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ANTIFUNGAL ACTIVITY OF SOME PLANT EXTRACTS AGAINST *Botrytis cinerea* Pers. IN THE BLACKCURRANT CROP (*Ribes nigrum* L.)

Tatiana Eugenia Şesan[1], Elena Enache[1], Beatrice Michaela Iacomi[2], Maria Oprea[3], Florin Oancea[4], Cristian Iacomi[2]

[1]University of Bucharest, Romania

[2]University of Agricultural Sciences and Veterinary Medicine Bucharest, Romania

[3]Research-Development Institute of Plant Protection Bucharest, Romania

[4]National Research-Development Institute for Chemistry and Petrochemistry Bucharest, Romania

Abstract. There were tested and screened, *in vitro* and *in vivo*, for the first time in Romania, nine respectively six plant extracts manufactured by Hofigal S.A. against Botrytis cinerea (strain Bc 27) isolated from blackcurrant (*Ribes nigrum* L.). The highest antibotrytis *in vitro* activity (efficiency between 80 and 100%) was obtained using the following extracts: Hyssopus officinalis (at 20, 10 and 5%), *Satureja hortensis*, *Allium sativum*, *Tagetes patula* (at 20 and 10%) and *Mentha* (at 20%). A moderate antibotrytis activity (efficiency between 35.7 and 65.7%) has been noticed for *Mentha* (at 10 and 5%), *Satureja hortensis*, *Allium sativum* and *Tagetes patula* (at 5%) extracts. The lowest antibotrytis activity or no efficiency was noticed using extracts obtained from *Achillea millefolium*, *Artemisia dracunculus* 'sativa', *Rosmarinus officinalis* and *Valeriana officinalis* even applied at 20%. Based on results obtained in *in vitro* tests, six plant extracts were tested and screened *in vivo*, under field conditions at Hofigal S.A. Bucharest. *Satureja hortensis*, *Allium sativum*, *Hyssopus officinalis*, *Mentha*and *Tagetes patula* extracts have been efficient in limiting gray mold severity in blackcurrant applied at 10% compared to untreated control. No *in vivo* activity was registered for *Valeriana officinalis* extract. Plant extracts with highly efficiency can be recommended as a non-polluting and environmental-friendly alternative (organic horticulture) in the protection of blackcurrant as medicinal crop against grey mould, the most economically important disease in Europe at present.

Key words: blackcurrant, *Botrytis cinerea*, plant extracts, organic horticulture, Romania

Corresponding author: Tatiana Eugenia Şesan, Department of Botany and Microbiology, Biology Faculty, University of Bucharest; 1–3 Portocalelor Alley, sector 6, 060101 Bucharest 35, Romania, e-mail: tatianasesan@yahoo.com

INTRODUCTION

Botrytis cinerea Pers. is a typical high risk necrotrophic parasite, responsible for grey mould, one of the most important plant diseases in Europe. The fungus has a broad host range and can infect over 230 plant species [Elad and Evenses 1995, Şesan 2003, Gonzáles-Collado et al. 2006, Şesan and Tănase 2007, 2011, Enache 2013]. This pathogen causes major economic damages both in pre- and post-harvest [Coley-Smith et al. 1980, Elad et al. 2004, El Oirdi and Bouarab 2007] sometimes reaching high levels, up to 55% of harvested grapes [Martínez-Romero et al. 2007].

The control of this pathogen remains a challenge and is still based upon multiple applications of fungicides. Chemical control is effective and efficient but, at the same time, can leads to the development of pathogen resistance, chemical residues in fruit, phytotoxicity to other organisms or environmental and public health problems [Beever et al. 1989, Brent and Hollomon 1998, Vali and Moorman 1992, Elad et al. 1992, Hébért et al. 2002, Leroux et al. 2002, Adebayo et al. 2013]. In order to minimize these factors and also to comply with food safety standards, there is an increased interest for bio-ecology studies of this pathogen and a worldwide trend to explore new alternatives to synthetic fungicides.

Among alternative methods of grey mould control, the use of natural compounds as plant extracts is one which can be characterised by lack of toxicity for humans and environment, selectivity, biodegradable activity and a great variety of chemical composition, with a large variety of secondary metabolites, most of them not yet studied in correlation with their fungicidal action.

Many researches focused on *B. cinerea* control: Shimoni et al. 1993, Saks and Barkai-Golan 1995, Arras et al. 1995, Carta et al. 1996, Reddy et al. 1997, Han et al. 2000, Ozcan and Boyraz 2000, Lee et al. 2001, 2005, Park et al. 2002, Sas-Piotrowska and Piotrowski 2002, Chebli et al. 2004, Plotto et al. 2003, Alkhail 2005, Romagnoli et al. 2005, Shen et al. 2005, Soylu et al. 2005, 2010, Martínez-Romero et al. 2007, Nikos and Economakis 2007, Tzortzakis and Economakis 2007, Coetzee et al. 2008, Mendoza et al. 2008, Patkowska 2008, Ribeira et al. 2008, Camele et al. 2010, Roy and Chatterjee 2010, Tao et al. 2010, Bi YaLing et al. 2011, Wahmare et al. 2011, Shaymaa et al. 2012, Vio-Michaelis et al. 2012, Adebayo et al. 2013, Mogle 2013 and many others.

Also, researches were done on some natural chemical components with antifungal activity to plant pathogens, these results being synthesized in book chapters or reference books [Cutler et al. 1996, Davidson and Naidu 2000, Toncea and Stoianov 2002, Huang and Chung 2003, Şesan 2003, Copping and Duke 2007, Rai et al. 2011, Dubey 2011], doctoral thesis [Ivănescu 2010] and numerous articles.

Although the volume of publications regarding experimental researches of plants extracts on grey mold is remarkable, few products were launched on the agricultural market to be used in agricultural practice. Among these can be mentioned BM-608 (essential oils from *Melaleuca alternifolia*) [Reuveni et al. 2009] or Gloves Off® from Thymus oils, manufactured by Planet People and Laboratoire M2, INC Sherbrooke, QC, Canada [Adebayo et al. 2013]. This direction for investigations and development is to be used for further research.

The objectives of the present study were: (i) find a non-pollutant alternative solution to synthetic fungicides used to control B. cinerea in blackcurrant, as medicinal crop; (ii) testing and selecting some plant extracts manufactured by Hofigal S.A. Bucharest which best fit the control of B. cinerea and (iii) extension of medical use of natural plant extracts, manufactured by Hofigal S.A. Bucharest, such as plant protection agents as an alternative to chemical fungicides.

MATERIALS AND METHODS

In vitro tests were conducted using one strain of Botrytis cinerea (Bc 27) isolated at ICDPP Bucharest from blackcurrant leaves (*Ribes nigrum* L.) from experimental station of Hofigal S.A.

Nine plant hydroalcoholic extracts were used in *in vitro* tests: *Hyssopus officinalis*, *Tagetes patula*, *Rosmarinus officinalis*, *Satureja hortensis*, *Allium sativum*, *Artemisia dracunculus* 'Sativa', *Valeriana officinalis*, *Achillea millefolium* and *Mentha* sp. (tab. 1). These plants were selected based on i) their antimicrobial action (reported in scientific and medical literature); ii) capacity to synthesize fungicide analogues; iii) amount of obtained biomass and iiii) reduced economical costs. The extracts were manufactured by Hofigal S.A. from stems, leaves, flowers, sprouts and bulbs, harvested at recommended time. Stock solutions were prepared for each plant extract. Aliquots of stock solutions were incorporated to PDA medium to provide final concentrations of 20, 10 and 5%.

The biological activity of plant extracts was evaluated on mycelial growth of Bc 27 isolate. Mycelial disks of pathogens (8 mm in diameter) removed from the margins of a 7 days old culture were transferred to PDA media containing the plant extracts at tested concentrations. Three replicates were used per treatment. For each plant extract and concentration, inhibition of radial growth compared with the untreated control was calculated after 7 days of incubation at 24ºC, in the dark.

Table 1. Plant species as source of extracts

Plant species	Part used	Harvesting	*In vitro* test	*In vivo* test
Achillea millefolium L.	flowers	VI–VII	+	
Allium sativum L.	bulbs	X–XI	+	+
Artemisia dracunculus 'Sativa' L.	stems, leaves	VI–VIII	+	
Hyssopus officinalis L.	stems, leaves	VI–VII	+	+
Mentha sp.	leaves	VI–VIII	+	+
Rosmarinus officinalis L.	stems, leaves	V–VI	+	
Satureja hortensis L.	stems, leaves	VII–VIII	+	+
Tagetes patula	flowers	VI–VII	+	+
Valeriana officinalis L.	stems, leaves	VI–IX	+	+

Results were expressed as efficiency of the plant extract (inhibition rate of mycelial growth compared to untreated control) and as effective concentrations EC50 and EC90 (the concentration which reduced mycelial growth by 50 or 90%) determined by regressing the inhibition of radial growth values (% control) against the values of the fungicide concentrations.

In vivo tests. The efficiency of plant extracts against B. cinerea was tested in the production and experimental fields of Hofigal S.A. and S.C.P.P. Baneasa-Bucuresti, during 2013. Extracts of *A. sativum*, *H. officinalis*, *Mentha* sp., *S. hortensis*, *T. patula* and *V. officinalis* were used, selected based on results obtained in *in vitro* tests. Three treatments were applied, at 10%, in correlation with plant phenophase: (i) after flowering, (ii) at fruit setting and (iii) beginning of fruit ripening. The degree of attack on leaves was calculated based on frequency and disease severity, in natural infection conditions. The efficiency of treatments has been calculated using Abbot formula: Efficiency % = 100 – Z; Z = attack degree of variant × 100/ attack degree of control.

RESULTS

Biological activity of plant extracts – *in vitro* assay. The mycelial growth of the Botrytis cinerea isolate has been influenced differently by the nine tested plant extracts (tab. 2, fig. 1).

The highest efficiency (100%) against B. cinerea was registered for *Hyssopus officinalis* extract, at all tested concentrations, followed by *Satureja officinalis* extract, with the same efficiency but only in higher concentrations (10 and 20%). The extracts of *Mentha*and *Allium sativum* have got maximum efficiency only in very high concentration (starting and above 20%). In all of the cases the mycelial growth of Bc 27 isolate was totally stopped and sporulation was absent. Very close to the maximum value of efficiency (95.7%) was obtained by *Artemisia dracunculus* 'Sativa' extract at 10%. High levels of efficiency (from 80 to 88.5%) were recorded when *Tagetes patula* extract was used, at 20 and 10% respectively. In all these cases mycelial growth values ranged from 8 to 14 mm and sporulation was absent.

A moderate efficiency (values from 60.0 to 65.7%) was obtained with *Satureja hortensis* (at 5%), *Mentha*(at 10%) and *Tagetes patula* (at 5%) extracts. A reduced efficiency (values from 35.7 to 42.8%) was registered using *A. sativum* and *Mentha*extracts (at 5%). In these cases the mycelial growth ranged from 40 to 45 mm.

The lowest or even no efficiency to B. cinerea, was obtained with *Rosmarinus officinalis*, *Valeriana officinalis*, *Achillea millefolium*, *Artemisia dracunculus* 'Sativa' extracts, at all tested concentrations.

The level of sensitivity of *B. cinerea* isolate to tested plant extracts was expressed as EC50 and EC90 concentrations (tab. 2). Botrytis cinerea Bc 27 isolate appeared to be the most sensitive, with EC50 values < 5% to *T. patula*, *S. hortensis*, *A. sativum* and *H. officinalis* (< 5) extracts. *Mentha*extract has an inhibitory efect on mycelial growth, with a EC50 value of 7.8. By contrast, the mycelial growth was not inhibited by *A. millefolium*, *A. dracunculus* 'Sativa', *R. officinalis* and *V. officinalis* extracts (EC50 and EC90 values > 20).

Table 2. Biological activity of plant extracts on mycelial growth and sporulation of Botrytis cinerea isolate

Plant extract	Concentration (%)	Colony diameter (mm)	Efficiency (%)	EC50 values for mycelial growth (%)	
				EC 50	EC90
Achillea millefolium L.	20	70	0	> 20	> 20
	10	70	0		
	5	70	0		
Allium sativum L.	20	0	100	2.8	14.8
	10	3	95.7		
	5	40	42.8		
Artemisia dracunculus 'Sativa' L.	20	70*	0	> 20	> 20
	10	70*	0		
	5	70*	0		
Hyssopus officinalis L.	20	0	100	< 5	< 5
	10	0	100		
	5	0	100		
Mentha sp.	20	0	100	7.8	17.3
	10	26	62.8		
	5	45	35.7		
Rosmarinus officinalis L.	20	70*	0	> 20	> 20
	10	70	0		
	5	70	0		
Satureja hortensis L.	20	0	100	2.7	8.5
	10	0	100		
	5	24	65.5		
Tagetes patula	20	5	92.8	2.5	12.5
	10	20	71.4		
	5	30	51.7		
Valeriana officinalis L.	20	70	0	> 20	> 20
	10	70	0		
	5	70*	0		
Control (untreated)	–	70	–		

* – good sporulation

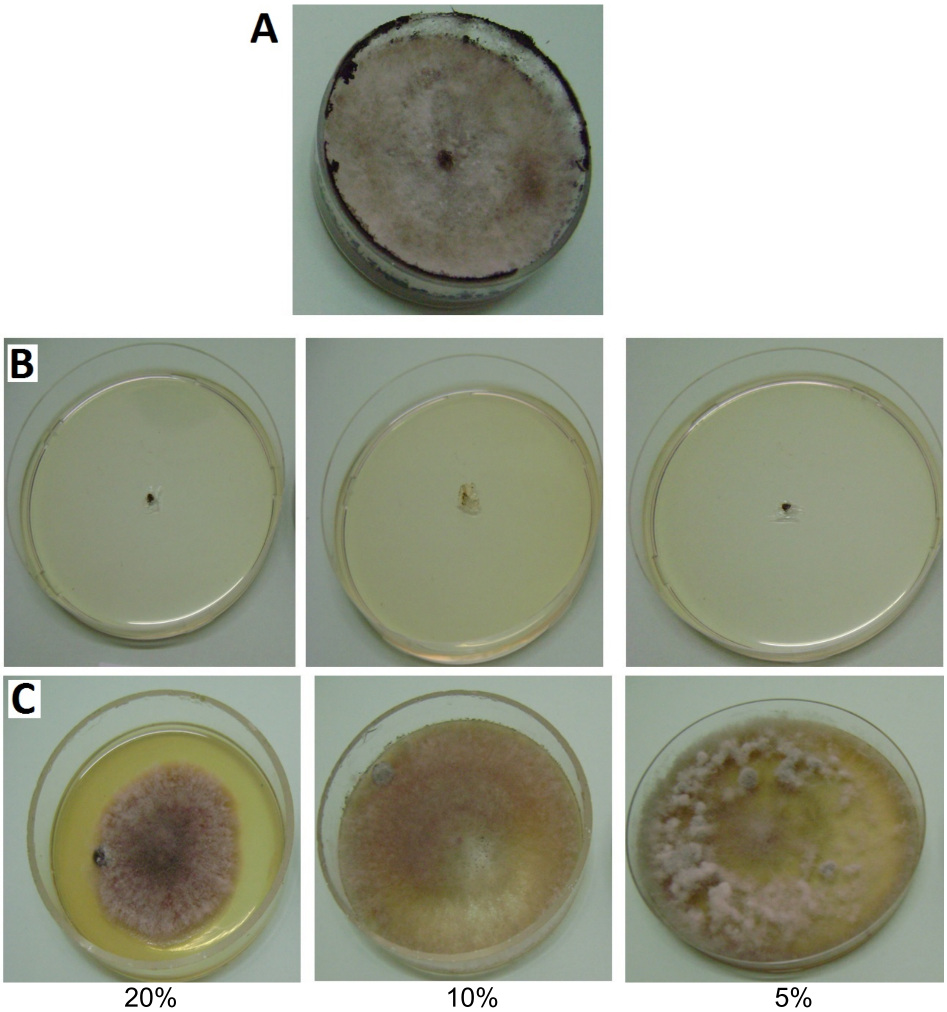

Fig. 1. *In vitro* biological activity of some plant extracts against Botrytis cinerea (Bc 27) from blackcurrant. A – control, B – *Hyssopus officinalis* extract; C – *Valeriana officinalis* extract

Biological activity of plant extracts – *in vivo* assay. Typical symptoms of grey mould were observed in the field in the first decade of July, after a period of 3–5 days of rain. Through laboratory examinations, at post-flowering and fruit development, under RH > 90%, the pathogen was detected however macroscopic manifestation. After the beginning of fruit ripening, first symptoms were developed and grey mould was present on berries, as mycelium and spores. The level of attack was variable in treated variants compared to untreated control, depending on the efficiency of plant extract (fig. 2).

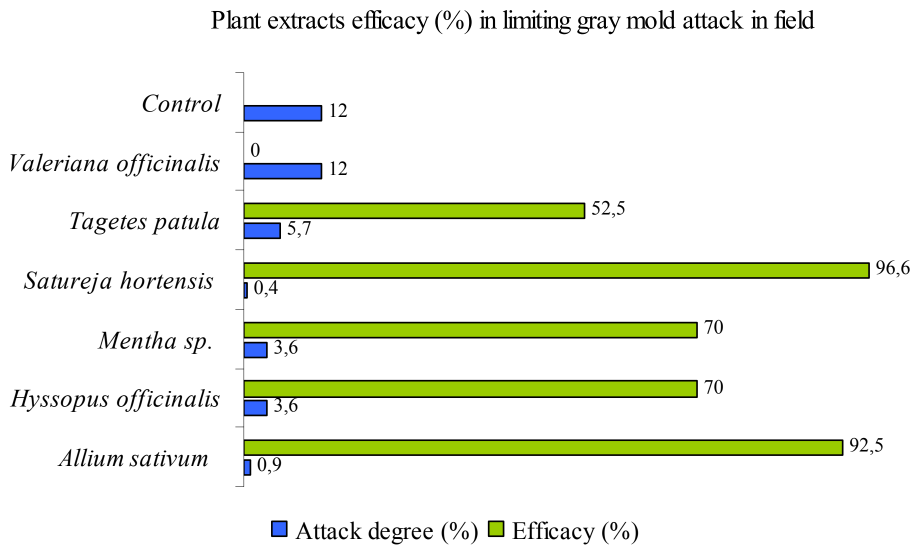

Fig. 2. Plant extracts efficiency in grey mould control, at the beginning of fruit ripening

Table 3. *In vitro* and *in vivo* efficiency (%) of plant extracts on gray mold *Botrytis cinerea* in blackcurrant

	In vitro activity	*In vivo* activity
Strong activity	*Hyssopus officinalis* L. (100) *Satureja hortensis* L. (100–65.7) *Allium sativum* L. (100–42.8)	*Satureja hortensis* L. (96.6) *Allium sativum* L. (92.5)
Good activity	*Mentha* (100–35.7) *Tagetes patula* (88.5–60)	*Hyssopus officinalis* L. (70) *Mentha* (70) *Tagetes patula* (52.5)
No activity	*Valeriana officinalis* L. (0)	*Valeriana officinalis* L. (0)

So, application of all extracts, except that from *V. officinalis*, highly reduced the level of grey mould attack compared to the untreated control (attack degree 12%). Extracts from *S. hortensis* and *A. sativum* have had almost 100% efficiency at 10% concentration (96.6 and 92.5% respectively). Extracts from *H. officinalis* and *Mentha sp.*, have had a relatively high efficiency (70%). The extract from *Tagetes patula* reached a 52.5% efficiency while the extract from *V. officinalis* had no efficiency against grey mould in blackcurrant.

Comparison of *in vivo* and *in vitro* efficiency of plant extracts confirmed the results obtained in laboratory assays with those obtained in the field. The results from *in vitro* and *in vivo* tests permitted us to classify the tested plant extracts into three main categories (tab. 3): extracts with strong biological activity at all concentrations (mainly at 20 and 10 but some at 5%) (*S. hortensis*, *A. sativum*, *H. officinalis*); extracts with good biological activity (*H. officinalis*, *Menta sp.*, *T. patula*) and extracts with no biological activity (*V. officinalis*).

DISSCUSION

***Allium sativum* extract.** Our results on the efficiency of *A. sativum* extract in the growth inhibition of *B. cinerea* Bc 27 isolate of blackcurrant confirmed previous results [Şesan 2003, Şesan and Ştefan 2005] with the same pathogen but different isolates (sunflower, grapevine and Geranium). Also, other results are confirmed [Saniewska 1996, Wilson et al. 1997 quoted by Şesan 2003, Alkhail 2005]. Similar results have been obtained against *B. cinerea* of ornamental plants using extracts from other varieties of Allium, such as *A. ursinum* L., *A. fistulosum* L., *A. obliquum* L., *A. senescens* L. ssp. montanum (Fries) Holub. [Pârvu et al. 2009, 2010a, b, 2011a, b, Pârvu and Pârvu 2011]. Also, the well known *A. cepa* is mentioned as being effective against *B. cinerea* [Toncea and Stoianov 2002].

***Achillea millefolium* extract.** In our tests this extract has a low inhibitory activity on *B. cinerea* in blackcurrant. A powerful inhibitory *in vitro* effect of this extract was previously reported [Iacomi et al. 2000] for a *B. cinerea* isolate from eggplant. The differences in behaviour of the same extract are possible due to a complex of factors, such as origin, specificity and virulence of the isolate or the extraction methodology. The literature quotes extracts from different varieties of Achillea, which are not present in the flora of Romania, such as *A. gypsicola* Hub-Mor. and *A. biebersteinii* Afan., from Turkey, which are very active against *B. cinerea* [Kordali et al 2009].

***Mentha* spp. extract.** The moderate activity of *Mentha*extract observed in our study (variety not mentioned by producer, Hofigal S.A.) has been reported by other authors. It was reported that this extract had a good activity on *B. cinerea* in harvested plums [Aminifard and Mohammadi 2013]. Extracts from *M. piperita* L. and *M. pulegium* L. have had a good efficiency against B. cinerea also [Cutler et al. 1996, Antonov et al. 1997, Daferera et al. 2003].

***Hyssopus officinalis* extract.** Our study reports a powerful inhibitory activity of this extract on mycelial growth of *B. cinerea* isolate, in all tested concentrations (20, 10 and 5%). Our results highlight, for the first time in Romania, the biological activity of *H. officinalis*. This new observation confirms previous results [Cutler et al. 1996, Antonov et al. 1997].

***Tagetes patula* extract** had a good efficiency (88.5 and 80%) at 20%, respectively 10% concentration and a moderate one (60%) at 5%, which is a confirmation of previous results [Antonov et al. 1997]. In India, recent results [Wahmare et al. 2011] confirmed a strong inhibitory activity of *Tagetes erecta* L. extracts on *B. cinerea* of rose. Also, other extracts from various plants, which we have not worked with, such as *Melia azedarach* L., *Clerodendrum inerme* L., *Hyptis suaveolens* (L.) Poit., *Swietenia macrophylla* King, *Melia azedarach* L., *Clerodendrum inerme* L., *Hyptis suaveolens* (L.)

Poit., *Swietenia macrophylla* King are reported to have inhibitory effect against *B. cinerea*. Some essential oils from *Tagetes patula* L., are efficient against *B. cinerea* [Romagnoli et al. 2005]. Recently, in Romania, Teodorescu et al. [2008] have tested an extract from *T. patula* and a mixture of extracts of *T. patula* and *Cynara scolymus* L., to protect apples in grrenhouses, with positive results only for the mixture.

Valeriana officinalis **extract.** We did not find any data in literature to compare our results regarding the effect of this extract against *B. cinerea*. Our results showed no activity against *B. cinerea* in blackcurrant.

Satureja hortensis **extract.** In our study this extract was very active (100%) against *B. cinerea* in high concentrations (20 and 10%) and had a moderate efficiency (65.7%) at 5%. This new set of data confirms previous results [Shimoni et al. 1993, quoted by Şesan 2003], which reported the inhibition of grey mould on some ornamental plants using essential oils from *S. thymbra* L. variety, which is different from *S. hortensis* L.

Rosmarinus officinalis **extract.** The lowest efficiency against *B. cinerea* was obtained using *R. officinalis* extract, at all tested concentrations, which confirms previous results [Daferera et al. 2003].

Artemisia dracunculus **'Sativa' extract.** In Romania, recent studies on extracts from spontaneous Artemisia species (*A. absinthium*, *A. annua*, *A. vulgaris*) underlined their antimicrobial activity [Ivănescu 2010]. No studies on *A. dracunculus* 'Sativa' (french tarragon) and its antimicrobial activity were found, which once again demonstrates the novelty of our research. The antifungal activity of *A. annua* against *B. cinerea* has been reported [Soylu et al. 2005, 2010].

Positive results in the inhibition of *B. cinerea* from different crops but not from blackcurrant are known and also some other plant extracts which we were not working with, yet are reported. We can mention, selectively: *Thymus* spp., *Thymus vulgaris* L. [Reddy et al. 1997, Daferera et al. 2003, Plotto et al. 2003, Martínez-Romero et al. 2007, Kumar et al. 2008, Camele et al. 2010, Adebayo et al. 2013] and *Thymus capitatus* L. [Arras et al. 1995, Shaymaa et al. 2012]; *Salvia officinalis* L. [Carta et al. 1996, Daferera et al. 2003]; *Lavandula angustifolia* L., syn. L. officinalis Chaix ex Vill. [Daferera et al. 2003]; *Carum carvi* L. [Alkhail 2005], *Verbena officinalis* L. [Camele et al. 2010], *Polygonum* spp. [Sas-Piotrowska and Piotrowski 2002]; *Origanum vulgare* L. [Daferera et al. 2003, Plotto et al. 2003, Martínez-Romero et al. 2007, Camele et al. 2010, Adebayo et al. 2013], *Coriandrum sativum* L. [Plotto et al. 2003]; *Catharanthus roseus* (L.) G. Don. [Roy and Chatterjee 2010, Mogle 2013], *Chelidonium majus* L. [Pârvu and Şesan 1997, Pârvu et al. 2008], *Berberis vulgaris* L. [Pârvu and Şesan 1997, Pârvu et al 2010c].

This literature data can be very useful for further research, some varieties being reported in Romanian flora. Many of these plants used to obtain extracts came from Mediterranean area, an area which we must focus on because of climate change and modifications of the biodiversity [Shimoni et al. 1993, Sivropoulou et al. 1996, Mrabet et al. 1999, Bouchra et al. 2003, Daferera et al. 2003, Soylu et al. 2005, 2010, Kordali et al. 2009, Camele et al. 2010].

CONCLUSIONS

In conclusion, our results represent new contributions on the antifungal effect of plant extracts in Romania, completing those which have *B. cinerea* as target pathogen [Iacomi et al. 2000, Toncea and Stoianov 2002, Şesan 2003, Şesan and Ştefan 2005, Roşca-Casian et al. 2007, Pârvu and Şesan 1997, Pârvu et al 2008, 2009, 2010a, b, c, 2011a, b], and with other extracts [Pârvu and Şesan 1997, Roşca-Casian et al 2007, Pârvu and Pârvu 2011].

Field results, from Hofigal Company and SCDA Baneasa, have demonstrated a powerful drop of grey mould severity in blackcurrant crops compared to control when using extracts at 10%. Comparing the efficiency of tested plant extracts both *in vivo* and *in vitro*, our results showed a confirmation of *in vitro* tests with those obtained in field, permitting us to divide the extracts into three main categories, based on their biological activity: extracts with strong biological activity (*S. hortensis*, *A. sativum*, *H. officinalis*); extracts with good biological activity (*H. officinalis*, *Menta sp*., *Tagetes sp.*) and extracts with no biological activity (*V. officinalis*).

These data are very useful for plant protection practice, particulary for medicinal plants, as blackcurrant, which demands for non-pollutant and environmental friendly alternative methods to fungicides.

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REFERENCES

Adebayo, O., Dang, T., Belanger, A., Khanizadeh, S. (2013). Antifungal studies of selected essential oils and a commercial formulation against *Botrytis cinerea*. J. Food Res., 2(1), 217–226.

Alkhail Aba, A.A. (2005). Antifungal activity of some extracts against some plant pathogenic fungi. Pakistan J. Biol. Sci., 8(3), 413–417.

Aminifard, M.H., Komodammadi, S. (2013). Essential oils to control *Botrytis cinerea in vitro* and *in vivo* on plum fruits. J. Sci. Food Agric., 93(2), 348–353.

Antonov, A., Stewart, A., Walter, M. (1997). Inhibition of conidium germination and mycelial growth of *Botrytis cinerea* by natural products. Proceedings of the 50th New Zealand Plant Protection Conference, New Zealand Plant Protection Society (Inc.), 159–164.

Arras, G., Agabbio, M., Piga, A., D'Hallewn, G., Gerasopoulos, D., Olympos, C., Passam, H. (1995). Fungicide effect of volatile compounds *Thymus capitatus* essential oil. Acta Horticult., 379, 593–600.

Beever, R.E., Laracy, E.P., Pak, H.A. (1989). Strains of *Botrytis cinerea* resistant to dicarboximide and benzimidazole fungicides in New Zealand vineyards. Plant Pathol., 38, 427–437.

Bi, Ya Ling, Wang, Bo, Huang, Bao Hong, Zhanng, Wen Tong, Zhang, Yi Hui (2011). Antifungal actyivity of botanical extracts against *Botrytis cinerea* and *Alternaria solani*. J. Agricult. Sci. Technol. Hunan, 12(6), 862–864.

Bouchra, C., Achouri, M., Hassani, L.M.I., Hmamouchi, M., 2003. Chemical composition and antifungal activity of essential oils of seven *Moroccan Labiatae* against *Botrytis cinerea*. Pers. J. Ethnopharm., 89, 165–169.

Brent, K.J., Hollomon, D.W. (1998). Fungicide resistance: the assessment of risk. FRAC. Global Crop Prot. Fed., Brussels, Monograph, 2, 1–48.

Camele, I., De Feo, V., Altieri, L., Mancini, E., De Martino, L., Rana, A G.L. (2010). An attempt of postharvest orange fruit rot control using essential oils from *Mediterranean plants*. J. Med. Food, 13(6), 1515–1523.

Carta, C., Moretti, M.D.L., Peana, A.T. (1996). Activity of the oil of *Salvia officinalis* L. against *Botrytis cinerea*. J. Essent. Oil Res., 8(4), 399–404.

Chebli, B., Hmanouchi, M., Achouri, M., Hassani Idrissi, I.M. (2004). Composition and *in vitro* fungitoxic activity od 19 essential oils against two post-harvest pathogens. J. Essent. Oil Res., 16(5), 507–511.

Coetzee, G., Marx, I.J., Pengilly, M., Bushula, V.S., Joubert, E., Bloom, M. (2008). Effect of rooibos and honeybush tea extracts against Botrytis cinerea. http://hdl.handle.net/10019.1/8434

Coley-Smith, J.R., Verhoeff, K., Jarvis, W.R. (1980). The biology of Botrytis. London, Academic Press, 181–218.

Copping, L.G., Duke, S.O. (2007). Natural products that have been used commercially as crop protection agents. Pest Manag. Sci., 63, 117–153.

Cutler, H.G., Hill, R.A., Ward, B.C., Rohitha, B.H., Stewart, A. (1996). Antimicrobial, insecticidal and medicinal properties of natural products, flavours and fragrances. In: Biotechnologies for improved foods and flavors, Takeoka, G.R., Teranishi, R., Williams, P.J., Kobayashi, A. (eds). Am. Chem. Soc., 51–66.

Daferera, D.J., Ziogas, B.N., Polissiou, M.G. (2003). The effectiveness of plant essential oils on the growth of *Botrytis cinerea*, *Fusarium*and *Clavibacter michiganense* subsp. michiganensis. Crop Prot., 22(1), 39–44.

Davidson, P.M., Naidu, A.S. (2000). Phyto-phenols. In: Natural Food Antimicrobial System Naidu A.S. (ed.). Boca Raton, FL, CRC Press, 265–294.

Dubey, N.K. (2011). Natural products in plant pest management. UK, 293 pp.

Elad, Y., Evenses, K. (1995). Physiological aspects of resistance to *Botrytis cinerea* II. Phytopathology, 85, 637–643.

Elad, Y., Yunis, H., Katan, T. (1992). Multiple resistance to benzimidazoles, dicarboximidea and diethofenocarb in field isolates of *Botrytis cinerea* in Israel. Plant Pathol., 41, 41–46.

Elad, Y., Williamson, B., Tudzynski, P., Delen, N. (2004). *Botrytis* spp. and diseases they cause in agricultural systems an introduction. In: Botrytis: Biology, pathology and control, Elad, Y., Williamson, B., Tudzynski, P., Delen, N. (eds). The Netherlands: Kluwer Academic Publishers Dordrecht, 1–8.

Enache, E. (2013). Biology, ecology and control of pathogenic fungi for blackcurrant cultivated as medicinal plant in the South of Romania. PhD thesis, University of Bucharest, Biology Faculty, 328 pp. (in Romanian)

El Oirdi, M., Bouarab, K. (2007). Plant signalling components EDS1 and SGT1 enhance disease caused by the necrotrophic pathogen *Botrytis cinerea*. New Phytol., 175, 131–139.

Gonzales-Collado, I., Macias-Sachez, A.J., Hanson, J.R. (2006). Fungal terpene metabolites: biosynthetic relationships and the control of phytopathogenic fungus *Botrytis cinerea*. Nat. Prod. Rep., 24, 674–688.

Han, Y., Xiao, D., Xiang, Y., Ye, L., Cheng, C. (2000). Study on the volatile oil of Nardostachys chinensis. Zhong Yao Cai, 23, 34–35.

Hébért, C., Charles, M.T., Gauthier, L., Willemot, C., Khanizadeh, S., Cousineau, J. (2002). Strawberry proanthocyanidins: biochemical markers for *Botrytis cinerea* resistance and self-life predicitability. Acta Horticult., 567, 659–662.

Huang, Jenn-Wen, Chung, Wen-Chuan (2003). Management of vegetable crop diseases with plant extracts. In: Advances in plant management, Huang, H.C., Surya A. (eds), 153–163.

Iacomi, B., Gherghieş, C., Enciu, S., Manole, M. (2000). Phyto-extracts with antifungal activity, Lucrări Ştiinţifice. USAMV Buc., ser. B, vol. 18, 137–139.

Ivănescu, B. (2010). Studiul fitochimic al unor compuşi din speciile *Artemisia absinthium*, *A. vulgaris* şi *A. annua* recoltate din flora spontană. Rezumat teza de doctorat. Univ. de Medicină şi Farmacie „Gr. T. Popa", Facultatea de Farmacie, Iaşi.

Kordali, S., Cakir, A., Akcin, T.A., Mete, E., Abcin, A., Aydin, T., Kilic, H. (2009). Antifungal and herbicidal properties of essential oils and n-hexane extracts of *Achillea gypsicola* Hub-Mor. and *A. biebersteinii* Afan. (Asteraceae). Indust. Crops Prod., 29, 562–570.

Kumar, A., Shukla, R., Singh, P., Prasad, C.S., Dubey, N.K. (2008). Assessment of *Thymus vulgaris* L. essential oil as a seif botanical preservative against post harvest fungal infestation of foof commodities. Innov. Food Sci. Emerg. Technol., 4, 575–580.

Lee, H.C., Cheng, S.S., Chang, S.T. (2005). Antifungal property of the essential oils and their constituents from *Cinnamomum osmophloem* leaf against tree pathogens fungi. J. Sci. Food Agric., 85, 2047–2053.

Lee, S.E., Park, B.S., Kim, M.K., Choi, W.S., Kim, H.T., Kho, K.Y., Lee, S.G., Lee, H.S. (2001). Fungicidal activity of pipernonaline, a piperidine alkaloid derived from long pepper, *Piper longum* L. against phytopathogenic fungi. Crop Protect., 20(6), 523–528.

Leroux, P., Fritz, R., Debieu, D., Albertini, C., Lanen, C., Bach, J., Chapeland, F. (2002). Mechanisms of resistance to fungicides in field strains of *Botrytis cinerea*. Pest Manag. Sci., 58, 876–888.

Martinez-Romero, D., Guillen, F., Valverde, J.M., Bailen, G., Zapata, P., Serrano, M., Castillo, S., Valero, D. (2007). Influence of carvacrol on survival of *Botrytis cinerea* inoculated in table grapes. Int. J. Food Microbiol., 115(2), 144–148.

Mendoza, L., Modak, B., Torres, R., Cotoros, M. (2008). *In vitro* sensitivity of *Botrytis cinerea* to resinous exudates of *Heliotropium filifolium* and geranyl derivatives compounds. J. Chil. Chem. Soc., 53(1), 10 pp.

Mogle, U.P. (2013). Efficiency of leaf extracts against the post harvest fungal pathogens of cowpea. Biosci. Disc., 4(1), 39–42.

Mrabet, N., Lahlou, H., Brnjilali, B. (1999). Effect of Moroccan *Cistus ladaniferus* L. (rockrose) extracts on the growth of four fungi. Cryptogam. Mycol., 20, 23–33.

Nikos, G.T., Economakis, C.E. (2007). Antifungal activity of lemongrass (*Cymbopogon citrates* L.) essential oil against key postharvest pathogens. Glob. J. Biotechnol. Biochem., 3(2), 56–59.

Ozcan, M., Boyraz, N. (2000). Antifungal properties of some herb decoctions. Europ. Food Res. Technol., 212, 86–88.

Park, I.K., Lee, S.G., Shing, S.C., Park, D.J., Ahn, Y.J. (2002). Larvicidal activity of isobutylamides identified in *Piper nigrum* fruits against three mosquito species. J. Agricult. Food Chem., 50, 1866–1870.

Patkowska, E. (2008). The application of chitosan, *Pythium oligandrum* and grapefruit extract in the protection of common bean (*Phaseolus vulgaris* L.) from soil-borne phytopathogens. Progress on chemistry and application of chitin and its derivatives, vol. 13, 133–139.

Pârvu, M., Pârvu, A. (2011). Antifungal plant extracts. In: Science against microbial pathogens: comunicating current research and technological advances, Méndez-Vilas, A. (ed.). Formatex, Microbiol. Ser.3, 1055–1062.

Pârvu, M., Şesan, T.E. (1997). *In vitro* action of plant extracts on *Botrytis species* from ornamental plants. Rév. Roum. Biol. Biol. Végét. 42(1–2), 103–110.

Pârvu, M., Pârvu, A.E. Crăciun, C., Barbu-Tudoran, L., Tămaş, M. (2008). Antifungal activieties of *Chelidonium majus* extract on *Botrytis cinerea in vitro* and ultrastructurral changes in its conidia. J. Phytopathol., 156, 550–552.

Pârvu, M., Roşca-Casian, O., Puşcaş, M., Groza, G. (2009). Antifungal activity of *Allium fistulosum* L. Contribuţii Bot., 44, 125–129.

Pârvu, M., Pârvu, A.E. Roşca-Casian, O., Vlase, L., Groza, G. (2010a). Antifungal activity of *Allium obliquum*. J. Med. Plants Res., 4, 138–141.

Pârvu, M., Toiu, A., Vlase, L., Pârvu, A.E. (2010b). Determination of some polyphenolic compounds from Allium species by HPLC-UN-MS. Nat. Prod. Res., 24, 1318–1324.

Pârvu, M., Pârvu, A.E., Crăciun, C., Barbu-Tudoran, L., Vlase, L., Tămaş, M., Roşca-Casian, O., Tripon, S.C., Persecă, C., Molnar, A.M. (2010c). Changes in *Botrytis cinerea* conidia caused by *Berberis vulgaris* extract. Notulae Bot., 38(3), 15–20.

Pârvu, M., Pârvu, A., Vlase, L., Roşca-Casian, O., Pârvu, O. (2011a) Antifungal properties of *Allium ursinum* L. ethanol extract. J. Med. Plants Res., 5(10), 2041–2046.

Pârvu, M., Pârvu, A.E., Vlase, L., Roşca-Casian, O., Pârvu, O., Puşcaş, M. (2011b). Allicin and alliin content and antifungal activity of *Allium senescens* L. ssp. montanum (F.W. Schmidt) Holub ethanol extract. J. Med. Plants Res., 5(29), 6544–6549.

Plotto, A., Roberts, R., Roberts, D. (2003). Evaluation of plant essential oils as natural postharvest disease control of tomato (*Lycopersicon esculentum*). Acta Horticult., 628, 737–745.

Rai, M., Acharya, D., Rios, H.L. (2011). Ethnomedical plants. Revitalization of traditional knowledge of herbs. Sci. Publ. Enfield, New Hampshire, CRC Press, Taylor & Francis, LLC, USA, 507 pp.

Reddy, M.V.B., Angres, P., Gosselin, A., Arul, J. (1997). Characterization an duse of essential oil from *Thymus vulgaris* against *Botrytis cinerea* and *Rhizopus stolonifer* in strawberry fruit. Phytochemistry, 97, 1515–1520.

Reuveni, M., Neifeld, D., Dazan, D., Kotzer, Z. (2009). BM-608-a noovel organic product base don essential tea tree oil for the control of fungal diseases in tomato. US Department of Agriculture, AGRICOLA on-line catalog of the National Agricultural Library (NAL): 2 pp.

Ribera, A., Cotoras, M., Zuniga, G.E. (2008). Effect of extracts from *in vitro*-grown shoots of *Quillaja saponaria* Mol. on *Botrytis cinerea* Pers. World J. Microbiol. Biotechnol., 24(9), 1803–1811.

Romagnoli, C., Bruni, R. Andreotti, E., Rai, M.K., Vicentini, C.B., Mares, D. (2005). Chemical characterization and antifungal activity of essential oil of capitula from wild Indian *Tagetes patula* L.. Protoplasma, 225(1–2), 57–65.

Roşca-Casian, O., Pârvu, M., Vlase, I., Tămaş, M. (2007). Antifungal activity of *Aloë vera* leaves. Fitoterapia 78, 219–222.

Roy, S., Chatterjee, P. (2010). A non-toxic antifungal compound from the leaves of *Catharanthus roseus* characterized as 5-hydroxy flavone by UV spectroscopic analysis and evaluation of its antifungal properties by agar-cup method. Industr. Crops Prod., 32(3), 375–380.

Saks, Y., Barkai-Golan, R. (1995). *Aloe vera* gel activity against plant pathogenic fungi. Postharv. Biol. Technol., 6, 159–165.

Saniewska, A. (1996). Potential use of garlic compounds and fungicides in the control on fungi on seeds of some ornamental plants. Polish Phytopathol. Soc., Biol. Contr. of Plant Dis., Skierniewice (Poland), 141–147.

Sas-Piotrowska, B., Piotrowski, W. (2002). Plant extracts in protection of strawberry (*Fragaria vesca* L.) against *Botrytis cinerea* Pers.. Rocz. Ochr. Środ., 4, 545–553.

Shaymaa, M.R., Abou-Zaid, M.A., Aly, A.Z., Tohamy, M.R.A. (2012). Safety control of strawberry fruit gray mold fungus by volatile oils. Zagazig J. Agricult. Res., 39(2), 181–187.

Shen, Y.H., Weng, Z.Y., Zhao, Q.S., Zeng, Y.Q., Rios, J.L., Xiao, W.L., Ku, G., Sun, H.D. (2005). Five new triterpene glycosides from *Lysimachia foenum-graecum* and evaluation of their effect on the arachidonic acid metabolizing enzyme. Planta Med., 71, 770–775.

Shimoni, M., Putiewski, E., Ravid, U., Reuven, R. (1993). Antifungal activity of volatile fractions of essential oils from four aromatic wild plants in Israel. J. Chem. Ecol., 19(6), 1129–1133.

Sivropoulou, A., Papanikolaou, E., Nikolaou, C., Kokkini, S., Lanaras, T., Arsenakis, M. (1996). Antimicrobial and cytotoxic activities od *Origanum essential* oils. J. Agricult. Food Chem., 44(5), 1202–1205.

Soylu, E.M., Yigitbas, H., Tok, F.M., Sozlu, S., Kurt, Ş., Baysal, Ő., Kaya, A.D. (2005). Chemical composition and antifungal activity of the essential oil of *Artemisia annua* L. against foliar and soil-borne fungal pathogens. Z. Pflanzenkrankh. Pflanzenschutz, 112(3), 229–239.

Soylu, E.M., Kurt, S., Soylu, S. (2010). *In vitro* and *in vivo* antifungal activieties of the essential oils of various plants against tomato grey mould disease agent *Botrytis cinerea*. Internat. J. Food Microbiol. 143(3), 183–189.

Şesan, T.E. (2003). Sustainable management of gray mould (*Botrytis cinerea*) on grapevine, strawberry and ornamentals. In: Chapter in advances in plant managenent, Huang, H.C., Surya A. (eds.). Res. Signpost Publ. House, 121–152.

Şeasan, T.E., Ştefan, A.L. (2005). Acţiunea biologică *in vitro* a unor extracte vegetale faţă de ciuperca *Botrytis cinerea* Pers. Sănătatea Plantelor, ediţie specială, Lucrările celei de al 14-lea Simpozion Naţional de Micologie, Sinaia, România, 2004, 111–115.

Şeasan, T.E., Tănase, C. (2007). Ciuperci anamorfe fitopatogene. Univ. Bucureşti, 44–66.

Şesan, T.E., Tănase, C. (2011). Ascomicete fitopatogene. Univ. Bucureşti, 123–132.

Teodorescu, G., Marin, F.C., Sumedrea, M., Murariu, F. (2008). Preliminary results regarding the effect of vegetal extracts on storage diseases. International Workshop on Sustainable Fruit Growing, RIFG Piteşti-Mărăcineni, Romania, 109–119.

Tao, S., Zhang, G.S., Tsao, R., Charles, M.T., Yang, R., Khanizadch, S. (2010). *In vitro* antifungal activity and mode of action of selected polyphenolic antioxidants on *Botrytis cinerea*. Arch. Phytopathol. Plant Protect., 43(18), 1564–1578.

Toncea, I., Stoianov, R. (2002). Metode ecologice de protecţie a plantelor. Ed. Ştiinţelor Agricole, Bucureşti.

Tzortzakis, N.G., Economakis, C.E. (2007). Antifungal activity of lemongrass (*Cymbopogon citratus* L.) essential oil against key postharvest pathogens. Innovat. Food Sci. Emerg. Technol., 8(2), 253–258.

Vali, R.J., Moorman, G.W. (1992). Influence of selected fungicides regimes on frequency of dicarboximide-resistant and dicarboximide-sensitive strains of *Botrytis cinerea*. Plant Dis. 76(9), 919–924.

Vio-Michaelis, S., Pablaza-Hidalgo, G., Gomez, M., Pena-Vera, R., Montenegro, G. (2012). Antifungal activity of three Chilean plant extracts on *Botrytis cinerea*. Bot. Sci., 90(2), 179–183.

Wahmare, M.B., Wahmare, R.M., Kamble, S.S. (2011). Bioefficiency of plant extracts on growth of *Botrytis cinerea* causing leaf blight of rose. The Bioscan, 6(4), 643–645.

Wilson, C.L., Solar, J.M., El-Ghaouth, A., Wisniewski, M.E. (1997). Rapid evaluation of plant extracts and essentials oils for antifungal activity against *Botrytis cinerea*. Plant Dis., 81, 204–210.

GRZYBOBÓJCZE DZIAŁANIE NIEKTÓRYCH WYCIĄGÓW ROŚLINNYCH PRZECIW *Botrytis cinerea* Pers. W PLONIE CZARNEJ PORZECZKI (*Ribes nigrum* L.)

Streszczenie. Po raz pierwszy w Rumunii przetestowano i dokonano przesiewu *in vitro* oraz *in vivo* dziewięciu wyciągów roślinnych produkowanych przez Hofigal S.A. przeciwko *Botrytis cinerea* (szczep Bc 27) wyizolowanego z czarnej porzeczki (*Ribes nigrum* L.). Najlepszy efekt przeciw Botrytis *in vitro* (wydajność między 80 a 100%) uzyskano przy użyciu następujących wyciągów: *Hyssopus officinalis* (przy 20, 10 i 5%), *Satureja hortensis*, *Allium sativum*, *Tagetes patula* (przy 20 i 10%) oraz *Mentha* sp. (przy 20%). Umiarkowany efekt przeciw Botrytis (wydajność między 35,7 a 65,7%) zaobserwowano dla wyciągów *Mentha* sp. (przy 10 i 5%), *Satureja hortensis*, *Allium sativum* and *Tagetes patula* (przy 5%). Najsłabszy efekt przeciw Botrytis lub zero efektu stwierdzono przy użyciu wyciągów otrzymanych z *Achillea millefolium*, *Artemisia dracunculus* 'Sativa', *Rosmarinus officinalis* i *Valeriana officinalis*, zastosowanych nawet w 20%. Na podstawie wyników otrzymanych *in vitro* przetestowano i dokonano przesiewu *in vivo* sześciu wyciągów roślinnych w warunkach polowych w Hofigal S.A. Bucharest. Wyciągi *Satureja hortensis*, *Allium sativum*, *Hyssopus officinalis*, *Mentha* sp. i *Tagetes patula* były skuteczne przy 10% w ograniczaniu nasilenia szarej pleśni w porzeczce w porównaniu z kontrolą. Nie zarejestrowano żadnego działania *in vivo* dla wyciągu *Valeriana officinalis.* Wyciągi roślinne o dużej skuteczności mogą być rekomendowane jako alternatywne środki przyjazne dla środowiska, niepowodująca zanieczyszczenia (ogrodnictwo organiczne) w ochronie czarnej porzeczki jako rośliny leczniczej przeciwko szarej pleśni, która jest obecnie gospodarczo najważniejszą chorobą w Europie.

Słowa kluczowe: czarna porzeczka, *Botrytis cinerea*, wyciągi roślinne, ogrodnictwo organiczne, Rumunia

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