Effect of Fungicides on the Growth of Fungi Isolated from *in vitro* Propagated Fruit-Bearing Plants

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Abstract

The aim of the work was to determine the effect of fungicides: Topsin M 70 WG, Bravo 500 SC, and Sportak 45 EC in two concentrations on the growth of fungi: *Aspergillus niger*, *Epicoccum purpurascens*, *Cladosporium sphaerospermum, Mortierella isabellina* and *Penicillium verrucosum* var. *verrucosum* on PDA and Murashige-Skoog medium (MS). The smallest index of linear growth rate of the fungi had Topsin M 70 WG for fungi: *E. purpurascens* and *P. verrucosum* var. *verrucosum* on PDA and MS medium and for *M. isabellina* and *C. sphaerospermum* on MS in concentrations of 71.42 mg/l and 107.13 mg/l. The highest index of linear growth had Bravo 500 SC in concentrations of 0.5 ml/l and 0.75 ml/l on PDA for *E. purpurascens* and *A. niger*. The lowest index of linear growth on MS for *E. purpurascens*, *C. sphaerospermum* and *P. verrucosum* var. *verrucosum* was noted down. The highest effectiveness in control of linear growth all tested species of fungi was found for fungicide Sportak 45 EC in concentration of 0.55 ml/l and 0.825 ml/l on MS and PDA. Among all tested fungicides used *in vitro* propagation fungicides from imidazoles group are recommended.

Keywords: fungi; fungicides; *in vitro*; micropropagation

INTRODUCTION

In specific conditions of tissue cultures saprophytic species of fungi behave like "vitropathogens". Their metabolites change the pH value and the osmotic potential of the medium, the toxins poison the explant, and the enzymes macerate tissues. The Murashige-Skoog medium is most frequently used in *in vitro* cultures. The components of this medium can limit the growth of fungi (LEIFERT *et al*. 1994). The growth rate of fungi depends on the concentration of sugars in the medium (LILLY & BARNETT 1959). Fungicides help in the limitation or elimination of fungi in tissue cultures. The efficiency of a fungicide in protecting *in vitro* cultures is measured by the extent of its spectrum and lack in phytotoxicity (ZENKTELER 1998). Imidazoles show good fungistatic effects (SHIELDS *et al*. 1984).

The aim of the work was to determine the effect of fungicides added to media on the growth of fungi isolated from infected *in vitro* cultures of fruit-bearing plants.

MATERIAL AND METHODS

Five species of fungi were tested: *Aspergillus niger, Epicoccum purpurascens, Cladosporium sphaerospermum, Mortierella isabellina,* and *Penicillium verrucosum* var. *verrucosum* which infect explants of *in vitro* propagated fruit-bearing plants. The fungicides Topsin M 70 WG, Bravo 500 SC, and Sportak 45 EC at two concentrations (C_1 lower and C_2 higher one) and the potato-dextrose (PDA) and Murashige-Skoog (MS) media were used in the investigation. The prepared solutions of fungicides (Table 1) were introduced to the media. The PDA and MS media without fungicides were used as control combinations. Fungal inoculum was sampled from a two-week old colony and inoculated pointwise on the media. The size of colonies was measured at 48-hour intervals. The index of the linear growth rate of mycelium was calculated. Statistical calculations were carried out using analysis of variance for two-factor experiment. The significance of differences between averages was evaluated using the Duncan *t*-test.

RESULTS

Varied effects on the growth of the tested fungi characterized the fungicides introduced to the PDA and MS media. In comparison with the control both concentrations of Sportak 45 EC limited the growth of all the tested fungi on PDA and MS media.

The fungus *E. purpurascens* attained the highest value of the growth rate index on PDA medium with Bravo 500 SC at two concentrations and the lowest with Sportak 45 EC at C_2 concentration on MS and PDA media.

The fungus *C. sphaerospermum* attained the highest values of the linear growth index in the control combination on PDA and in combinations with Topsin M 70 WG and Bravo 500 SC at lower concentrations. The lowest value of the index was found in all the combinations with Sportak 45 EC.

The Topsin M 70 WG fungicide stimulated the growth of the fungus *A. niger* while only Sportak 45 EC decisively limited the growth of this vitropathogen on PDA and MS media.

The fungus *M. isabellina* showed the highest value of the linear growth rate index in control combinations and on PDA medium with the fungicide Bravo 500 SC and the lowest one on MS medium with Sportak 45 EC at both concentrations.

For the remaining fungi the values of the linear growth rate index are given in Table 1.

DISCUSSION

In the conducted experiments only Sportak 45 EC stunted the growth of all the tested fungi and Topsin M 70 WG limited the development of most of them. Imidazoles – among them Sportak 45 EC are characterized by a proper fungistatic effectiveness, a wide spectrum of effects, lack of phytotoxicity with regard to explants, and hence can be used in *in vitro* cultures (SHIELDS *et al*. 1984). In the *in vitro* cultures of *Synnignia* × *hybrida* Bravo 450 SC and Topsin M 70 WG showed fungicidal and bacteriostatic effects while Sportak 450 EC did not eliminate fungal infections (REBY 2001). In the investigations carried out by KOWALIK and GRÓDEK (2000) the fungicides Sportak 45 EC, Bravo 500 SC, and Topsin M 70 WG used for surface disinfection of strawberry explants prevented the occurrence of fungal infections on the explants and the MS medium. As HERMAN (1990) reports the MS medium is most frequently used at the initial stages of

Table 1. Effect of fungicides on the growth of fungi expressed by the index of the linear growth rate

Fungus	Medium	Topsin M 70 WG		Sportak 45 EC		Bravo 500 SC		Control	
		*C_1 71.42 mg	*C_2 107.13 mg	C_1 0.55 ml	C_2 0.825 ml	C_1 0.5 ml	C_2 0.75 ml	*K_1	*K_2
Epicoccum purpurascens	PDA	2.13 abc	2.56 abcd	1.56 ab	0.75 a	34.74 lm	36.67 lm	14.01 gh	14.01 gh
	MS	4.7 bcd	6.73 bcde	1.36 ab	0.46 a	34.74 lm	3.18 abcd	5.52 cde	5.52 abcde
Mortierella isabellina	PDA	6.98 def	6.9 cde	1.72 ab	0.48 a	17.06 hi	17.30 hi	31.57 l	31.57 k
	MS	3.81 abcd	1.81 abc	0.99 a	0.35 a	6.99 def	7.99 def	21.99 j	21.99 ij
Cladosporium sphaerospermum	PDA	17.82 i	12.88 fgh	0.36 a	0.14 a	13.56 g	8.88 efg	23.99 j	23.99 j
	MS	2.27 abc	0.72 a	0.24 a	0.16 a	2.92 abc	1.13 ab	9.24 f	9.24 efg
Aspergillus niger	PDA	43.89 o	41.71 m	0.13 a	0.67 a	32.59 l	33.79 kl	37.28 mn	37.28 lm
	MS	8.3 ef	17.75 hi	0.89 a	0.24 a	27.1 k	19.28 ij	38.79 n	38.79 lm
Penicillium verrucosum var. *verrucosum*	PDA	0.64 a	1.03 ab	0.67 a	0.84 a	2.73 abc	1.61 abc	2.89 abc	2.89 abcd
	MS	0.95 a	0.37 a	0.16 a	0.14 a	0.95 a	0.87 a	1.91 abc	1.91 abc

C_1 – the lower concentration of the fungicide in 1 l of the medium
C_2 – the higher concentration of the fungicide in 1 l of the medium
K_1 – control C_1
K_2 – control C_2

micropropagation and numerous species of fungi grow well on this medium. However, the results obtained by REBY and KOWALIK (2000) show that the MS medium efficiently limited the growth of fungi of the genera *Cladosporium, Mortierella, Epicoccum, Aspergillus, Fusarium,* and *Botrytis*. The faster growth of fungi of the genera *Alternaria, Aspergillus,* and *Penicillium* can be explained by their ability to metabolize components of the medium and reduce the pH value by producing phytotoxic metabolites. The results of the present study confirmed that the MS medium limited the growth of most fungi. Sportak 45 EC (at two concentrations tested) introduced to this medium efficiently limited the growth rate of vitropathogens.

Conclusion

In was determined that Sportak 45 EC controlled the growth of fungi isolated from *in vitro* cultures of fruit-bearing plants, showing that it could be introduced directly to the media at the stage of stabilization and multiplication of explants.

On PDA and MS media Sportak 45 EC showed strong fungicidal effects to the fungi *Aspergillus niger*, *Epicoccum purpurascens, Mortierella isabellina,* and *Penicillium verrucosum* var. *verrucosum* most frequently infecting explants of fruit-bearing plants.

Topsin M 70 WG introduced to MS medium showed controlling effects on the growth of most fungi tested.

Fungicides of the imidazole group can be recommended for use in tissue cultures.

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