated with the values of the coefficient x, in this case the relationship was the antagonism.

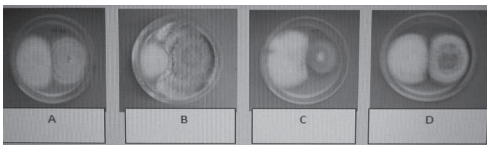

Fig. 3 Macroscopic *in vitro* aspects of the relationship between phytopathogen *Fusarium oxysporum* and *Trichothecium roseum* (A), *Trichoderma viride*(B), *Chaetomium globosum* (C) and *Epicoccum nigrum* (D), on PDA medium

Analysis of x coefficient value showed that only *T. viride* manifested a considerable antagonistic activity against the fungus *B. cinerea*. The fungus *T. roseum* had not manifested any antagonistic effect and the strains of *C. globosum* and *E. nigrum* showed a very slight action of inhibition towards *B. cinerea* at 3 days after inoculation, and then no antagonistic effect (Table 1, Table 2).

Table 1. The *in vitro* relationships between *Botrytis cinerea* and some saprophytic fungi on PDA medium, expressed by the x coefficient, calculated at 3 and 6 days, after Jouan et al. (1964)

Variant	at 3 days		at 6 days	
	x	relationship	x	relationship
B.cinerea/T. roseum	1.099	NA	1.958	NA
B. cinerea/T. viride	0.805	A	0.625	A
B.cinerea/ C. globosum	0.894	A	1.841	NA
B. cinerea/E. nigrum	0.939	A	1.261	NA
B. cinerea/B. cinerea	1.000	I	1.000	I

A – antagonistic, I- indifferently, NA- nonantagonistic

Table 2. The *in vitro* relationships between *Botrytis cinerea* and some saprophytic fungi on PDA medium, expressed by inhibition percent (I%) and category of growth inhibition on scale (0-4)

Variant	at 3 days		at 6 days	
	I%	category	I%	category
B. cinerea/T. viride	29.82	2	39.66	2
B.cinerea/ C. globosum	10.52	1	0	0
B. cinerea/E. nigrum	12.27	1	0	0
B. cinerea/B. cinerea	0.000	0	0.000	0

Towards the fungus *F. oxysporum*, the fungi which showed antagonism, in range of the degree of antagonism appreciated by x coefficient and inhibition percent were: *T. viride*, *T. roseum*, *E. nigrum* [table 3, table 4]. The most powerful antagonistic action manifested the strain of *T. viridae*. In this case the average of coefficient x at 6 days was 0.410 (Table 3) and inhibition percent was 57.3 (Table 4). The isolate of *C. globosum* showed no antagonistic effect. The other fungi showed only a slight antagonistic action (Table 3, Table 4).

Table 3 The *in vitro* relationships between *Fusarium oxysporum* and some saprophytic fungi on PDA medium, expressed by the x coefficient, calculated at 3 and 6 days, after Jouan et al. (1964)

Variant	at 3 days		at 6 days	
	x	relationship	x	relationship
F. oxysporum/T. roseum	0.881	A	0.829	A
F. oxysporum/T. viride	0.729	A	0.410	A
F. oxysporum/ C. globosum	0.935	A	1.435	NA
F. oxysporum/E. nigrum	0.890	A	0.892	A
F.oxysporum/F. oxysporum	1.000	I	1.000	I

A – antagonistic, I- indifferently, NA- nonantagonistic

Table 4 The *in vitro* relationships between *Fusarium oxysporum* and some saprophytic fungi on PDA medium, expressed by inhibition percent (I%) and category of growth inhibition on scale (0-4)

Variant	at 3 days		at 6 days	
	I%	category	I%	category
F. oxysporum/T. roseum	14.66	1	36	2
F. oxysporum/T. viride	24.33	1	57.3	3
F. oxysporum/ C. globosum	6.40	1	0	0
F. oxysporum/E. nigrum	5.60	1	28.99	2
F.oxysporum/F. oxysporum	0.00	0	0.00	0

CONCLUSIONS

Against the strain of fungus *Botrytis cinerea* isolated from blackurrant, only the strain of *Trichoderma viride* showed *in vitro* antagonism. The strains of *Trichothecium roseum*, *Chaetomium globosum* or *Epicoccum* nigrum have no antagonistic effect.

Against the fungus *Fusarium oxysporum*, the fungi which manifested antagonism, in range of the degree of their antagonistic action, were *T. viride*, *T. roseum* and *Epicoccum nigrum*. The fungus *C. globosum* was no antagonistic effect against *F. oxysporum*.

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