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Original Article

In vitro Antifungal Activity of a New Bioproduct Obtained from Grape Seed Proanthocyanidins on *Botrytis cinerea* Mycelium and Spores

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Abstract

Botrytis cinerea is a necrotrophic fungus that affects over 200 plant species. In vineyards, this pathogen is responsible for one of the most important diseases, commonly known as botrytis bunch rot or grey mould. Keeping infection under control with synthetic fungicides leads to an increased biological resistance of pathogen populations. An alternative way to synthetic products is to obtain natural fungicides by using bioactive compounds of plants. This study focuses on the antifungal properties of a new bioproduct obtained from polymeric proanthocyanidins extracted from grape seeds of 'Fetească neagră' variety. The bioproduct in solid state presented a total content of polyphenols of 0.625 mg GAE mg^{-1}, a polyphenolic index of 17.40 and an antioxidant activity of 91.27% scavenged DPPH. The bioproduct with polyphenolic structure showed a moderate effect on the radial growth of fungal mycelium, at EC_{50} values between 11.23 and 12.15 mg mL^{-1}. Effective antifungal activity was showed in the inhibition of spore germination, where the EC_{50} values varied from 1.14 to 1.47 mg mL^{-1}. These *in vitro* results sustain the possibility of including the bioproduct in the category of natural fungicides for biological control against *Botrytis cinerea* fungus.

Keywords: biological activity; minimum fungicidal concentration; natural fungicide; necrotrophic fungus; polyphenolic compounds

Introduction

In vineyards, among other pathogenic species, *Botrytis cinerea* Pers. is the most aggressive specie, causing significant economic damages (Gatto, 2011). Control of infection with *Botrytis cinerea* is currently performed with synthetic fungicides, but their danger has been proven, both for consumers and the environment due to the long time required for degradation (Lingk, 1991). Also, their frequent use in treatments promotes the emergence of resistant strains of *Botrytis cinerea* (Elad and Evensen, 1995). An alternative path to the chemical control of pathogenic fungi was outlined by focusing the research on bioactive components of plants, especially essential oils, to obtain extracts whose antifungal activity has been proven (Tripathi and Dubey, 2004, Lee *et al*., 2005; Tripathi *et al*., 2008; Sokovic *et al*., 2009; Wang *et al*., 2010; Vitoratos *et al*., 2013; Kocić-Tanackov *et al*., 2013; Seșan *et al*., 2015).

In plant defense system against pathogenic fungi, phenolic compounds are directly involved. These bioactive compounds are secondary metabolites synthesized by plants under stress conditions (i.e. injuries, infections, UV radiation), showing different chemical structures, from simple molecules (phenolic acids) to complex structures (i.e. flavanols, flavonols, anthocyanins, stilbens) (Ahmed *et al*., 2017). In grapes, phenolic compounds are found mainly in skins and seeds (Ky *et al*., 2012). In response to *B. cinerea*

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infection, phenolic phytoalexins are synthesized in grapes, mainly stilbens, with high antifungal activity (Goetz *et al*., 1999; Chong *et al*., 2009; Xu *et al*., 2018a).

In vitro antifungal activity of phenolic compounds on *B. cinerea* spore germination and mycelium growth has been extensively studied. Recent studies suggest that plant proanthocyanidins maintain *B. cinerea* in a quiescent stage, leading to delayed development of the symptoms (Van Baarlen *et al*., 2007). Moreover, plant proanthocyanidins may act as competitive inhibitors of *B. cinerea* laccase, thereby preventing detoxification of the phenolic phytoalexin (Goetz *et al.* 1999; Ky *et al*., 2012).

Mendoza *et al*. (2013) demonstrated *in vitro* inhibitory effect of polyphenols extracted from Cabernet Sauvignon grape pomace (skins and seeds) on *B. cinerea* mycelium growth and germ tube elongation. Tao *et al*. (2010) have evaluated *in vitro* six phenolic compounds for their antifungal activity and mode of action on *B. cinerea*. Catechin and quercetin-3-galactoside showed linear inhibitory effects on germ tube elongation, while gallic acid showed very strong and linear inhibition on spore germination, but the effect diminished after spore germination.

Patzke and Schieber (2008) used five phenolic compounds as active ingredients in the preparation of a bioactive emulsion and screened for their ability to inhibit the growth of phytopathogenic fungi. Ferulic acid was identified as highly effective against the growth of *B. cinerea* in very low concentrations (0.085%).

Pathogen inhibition mechanisms vary depending on phenolic compounds tested, effective results can be obtained in the future by associating the ability of these compounds to inhibit both mycelium growth and spore germination.

Teixeira *et al*. (2014) highlighted the importance of phenolic compounds resulting as waste of winemaking industry, recommending their use in preparations with multiple biologically active properties. Grape seeds are a rich source of extractable phenolic compounds of which polymeric proanthocyanidins (PA) or condensed tannins, represent 75-81% of the total flavan-3-ols (Monagas *et al*., 2003). Our work aimed the recovery of this complex group of phenolic compounds from grape seeds of 'Fetească neagră' variety. As a result of the extraction procedures, was obtained a polyphenolic preparation in solid state, insoluble in water, but soluble in alcohol, with the yield of 30 g kg^{-1} dry weight. The phenolic structure of the preparation was determined by fractionation on C18 cartridges and thin layer chromatography (TLC), which allowed the identification and quantification of three fractions of proanthocyanidins: monomers (5.20 mg 100 g^{-1} d.w.), oligomers (11.05 mg 100 g^{-1} d.w.) and polymers (74.90 mg 100 g^{-1} d.w.) (Filimon *et al*., 2017). Vegetal waste resulting from extraction of phenolic compounds was used as organic fertilizer in soil (Nechita *et al*., 2017b).

The solid water-insoluble polyphenolic preparation was treated with hydrogen peroxide. After this treatment, polymers fragmentation resulted in a new water-soluble bioproduct with phenolic structure. The induction of water solubility properties allowed the evaluation of the antibacterial (Nechita *et al*., 2017a) and antitumoral activities of the new bioproduct.

The aim of this paper was to evaluate the *in vitro* antifungal activity of the water-soluble bioproduct obtained from grape seed proanthocyanidins, on the mycelial growth and spore germination of three *B. cinerea* strains.

Materials and Methods

Plant material

Grape seeds resulting from the winemaking process of the 'Fetească neagră' grapes were manually extracted from the grape pomace, dried at room temperature, milled and defatted with n-hexane, in order to extract the phenolic compounds.

Extraction of polymeric proanthocyanidins (PA)

The extraction of polymeric PA was done with ethanol 96%, in a solid:solvent ratio of 1:4 (w/v), in three stages, at 30 °C. The polyphenolic extracts were cumulated and concentrated in vacuum at 37 °C, up to a density of 1.0 g cm^{-3}. Precipitation of polymeric PA was performed with 500 mL of 99% diethyl ether. The crude preparation of polymeric PA was soluble in 96% ethanol and insoluble in water. The induction of water solubility properties was conducted by the procedure proposed by Lupaşcu and Lupaşcu (2006), respectively, the treatment of the purified preparation of polymeric PA with 30% hydrogen peroxide under determined conditions: ratio preparation (PA) / oxidizing agent 1:2.5 (w/w), temperature 67.5 °C, time of contact 45 min, and removal of the oxidizing agent at 40 °C to constant weight. Obtained bioproduct was stored at -18 °C until assessing its biologically active properties.

For the characterization of the water-soluble bioproduct were determined its total polyphenolic content (TPC), polyphenolic index (PI) and antioxidant activity (AA).

Total polyphenolic content was analysed by Folin-Ciocalteu reagent, according to the protocol proposed by Singleton *et al*. (1999). Gallic acid was used as standard ($y = 1.3243 x + 0.0162$; $R^2 = 0.9991$), and the results were expressed as mg gallic acid equivalent (GAE) mg^{-1} bioproduct.

Polyphenolic index (A_{280}) was determined by measuring the absorption of phenol benzene cycles at 280 nm, using a Shimadzu UV-VIS spectrophotometer (UV-VIS Mini Series 1240). Extracts were diluted 1/100 and optical density (OD) was measured on a 10 mm optical path: $A_{280} = OD_{280} \times$ dilution (Ribéreau-Gayon *et al*., 2006).

Antioxidant activity was determined according to the method proposed by Brand-William *et al*. (1995). Stock solution: 4.00 mg of 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical in 100 mL of 96% ethanol. From the 1 mg mL^{-1} solution were taken 100 µL to which 3.9 mL of DPPH solution was added. The control was obtained from 100 µL of 96% ethanol and 3.9 mL of DPPH. The homogenized samples were incubated at 35 °C for 30 minutes. Absorbance of control samples and analysed samples were determined spectrophotometrically at 517 nm. Antioxidant activity (AA) was calculated according to the formula: $AA = [(A_{control}-A_{sample})/A_{control}] \times 100$; where A represents the absorbances at 517 nm.

HPLC procedure

Characterization and quantification of individual polyphenolic compounds was conducted using the protocol presented by Castelari *et al.* (2002), by means of a Shimadzu LC-20 ultra-high-performance liquid chromatography system (UHPLC), coupled with a diode array detector (DAD) Shimadzu SPD-M30A. All samples were filtered through 0.45 μm syringe filters. The chromatography precolumn used was a C18 Security Guard® Ultra 4.6 mm, coupled with a capillary C18 column Kinetex® Phenomenex 2.6 μm, 150×4.6 mm, thermostated at 20 °C, 10 μL of injection volume. Mobile phase (pH 2.15) was represented by 65 mM TFA in 1% methanol as eluent A and 65 mM TFA in 50% methanol as eluent B, with a flow rate of 0.85 mL/min. The HPLC gradient was performed as follows: 100% solvent A for the first 11 min, 82% A / 18% B from 11 to 19 min, 70 % A / 30% B from 19 to 25 min, 65% A / 35% B from 25 to 33 min, 40% A / 60% B from 33 to 43 min, 20% A / 80% B from 43 to 46 min, 100% B from 46 to 53 min, 100% A from 53 to 60 min. Detection was done at 280 nm based on spectral characteristics, while compounds quantification was performed by using pure external standards.

In vitro evaluation of antifungal activity

The antifungal activity of the bioproduct was determined *in vitro* by mycelial growth and spore germination inhibition. The assays were performed on three strains of *Botrytis cinerea*: BC_5, BC_6 and BC_8, from the collection of microorganisms of Laboratory of Wine Microbiology belonging to the Research and Development Station for Viticulture and Winemaking Iasi, Romania.

The inhibition of mycelium radial development was performed in Petri dishes with potato dextrose agar (PDA) medium, containing different concentrations of bioproduct between 1 and 21 mg mL^{-1}. For each *B. cinerea* strain, from the margins of the seven days old colonies, were collected 5 mm diameter mycelium discs and placed in the center of the Petri dishes with PDA medium supplemented with the bioproduct concentrations and at the center of the control plates with PDA medium without bioproduct. Three dishes were used for each test concentration. Petri dishes were incubated at 24 °C for five days. After this time, the diameter (mm) of the colonies were measured in the control dishes and in the dishes supplemented with bioproduct. Inhibition (%) of the radial development of the colonies was calculated according to the formula: $I\% = [(dc-dt)/dc] \times 100$, where: dc represents fungal diameter of the control and dt is the fungal diameter of the tested concentrations.

The spore suspension was obtained from PDA cultures of *B. cinerea* strains developed at room temperature, under fluorescent light, for 14 days (Richard *et al.*, 2002). Preparation of the spore suspension was performed by introducing 20 mL of sterile distilled water containing 0.1 mL L^{-1} Tween 20 on the Petri dishes, and tender displacement of the spores with an inoculating loop. The spore suspension was filtered through four layers of sterile gauze to remove mycelial remains. In the spore germination inhibition test, was used a spore suspension of 10^3 spores mL^{-1} numbered using a haemocytometer (Vitoratos *et al.*, 2013). Thus, 0.1 mL of the 10^3 spores mL^{-1} suspension roughly contained 100 spores that were placed on the surface of the PDA medium, in Petri dishes containing the bioproduct in concentrations of 1 to 12 mg mL^{-1}. Three plates were used for each test concentration. All Petri dishes were incubated at 24 °C until visible spore germination (48 h). The germinated spores were counted for each sample, as well as for the control.

Antifungal activity of the bioproduct was evaluated as minimum fungicidal concentration (MFC), determined as the lowest concentration of bioproduct that inhibited mycelial growth or spore germination, and as the effective concentrations that inhibits 50% of the mycelium growth or germinated spores (EC_{50}).

Statistical procedures

Data were reported as mean of minimum three replicates. Analysis of variance ANOVA test was initiated to investigate significant differences between strains. Tukey's test was used to discriminate among the means (XLStat® software). Different letters indicate significant differences ($p \leq 0.05$). Regression analysis was performed to look for relationships between data.

Results and Discussion

Bioproduct characterisation

The physico-chemical characteristics of the phenolic bioproduct are showed in Table 1. The bioproduct, obtained through controlled oxidation of the water-insoluble polymeric PA preparation appears as yellow-brown crusts, soluble in distilled water, with a total amount of polyphenolic compounds of 0.625 mg GAE mg^{-1}, with an antioxidant activity of 91.37% scavenged DPPH per 100 μg.

The UHPLC analysis highlighted a large number of signals represented by peaks of different amplitudes (Fig. 1). Based on the specific standards, were identified and quantified six phenolic compounds: gallic acid (17.53 mg g^{-1}), protocatehuic acid (5.29 mg g^{-1}), para-hydroxybenzoic acid (0.89 mg g^{-1}), syringic acid (4.09 mg g^{-1}), ferulic acid (0.48 mg g^{-1}) and trans-resveratrol (0.52 mg g^{-1}).

Antifungal activity of the bioproduct on Botrytis cinerea mycelium growth

Control colonies of *B. cinerea* strains showed different diameters on simple PDA medium: 8.56 cm for BC_5, 9.03 cm for BC_6 and 8.68 cm for BC_8. Depending on the measured diameter for the control and each tested concentration of bioproduct, was calculated the mycelium growth inhibition (%) for each fungal strain (Table 2).

Table 1. Physico-chemical characteristics of the phenolic bioproduct

Bioproduct	Physical condition	Colour	Solubility in distilled water	TPC (mg GAE mg^{-1})	PI 280 nm	AA (% DPPH•)
	solid	yellow - brown	completely	0.63 ± 0.08	18.40 ± 0.37	91.37 ± 0.68

Note: TPC: total polyphenolic content; PI: polyphenolic index; AA: antioxidant activity.

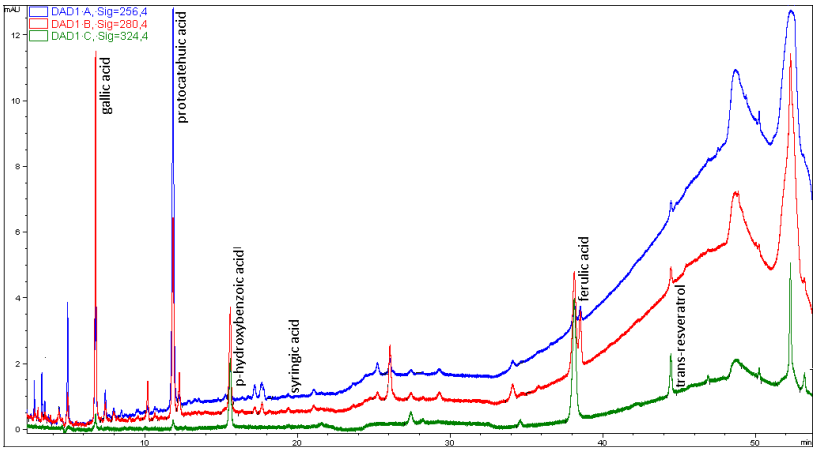

Fig. 1. HPLC chromatogram of the water-soluble bioproduct

Data analysis shows that the inhibition (%) of mycelium growth increased progressively as the bioproduct concentration increased. Inhibition of mycelium growth, assessed by MFC, was determined at bioproduct concentrations between 18 and 21 mg mL^{-1}. To determine the fungicidal spectrum, EC_{50} was calculated by linear regression. Coefficients of determination (R^2), indicating the relationship between tested concentrations and the inhibition values, were high, due to a high degree of linearity between variables (see Table 2).

The sensitivity of *B. cinerea* strains to tested bioproduct was significantly different ($p \leq 0.05$). Thus, the half maximal effective concentration (EC_{50}) for mycelium growth of BC_5 and BC_6 strains was 11.23 and 11.66 mg mL^{-1} respectively, while for BC_8 strain the EC_{50} was 12.15 mg mL^{-1}.

During the experiment, the observations made on the morphology of *B. cinerea* mycelium indicated that the increasing of bioproduct concentrations resulted in the occurrence of larger degenerative areas (Fig. 2). This effect can be attributed to the presence of some phenolic compounds that affect the structure of the hyphae. Morphological changes of mycelial hyphae have been showed by Wang *et al.* (2010), by testing the antifungal activity of eugenol (2-methoxy-4-allylphenol), a compound with phenolic structure. In its presence, occurred various structural modifications of the hyphae, leading to the clotting of the cell cytoplasm, contraction of the hyphae and alteration of cytoplasmic membrane permeability.

In our experiment, the evaluation of the bioproduct activity on the radial growth of *B. cinerea* mycelium was performed by placing the mycelial inoculum on PDA medium with different concentrations of bioproduct (Fig. 3). Thus, by direct contact with the phenolic structure of the bioproduct, the enzyme complex synthesized in the mycelium favoured its development. The invasive manifestation of fungal mycelium at low concentrations, but blocked at higher concentrations of the bioproduct, allows the assessment of its antifungal activity as moderate, this assumption being supported by the data presented in the literature. During *B. cinerea* infection on grapes, the development of mycelium on the surface and inside the skin leads to direct contact between their constituents. Numerous studies have shown that hyphae of *B. cinerea* synthesizes laccases (EC 1.10.3.2), extracellular enzymes that catalyse the oxidation of phenolic compounds (Bollag and Leonowickz, 1984; Oszmianski *et al.*, 1985; Pezet, 1998; Osman *et al.*, 2007). Ky *et al.* (2012) evaluated the impact of *B. cinerea* infection on phenolic compounds concentration, in healthy and naturally or artificially infected grape skins, and found a severe decrease in phenolic compounds in infected grapes.

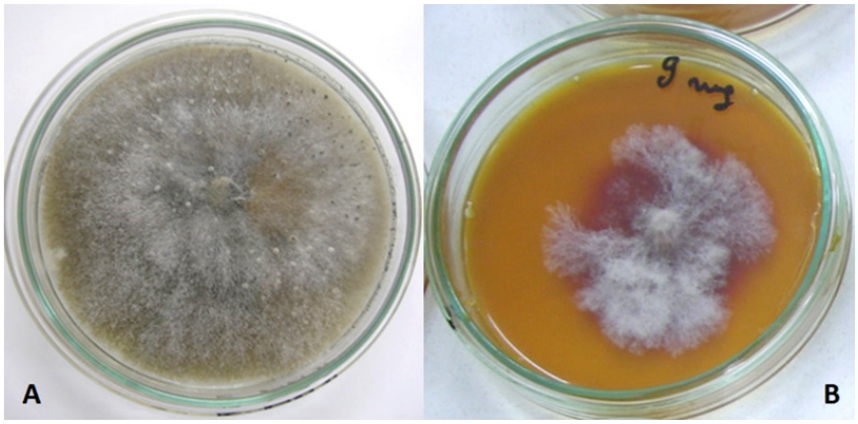

Fig. 2. Effect of the bioproduct on the development of *Botrytis cinerea* mycelium. Note: A: control (PDA medium); B: sample (PDA medium with bioproduct)

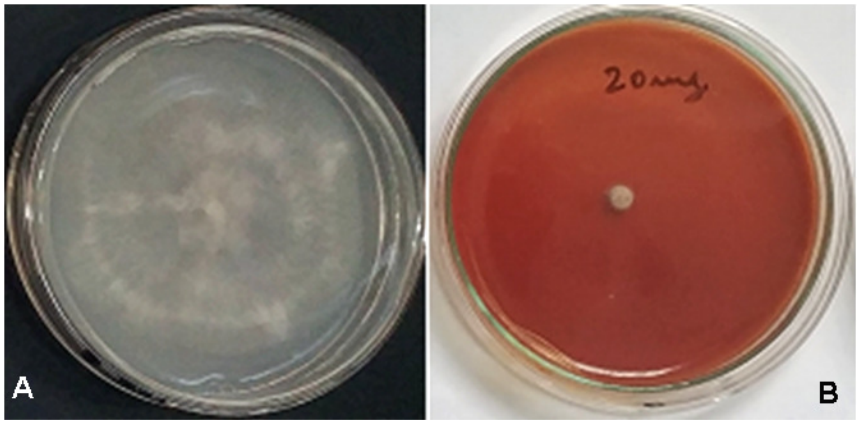

Fig. 3. Control (PDA medium) (A) and the concentration of bioproduct where the *Botrytis cinerea* mycelium growth was totally inhibited (20 mg mL^{-1}) (B)

Table 2. Biological activity of the bioproduct on *Botrytis cinerea* mycelium growth

Bioproduct concentration (mg mL^{-1})	BC_5		BC_6		BC_8		Significance
	Colony diameter (cm)	Inhibition (%)	Colony diameter (cm)	Inhibition (%)	Colony diameter (cm)	Inhibition (%)	
Control	8.56^{a}	0.00	9.03^{a}	0.00	8.66^{a}	0.00	***
7	5.75^{b}	0.00	6.80^{b}	0.00	5.85^{b}	32.44	***
8	5.25^{bc}	38.66	6.45^{bc}	28.57	5.70^{bc}	34.18	***
9	5.06^{bcd}	40.83	5.92^{bcd}	34.44	5.45^{bc}	37.06	**
10	4.74^{bcd}	44.58	5.23^{cde}	42.10	5.26^{bcd}	39.22	**
11	4.47^{cde}	47.75	4.87^{de}	46.09	4.91^{bcde}	43.30	*
12	4.13^{cde}	51.75	4.45^{ef}	50.75	4.65^{cde}	46.34	**
13	3.87^{def}	54.76	4.10^{efg}	54.62	4.25^{def}	50.92	**
14	3.53^{efg}	58.76	3.38^{fgh}	62.62	3.81^{fgh}	56.03	***
15	2.90^{fgh}	66.12	2.83^{ghi}	68.67	3.37^{gh}	61.12	**
16	2.50^{gh}	70.79	2.27^{hij}	74.82	3.06^{hi}	64.70	***
17	2.17^{hi}	74.64	1.84^{ij}	79.64	2.59^{hi}	70.04	***
18	1.70^{hi}	80.14	0.92^{jk}	89.85	2.25^{ij}	74.01	***
19	1.10^{ij}	87.14	0.00^{k}	100.00	1.90^{jk}	78.01	***
20	0.00^{j}	100.00	0.00^{k}	100.00	0.99^{jk}	88.52	**
21	0.00^{j}	100.00	0.00^{k}	100.00	0.00^{k}	100.00	n/s
LREq	y = 4.6882x - 2.6667		y = 6.0124x - 19.941		y = 4.5358x - 5.1078		-
CD	0.9707		0.9888		0.9653		-
EC_{50}	11.23		11.66		12.15		-

Note: n/s, *, **, *** indicate non-significant and significant differences between strains at $p \leq 0.05$, 0.01, 0.001 in ANOVA test. Different letters within the same column indicate significant differences in Tukey test ($p \leq 0.05$). LREq: Liniar regression equation; CD: coefficient of determination; EC_{50}: half maximal effective concentration.

Antifungal activity of the bioproduct on Botrytis cinerea spore germination

Spore germination of BC_5, BC_6 and BC_8 strains started after 20 hours, in the case of control (PDA without bioproduct). In Petri dishes with PDA medium, where the tested concentrations of the bioproduct were introduced, the spore germination was visible after 48 hours. These findings suggest that the tested bioproduct is particularly active on the inhibition of spore germination, mainly due to the strong action of phenolic compounds on the growth and elongation of spore germ tube, as was previously reported by Nassr and Barakat (2013).

Depending on the number of germinated spores for the control and each bioproduct concentration (Fig. 4), the percentage of spore germination inhibition was calculated for each tested strain (Table 3).

In the antifungal activity test on *B. cinerea* spore germination was found that the number of germinated spores decreased progressively with the increase of the bioproduct concentrations. MFC values varied into small limits, inhibition of spore germination being performed at 10 mg mL^{-1} for BC_5 and BC6 strains and 12 mg mL^{-1} for strain BC_8. Also, the EC_{50} varied depending on the strain of *B. cinerea* used. Half maximal effective concentration (EC_{50}) of bioproduct on spore germination was achieved at concentrations of 1.18 mg mL^{-1} for BC_5, 1.26 mg mL^{-1} for BC_6 and 1.42 mg mL^{-1} for BC_8 strain, confirming a superior resistance of BC_8 strain to bioproduct action (see Table 3).

Table 3. Biological activity of the bioproduct on *Botrytis cinerea* spore germination

Bioproduct concentration (mg mL^{-1})	BC_5		BC_6		BC_8		Significance
	Germinated spores	Inhibition (%)	Germinated spores	Inhibition (%)	Germinated spores	Inhibition (%)	
Control	115^{a}	0.00	110^{a}	0.00	125^{a}	0.00	**
1	71^{b}	38.26	69^{b}	37.27	82^{b}	34.40	***
2	51^{c}	55.65	53^{c}	51.82	62^{c}	50.40	**
3	42^{cd}	63.48	39^{cd}	64.55	48^{cd}	61.60	***
4	30de	73.91	28de	74.55	38de	69.60	**
5	24^{ef}	79.13	20^{ef}	81.82	32^{ef}	74.40	**
6	17^{fg}	85.22	12^{fg}	89.09	25^{efg}	80.00	***
7	10^{gh}	91.30	9^{fg}	91.82	18^{fgh}	85.60	***
8	5^{h}	95.65	4^{h}	96.36	11^{ghi}	91.20	***
9	2^{hi}	98.26	1^{hi}	99.09	8^{hi}	93.60	***
10	0^{i}	100.00	0^{i}	100.00	4^{hi}	96.80	**
11	0^{i}	100.00	0^{i}	100.00	2^{i}	98.40	n/s
12	0^{i}	100.00	0^{i}	100.00	0^{i}	100.00	n/s
LREq	y = 6.5033x + 42.319		y = 6.7493x + 41.515		y = 5.5015x + 42.182		-
COF	0.9323		0.9105		0.9145		-
EC_{50}	1.18		1.26		1.42		-

Note: n/s, *, **, *** indicate non-significant and significant differences between strains at $p \leq 0.05$, 0.01, 0.001 in ANOVA test. Different letters within the same column indicate significant differences in Tukey test ($p \leq 0.05$). LREq: Liniar regression equation; CD: coefficient of determination; EC_{50}: half maximal effective concentration.

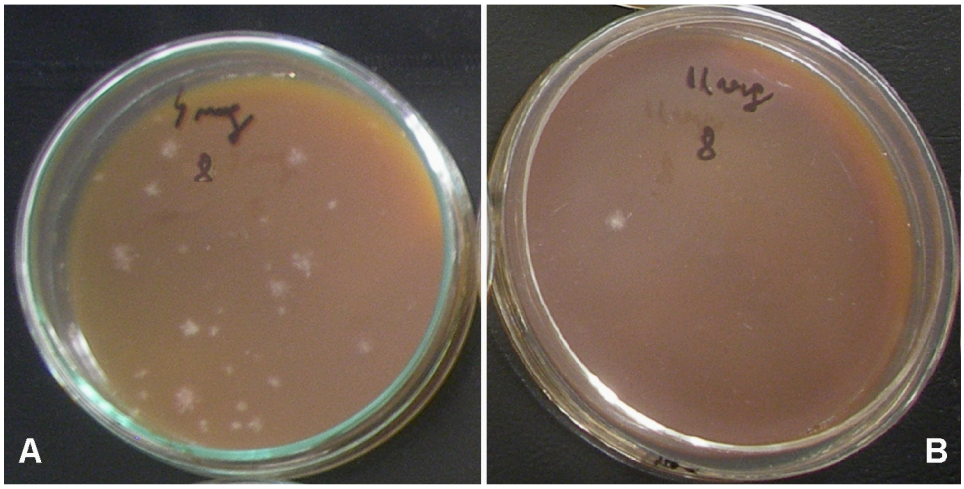

Fig. 4. *Botrytis cinerea* spores germination at low concentrations of bioproduct (A) and at minimum fungicidal concentration (B)

The coefficients of determination (R^2), obtained by correlating the bioproduct concentrations and the values for spore germination inhibition, were high (0.9323, 0.9105 and 0.9145), due to a high degree of linearity between variables.

Fourie and Holz (2001) studied the antifungal activity of the synthetic product Folpet, on a number of 71 *B. cinerea* strains. The authors showed a different manifestation of the synthetic product on mycelium and spores, the EC_{50} values for the inhibition of spore germination were significantly lower than those obtained in the inhibition of mycelium growth. This aspect was also showed in the present study, when the new phenolic bioproduct obtained from grape seeds was tested for its antifungal activity.

Increasing the antifungal potential of bioproduct against *B. cinerea* may be possible through a synergistic effect, achieved by its interaction with plant extracts, whose antifungal potential has been proven, or synthetic fungicides. A recent approach in this regard was performed by Xu *et al.* (2018b).

Conclusions

Polymeric proanthocyanidins extracted from grape seeds can be used as a biologically active product after inducing the water-solubilisation properties by oxidative treatment with hydrogen peroxide. Biological activity of the phenolic bioproduct on *Botrytis cinerea* mycelium growth was manifested at moderate MFC values, between 18 and 21 mg mL^{-1}. However, inhibition of spore germination was achieved at concentrations approximately ten times lower than those obtained for the mycelium growth inhibition, between 1.14 and 1.47 mg mL^{-1}, results that justify the possibility of using the phenolic bioproduct as potential natural agent for the biological control of *Botrytis cinerea*, as an alternative to currently used synthetic products that pose a risk to human health and the environment.

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