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ANTIFUNGAL ACTIVITY OF SOME PLANT EXTRACTS AGAINST *Alternaria alternata* (Fr.) Keissl. IN THE BLACK CURRANT CROP (*Ribes nigrum* L.)

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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 *Alternaria alternata* (strain Aa 20) isolated from blackcurrant (*Ribes nigrum* L.). The highest *in vitro* activity (efficacy between 70% and 100%) was recorded for *Satureja hortensis* and *Valeriana officinalis* extracts at 20 and 10% concentrations. A good inhibitory activity on mycelial growth (efficacy between 54.3 and 88.6%) has been noticed for *Allium sativum*, *Mentha* sp. *Rosmarinus officinalis*, *Tagetes patula* extracts (at 10 and 20%). No efficacy was noticed for *Artemisia dracunculus* 'sativa'. The extract obtained from *Hyssopus officinalis* inhibited the mycelial growth of *A. alternata* only at 20%, with 57.1% efficacy. In field trials, *Valeriana officinalis* and *Satureja hortensis* extracts have been very effective in limiting Alternaria disease severity in blackcurrant applied at 10%, compared to untreated control. This data confirmed their strong antifungal potential, both *in vitro* and *in vivo*. Based on our results, plant extracts with highly efficacy could be an alternative in the protection of blackcurrant as medicinal crop against Alternaria leaf spot and fruit rot especially in organic horticulture system.

Key words: Black currant, *Alternaria alternata*, plant extracts, organic horticulture, Romania

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INTRODUCTION

Black currant (*Ribes nigrum* L.) is a commercially important soft fruit crop with an annual turn over of 160,000 tonnes in Europe and 185,000 tonnes worldwide [Hedley et al. 2010]. In recent years there has been an increased scientific interest toward this crop, due to alimentary and therapeutic value of its fruits, young leaves and buds. Black currant fruits are known for their desired flavoure, the high content of tasty juice and as a source of bioactive compounds (vitamin C, polyphenols) with potential health promoting properties. Buds and leaves are also a valuable source of phenolic compounds. The leaf and bud extracts are of relevance as raw material for the food and health industry thereby making black currant a lucrative product for use as functional food ingredient [Vagiri 2012].

Since the quality of these products may be impaired by various pathogens, especially fungi, the establishment of biocontrol measures to protect such a medicinal crop is necessary. In addition, a rapid increase in organically grown black currants was observed [Anttonen and Karjalainen 2006].

Alternaria genus is ubiquitous, with cosmopolitan species found as saprophytes on different substrates and pathogenic on a wide range of crops, including vegetables, fruits, ornamentals and cereals. *Alternaria alternata* (Fr.) Keissl. is responsible for leaf spot and fruit rot in blackcurrant. In addition to causing economic losses, this pathogen is known as the most important mycotoxin producing species (e.g. alternariol, alternariol monomethyl ether) among *Alternaria* species.

To overrun the widespread public concern for long term health, environmental effects of pesticides and also to comply with food safety standards, there is a worldwide trend to explore new alternative to fungicides. Natural compounds as plant extracts have been shown to be effective against many plant pathogens, considered to be safe for humans and environment. Also, they have selectivity, biodegradable action and a great variety of chemical composition, with a large variety of secondary metabolites, most of them not yet studied in correlation with their fungicide action.

This study was focused on the efficacy of different plant extracts against *A. alternata*, responsible for leaf spot and fruit rot in black currant, *in vitro* and *in vivo*. The objective of the present study was to test and screen the biological activity of some plant extracts manufactured by Hofigal S.A. Bucharest against *A. alternata* in black currant both *in vitro* and *in vivo* trials.

MATERIAL AND METHODS

In vitro tests were conducted using one strain of *Alternaria alternata* (Aa20) isolated at Research-Development Institute for Plant Protection (RDIPP) Bucharest from infected blackcurrant leaves (*Ribes nigrum* L.) from production field of Hofigal S.A. The isolate was identified according to the cultural properties, morphological and microscopical characteristics, tested for pathogenicity on leaves and maintained on PDA for further studies.

The biological action of nine plants extracts was evaluated *in vitro* on mycelial growth of Aa 20 isolate through poisoned food technique. The plants screened in this study were: *Achillea millefolium* (yarrow), *Allium sativum* (garlic), *Artemisia dracunculus* 'sativa' (french tarragon), *Hyssopus officinalis* (hyssop), *Mentha* sp. (mint, variety not mentioned by producer, Hofigal S.A.), *Rosmarinus officinalis* (rosemary), *Satureja hortensis* (summer savory), *Tagetes patula* (marigold) and *Valeriana officinalis* (valerian). These plants were selected based on i) medicinal value and reported antimicrobial action; ii) capacity to synthesize fungicide analogues; iii) amount of obtained biomass and iv) availability in Romania and reduced economical costs. The hydroalcoholic extracts were manufactured by Hofigal S.A. from stems, leaves, flowers, sprouts and bulbs, harvested at recommended time (tab. 1). 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%.

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	✓	✓

Mycelial disks of pathogens (8 mm in diameter) removed from the margins of a 7 days old culture were transferred to PDA media amended with 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 24°C, in the dark.

Results were expressed as efficacy 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 efficacy of six most effective plant extracts against *A. alternata in vitro* was tested in the production and experimental fields of Hofigal S.A. and RDIPP Bucharest, during 2009–2012. Extracts of *A. sativum*, *H. officinalis*, *Mentha* sp., *S. hortensis*, *T. patula* and *V. officinalis* were used. Three treatments were applied, at 10% concentration: (i) after flowering, (ii) at fruit setting, (iii) at the beginning of fruit ripening. The degree of attack on leaves was calculated based on frequency and disease severity, in natural infections conditions. The efficacy of treatments has been calculated

using Abbot formula: Efficacy % = 100 – Z; Z = attack degree of variant ×100/attack degree of control.

RESULTS

***In vitro* antifungal activity of plant extracts.** The antifungal activity of nine plant extracts was investigated against the linear growth of *A. alternata* Aa20 isolate, at different concentrations (5, 10, and 20%). The efficacy of these extracts is shown in Table 2. The highest efficacy (100%) in inhibition of mycelial growth of *Alternaria alternata* isolate was recorded for *Satureja hortensis and Valeriana officinalis* extracts, at 20% concentration (fig. 1).

Table 2. Biological action of plant extracts on mycelial growth of *Alternaria alternata*

Plant extract	Concentration (%)	Colony diameter (mm)	Efficacy (%)	EC50 values for mycelial growth (%)	
				EC 50	EC90
Achillea millefolium	20	30	57.1	16.9	38.9
	10	47	32.8		
	5	48	31.4		
Allium sativum	20	19	72.8	2.8	33.9
	10	30	57.1		
	5	32	54.3		
Artemisia dracunculus 'sativa'	20	70	0	>20	>20
	10	70	0		
	5	70	0		
Hyssopus officinalis	20	30	57.1	19.2	29.1
	10	70	0		
	5	70	0		
Mentha sp.	20	16	77.1	2.3	24.8
	10	24	72.8		
	5	22	68.5		
Rosmarinus officinalis	20	8	88.6	7.8	21.2
	10	25	64.3		
	5	45	35.7		
Satureja hortensis	20	0	100	5.7	16.6
	10	24	72.8		
	5	40	42.8		
Tagetes patula	20	15	78.5	1.8	14.8
	10	20	71.4		
	5	22	68.5		
Valeriana officinalis	20	0	100	5.6	16.6
	10	24	72.8		
	5	40	42.8		
Control (untreated)	–	70			

A high level of efficacy (88.6%) was obtained with *R. officinalis* extract, at 20% also. A good inhibitory effect on mycelial growth (efficacy between 70 and 78.5%) was observed for *A. sativum*, *Mentha* sp., *T. patula* extracts, at 20% as well as for *S. hortensis*, *T. patula* and *V. officinalis* extracts at 10%.

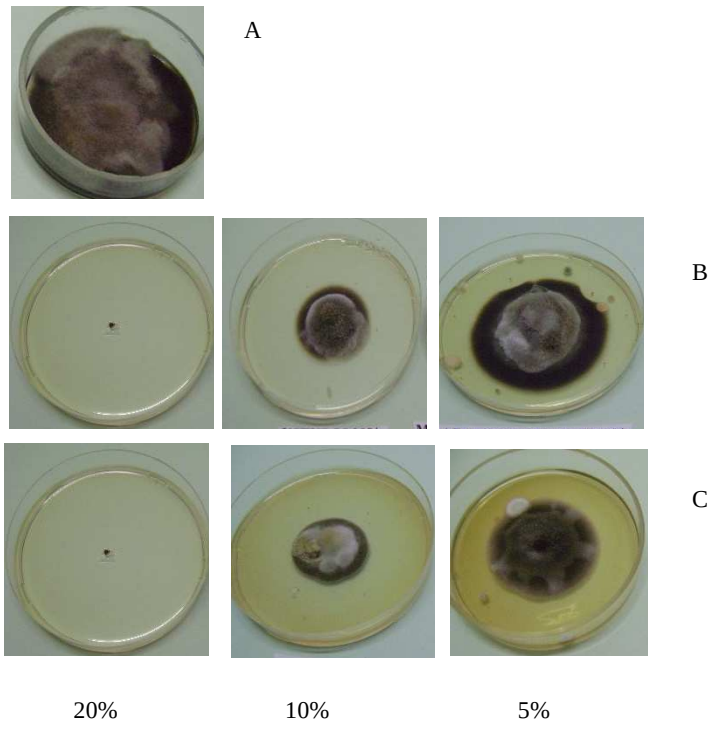

Fig. 1. *In vitro* biological activity of some plant extracts against development of *Alternaria alternata* (Aa 20) from blackcurrant. A – control; B – *Satureja officinalis* extract; C – *Valeriana officinalis* extract

No inhibitory effect was registered for *Artemisia dracunculus* 'sativa' extracts at all tested concentrations. The extract of *Hyssopus officinalis* had no inhibitory activity on *A. alternata* mycelial growth at 5 and 10% concentrations. At 20% concentrations, an inhibition in linear growth was recorded, with a moderate efficacy of 57.1%. At a concentration of 20%, the same moderate efficacy was registered for *A. millefolium* extract (57.1%). However, in the presence of this extract, even at 10 and 5% concentrations, it was recorded an inhibition of mycelial growth, with lower efficacy (32.8 and 31.4% respectively). The level of sensitivity of *A. alternata* was expressed as EC50 and EC90 concentrations (tab. 2). *Alternaria alternata* Aa20 isolate appeared to be the most sensitive to *T. patula* (EC50 value 1.8; EC90 value 14.8) followed by *Mentha* sp. (EC50 value 2.3), *A. sativum* (EC50 value 2.8), *V. officinalis* (EC50 value 5.6) and *S. hortensis* (EC50 value 5.7).

***In vivo* antifungal activity of plant extracts.** The efficacy of six plant extracts to limit Alternaria disease in blackcurrant was evaluated in the production and experimental fields of Hofigal S.A. and ICDPP Bucharest (2009–2012). These plant extracts were selected subsequently to *in vitro* bioassay.

Table 3. Biological action of plant extracts on *Alternaria alternata* – field assay

Plant exract	Attack degree (%)		
	I	II	III
Allium sativum	8.20	11.60	12.50
Hyssopus officinalis	7.80	10.60	11.90
Mentha sp.	9.80	11.00	13.50
Satureja hortensis	0.80	1.20	1.40
Tagetes patula	10.20	12.00	14.20
Valeriana officinalis	0.40	0.80	0.85
Control (untreated)	6.50	12.50	14.90
Limits	0.80–10.20	0.80–12.00	0.85–14.20
Average	6.2	7.86	9.05

I – after flowering; II – at fruit setting; III – beginning of fruit ripening

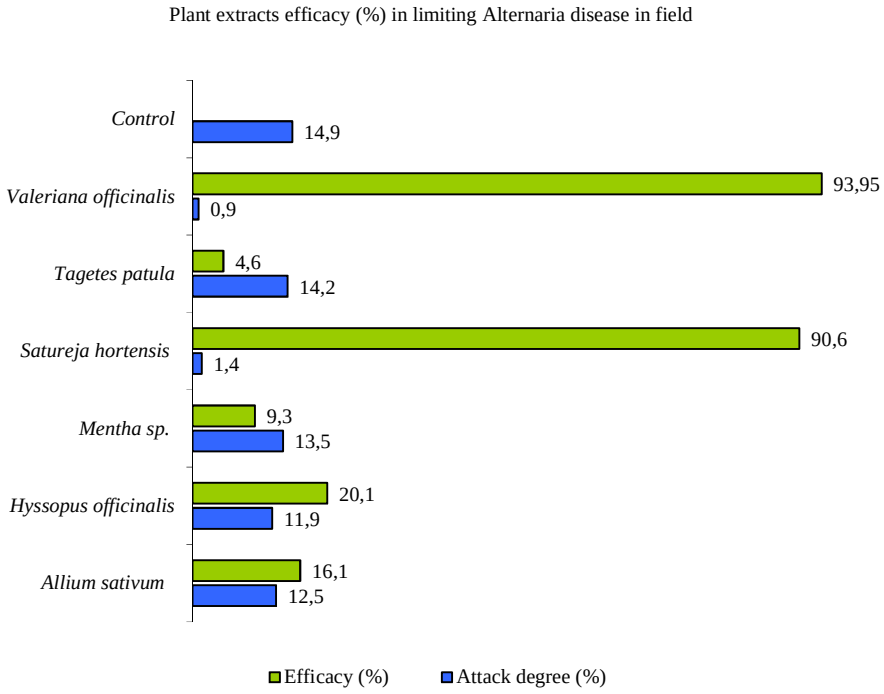

Fig. 2. Plant extracts efficacy in Alternaria disease control

Three treatments (after flowering, at fruit setting and at the beginning of fruit ripening) were applied with *A. sativum*, *H. officinalis*, *Mentha* sp., *S. hortensis*, *T. patula* and *V. officinalis* extracts, at 10% concentration. The efficacy of treatments has been calculated, based on the degree attack on leaves, in natural conditions of infection.

Typical symptoms of Alternaria leaf spot were observed in the field immediately after blooming. The level of attack was variable in treated variants compared to untreated control, depending on plant extract (tab. 3, fig. 2).

In field trials, *Valeriana officinalis* and *Satureja hortensis* extracts showed the highest efficacy (e.g. 94.3 and 90.6%, recorded at the beginning of fruit ripening) in controlling *Alternaria* disease. A reduced efficacy was recorded for the other tested extracts (values between 4.6 and 20.1%), despite their *in vitro* efficacy in inhibition of mycelial growth.

A comparison of *in vivo* and *in vitro* efficacy of plant extracts is presented in Table 4. The results from both tests permitted to classify the tested plant extracts into three main categories: extracts with *in vitro* and *in vivo* strong biological activity (mainly at 20% and 10% but some at 5%) (*S. hortensis*, *V. officinalis*), with *in vitro* good activity (*R. officinalis*, *T. patula*, *A. sativum*, *Mentha* sp.), and extracts with no biological activity (*Artemisia dracunculus* 'sativa', *H. officinalis* a.o).

Table 4. *In vitro* and *in vivo* efficacy (%) of plant extracts on *Alternaria alternata* in blackcurrant

	In vitro activity	*In vivo* activity
Strong activity	*Satureja hortensis* (100–72.8) *Valeriana officinalis* (100–72.8)	*Satureja hortensis* (90.6) *Valeriana officinalis* (93.9)
Good activity	*Rosmarinus officinalis* (88.6–64.3) *Tagetes patula* (78.5–68.5) *Allium sativum* (72.8–54.3) *Mentha sp.* (77.1–68.5)	
No activity/Reduced activity	*Artemisia dracunculus* 'sativa' (0)	*Hyssopus officinalis* (20.1) *Allium sativum* (16.1) *Mentha* sp. (9.3) *Tagetes patula* (4.6)

DISCUSSION

The efficacy of various herbal extracts in controlling pathogenic species of the genus *Alternaria* has been reported [Mesta et al. 2009, Raja 2010, Dellavalle et al. 2011, Chetana et al. 2012, Nashwa and Abo-Elyousr 2012, Ganie et al. 2013, Sobhy II et al. 2013, Waghmare 2014, Yazgi et al. 2015].

Previous reports have shown that several plant extracts may inhibit the mycelial growth of *Alternaria alternata* [Begum et al. 2010, Dellavalle et al. 2011, Ramjegatesh et al. 2011, Stangarlin et al. 2011, Swami and Alane 2013, Kantwa et al. 2014, Rodino et al. 2014, Singh et al. 2014, Rama Devi et al. 2015].

***Achillea millefolium* extract**. In our tests this extract has a low inhibitory activity on *A. alternata* in blackcurrant, both *in vitro* and *in vivo*. The lowest efficacy in inhibition of *A. alternata* of *A. millefolium* extract was previous reported [Rizatti et al. 2000, Itako et al. 2008].

***Allium* spp. extract.** Our results on the efficacy of *A. sativum* extract in the growth inhibition of *A. alternata* of blackcurrant, confirmed previous results. Fungicidal properties of garlic extracts has been reported against *A. alternata* strain obtained from potato [Chaudhary et al. 2003], different vegetables [Taskeen-Un-Nisa et al. 2010], *Phaseolus aureus* [Swami and Alane 2013], from senna (*Cassia angustifolia*) [Rama Devi et al. 2015], groundnut [Kantwa et al. 2014] and soybean [Bhosale et al. 2014]. Different concentrations of aqueous extracts of bulbs of onion (*A. cepa*) and garlic (*A. sativum*) were evaluated for their effect on the spore germination of *A. alternata* and *Rhizopus stolonifer*, well known post-harvest pathogens in various horticultural crops. The extract of *A. sativum* was the most effective in reducing the spore germination of these two important postharvest pathogens [Taskeen-Un-Nisa et al. 2010].

Garlic extract has been reported as having high efficacy in inhibiting mycelial growth and spore germination of different *Alternaria* species: *Alternaria brassicae* [Khurana et al. 2005, Neeraj and Verma 2010], *A. solani* [Hagag and El-Khair 2007, Nashwa and Abo-Elyousr 2012], *A. carthami* [Ranaware et al. 2010], *A. porri* [Chetana et al 2012], *A. helianthi* [Mesta et al 2009], *A. tenuissima* [Raja 2010], *A. zinniae* [Waghmare 2014].

***Artemisia dracunculus* 'sativa' extract**. Our results highlight a lack of efficacy of *Artemisia dracunculus* 'sativa' (french tarragon) extract in mycelial growth inhibition of *A. alternata* isolated from blackcurrant. In Romania, recent studies on extracts from spontaneous *Artemisia* species (*A. absinthium*, *A. annua*, *A. vulgaris*) underlined their antimicrobial activity [Ivănescu 2010]. A moderate inhibitory *A. annua* extract on mycelial growth of *A. alternata* in Murcott tangor fruit and no *in vivo* antifungal activity, when applied to fruits was reported [Carvalho et al. 2011]. Also, mycelial growth of *A. solani* causing early blight of potato was inhibited *in vitro* by *A. absinthium* extract, with a good efficacy [Ganie et al. 2013]. No studies on *A. dracunculus* 'sativa' and its antimicrobial activity were found on *Alternaria alternata*.

***Mentha* spp. extract.** A good *in vitro* activity of *Mentha* spp. extract was recorded in our study (68.5 to 78.1%). Our findings are in agreement with those of Taskeen-Un-Nisa et al. [2010] and Ramjegathesh et al. [2011] on *A. alternata*. Similar results were reported by Khurana et al. [2005] and Neeraj and Verma [2010] and on *Alternaria brassicae*.

***Hyssopus officinalis* extract**. Our study reports a moderate inhibitory activity of this extract on mycelial growth of *A. alternata* isolate, only at 20% concentration. No inhibitory effect on mycelial growth was recorded for 10 and 5% concentrations.

Satureja hortensis extract. In our study this extract was very active against *A. alternata* mycelial growth in high concentrations (20 and 10%): 100% and, respectively 72.8% and had a moderate efficacy (42.8%) at 5%. In field trials, three treatments with *S. hortensis* extract, at 10% concentration, had limited Alternaria disease with very high efficacy (90.6%). These results confirmed the reported antifungal potential of this plant extract against plant pathogens, including *Alternaria* species [Sadeghi-Nejad et al. 2010, Şesan et al. 2015].

Tagetes patula extract had a good *in vitro* efficacy (78.5% at 20%, 71.4 at 10% and 68.5 at 5%). Our results complete previous studies on biological action of *T. patula* extracts against other *Alternaria* species as *A. solani* and *A. tenuis* [Begum et al. 2010, Pattnaik et al. 2012, Saha et al. 2012]. A comparative study of the antifungal activity of essential oils/extracts from different parts of the *Tagetes minuta* plant showed moderate to high antifungal activity against a number of soil borne and foliar plant pathogens, including *A. alternata*, from tomato.

***Valeriana officinalis* extract**. Our study reports a very high efficacy of *V. officinalis* extract against *A. alternata*, no mycelial growth being recorded at 20% concentration. At 10%, the *in vitro* efficacy was still high (72.8%). The antifungal potential recorded *in vitro* was confirmed in field trials, where the efficacy in limiting Alternaria disease was 93.9%, the highest value compared to other treatments. We did not find any data in literature to compare our results regarding the effect of this extract against *Alternaria* species.

CONCLUSIONS

1. Our results are the first ones in Romania on plant extracts efficacy in controlling *Alternaria* disease in blackcurrant, as medicinal and horticultural crop. All tested plant extracts had different degrees of antifungal activity against *Alternaria alternata*.

2. The present study highlights the effectiveness of *Valeriana officinalis* and *Satureja hortensis* extracts in limiting *A. alternata*, both *in vitro* and *in vivo*. These extracts could be exploited for eco-friendly management of *Alternaria* leaf spot and fruit rot in black currant and other nutraceutical crops. The availability of these plants makes them an attractive potential candidate for the development of natural plant protection products, which will be an extension of their present medical use.

3. These results represent new and important contributions on *Alternaria alternata* control in blackcurrant, completing those which have *Alternaria* species as target pathogens.

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PRZECIWGRZYBICZE DZIAŁANIE NIEKTÓRYCH WYCIĄGÓW ROŚLINNYCH PRZECIWKO *Alternaria alternata* (Fr.) Keissl. U CZARNEJ PORZECZKI (*Ribes nigrum* L.)

Streszczenie. Po raz pierwszy w Rumunii przetestowano i zbadano *in vitro* i *in vivo* odpowiednio dziewięć i sześć wyciągów wyprodukowanych przez Hofigal S.A. w ich działaniu przeciwko *Alternaria alternata* (szczep Aa 20) wyizolowanego z czarnej porzeczki (*Ribes nigrum* L.). Najlepsze działanie *in vivo* (skuteczność między 70–100%) zanotowano dla *Satureja hortensis* i *Valeriana officinalis.* Dobre działanie inhibicyjne wobec wzrostu grzybni (skuteczność między 54,3–88,6%) zaobserwowano dla *Allium sativum*, *Mentha* sp. *Rosmarinus officinalis*, *Tagetes patula* (10 i 20%). Nie stwierdzono żadnej skuteczności dla *Artemisia dracunculus* 'sativa'. Wyciąg otrzymany z *Hyssopus officinalis* hamował wzrost grzybni *A. alternata* tylko przy 20% ze skutecznością 57.1%. W próbach polowych wyciągi z *Valeriana officinalis* i *Satureja hortensis* były bardzo skuteczne, ograniczając chorobę Alternaria u czarnej porzeczki przy 10% w porównaniu z kontrolą bez zabiegów. Dane te potwierdziły ich silny potencjał przeciwgrzybiczy zarówno *in vitro*, jak i *in vivo*. Opierając się na niniejszych wynikach, można powiedzieć, że wyciągi roślinne o wysokiej skuteczności mogą być alternatywą w ochronie czarnej porzeczki jako rośliny leczniczej przed plamistością liści i zgnilizną owoców spowodowanych przez Alternaria, zwłaszcza w ekologicznym systemie upraw ogrodniczych.

Słowa kluczowe: czarna porzeczka, *Alternaria alternata*, wyciągi roślinne, ogrodnictwo ekologiczne, Rumunia

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