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ORIGINAL PAPER

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In vitro ANTIFUNGAL ACTIVITY OF SOME PLANT EXTRACTS AGAINST *Fusarium oxysporum* IN BLACKCURRANT (*Ribes nigrum* L.)

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ABSTRACT

There were tested and screened, *in vitro*, nine plant extracts manufactured by Hofigal S.A., against *Fusarium oxysporum* (strain Fo 18) isolated from blackcurrant plants (*Ribes nigrum* L.). The highest *in vitro* activity (efficacy 78.6%) was recorded for *Allium sativum* extract, followed by *Satureja hortensis* and *Valeriana officinalis* extracts (71.4% efficacy), at 20% concentration. A good inhibitory activity on mycelial growth has been observed for *Mentha* sp., *Rosmarinus officinalis*, *Hyssopus officinalis* and *Artemisia dracunculus* 'Sativa' (62.8%, 58.6%, 57.1% and, respectively, 50% efficacy). *Achillea millefolium* extract had no effect on radial growth of *F. oxysporum* isolate. This report is the first in Romania regarding the *in vitro* antifungal activity of some plant extracts on *F. oxysporum* in blackcurrant. These data are very useful for plant protection practice, particularly for medicinal plants, as blackcurrant, which demands for non pollutant and environmental friendly alternative methods to fungicides. Locally plant extracts could have important roles in sustainable based management strategies of *Fusarium* disease in blackcurrant.

Key words: blackcurrant, *Fusarium oxysporum*, plant extracts, organic horticulture, Romania

INTRODUCTION

Black currant (*Ribes nigrum* L.), widely cultivated in the majority of European countries, it is a commercially important soft fruit crop which have grown in popularity and received an increased scientific interest, due to alimentary and nutraceutical value of its fruits. Black currant bushes are cultivated mainly for the juice-processing sector, but also for ornamental purposes. However, buds and leaves are also excellent sources of total phenols with antioxidant ability [Tabart et al. 2006, 2007]. Extracts from buds and leaves are important as raw material for the food and health industry thereby making black currant a lucrative product for use as functional food ingredient [Vagiri 2012].

Fusarium vascular wilts and Fusarium root and crown rots are some of the most widespread and destructive diseases of many horticultural crops. In blackcurrant, *Fusarium oxysporum* is a soil inhabit-

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ing pathogen of serious concern, which can rapidly build up and survive for many years, being a major limiting factor in this crop production.

Fusarium oxysporum populations are usually controlled through the use of resistant cultivars, cultural practices (as crop rotation, organic matter addition), soil solarization and disinfection, chemical control [Yucel et al. 2007, Bawa 2016]. However, management of this soilborne disease still remains difficult worldwide.

There is a trend to near zero-market tolerance for pesticide residues in fruits ad vegetables which enhanced the search for non-chemical means to control diseases [Reuveni et al. 2002, Dubey 2011]. Approaches to find environmentally friendly means to manage *Fusarium* disease have been made. Thus, the use of antagonistic microorganisms represents an alternative management strategy [Freeman et al. 2002, Lugtenberg and Kamilova 2009, Horinouchi et al. 2010, Petrescu, Şesan and Oprea 2012]. Plant extracts have proved to be complementary control means for soil-borne pathogens [Javaid and Iqbal 2014, Javaid and Rauf 2015]. Plant metabolites and plant based pesticides are considered to be another alternative as they are known to have minimal environmental impact and minimal danger to consumers in contrast to synthetic pesticides [Ramaiah et al. 2015].

The present study was designed to evaluate the antifungal activity of locally available plant extracts of *Achillea millefolium*, *Allium sativum*, *Artemisia dracunculus* 'Sativa', *Hyssopus officinalis*, *Mentha* sp., *Rosmarinus officinalis*, *Satureja hortensis*, *Tagetes patula* and *Valeriana officinalis* against *Fusarium oxysporum*, responsible for vascular wilt of blackcurrant.

MATERIAL AND METHODS

In vitro tests were conducted using one strain of *Fusarium oxysporum* Schltdl. (Fo 18) isolated at Research-Development Institute for Plant Protection (R-DIPP) Bucharest from infected blackcurrant plants (*Ribes nigrum* L.) from production field of Hofigal S.A. The isolate was identified according to the cultural properties, morphological and microscopical characteristics. Pure cultures were maintened on PDA medium.

The biological action of nine plants extracts was evaluated *in vitro* on mycelial growth of *F. oxysporum* 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). The hydroalcoholic extracts were manufactured by Hofigal S.A. from stems, leaves, flowers, sprouts and bulbs, harvested at recommended time (tab. 1).

Table 1. Plant species as source of extracts

Plant species	Part used	Harvesting	*In vitro* 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	✓

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%. 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, expressed as extract efficacy 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, as per cent inhibition as follows: E (%) = [(dc – dt)/dc] × 100, where dc = average diameter of fungal colony in control, and dt = average diameter of fungal colony for each extract and concentration) and as effective concentrations EC_{50} and EC_{90} (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).

RESULTS

Nine plant extracts were assessed for their potential to inhibit the mycelial growth of one isolate of *Fusarium oxysporum*. The radial growth of the tested isolate has been influenced differently by the plant extracts (tab. 2). The highest inhibition of mycelial growth (78.6% efficacy) was recorded for *Allium*

Table 2. Biological action of plant extracts on mycelial growth of *Fusarium oxysporum*

Plant extract	Concentration (%)	Colony diameter (mm)	Efficacy (%)
Achillea millefolium	20	70	0
	10	70	0
	5	70	0
Allium sativum	20	15	78.6
	10	35	50.0
	5	70	0
Artemisia dracunculus 'Sativa'	20	35	50.0
	10	45	35.7
	5	55	21.7
Hyssopus officinalis	20	30	57.1
	10	30	57.1
	5	70	0
Mentha sp.	20	26	62.8
	10	40	42.8
	5	55	21.4
Rosmarinus officinalis	20	29	58.6
	10	45	35.7
	5	70	0
Satureja hortensis	20	20	71.4
	10	25	64.3
	5	70	0
Tagetes patula	20	40	42.8
	10	45	35.7
	5	45	35.7
Valeriana officinalis	20	20	71.4
	10	45	35.7
	5	70	0
Control (untreated)	–	70	–

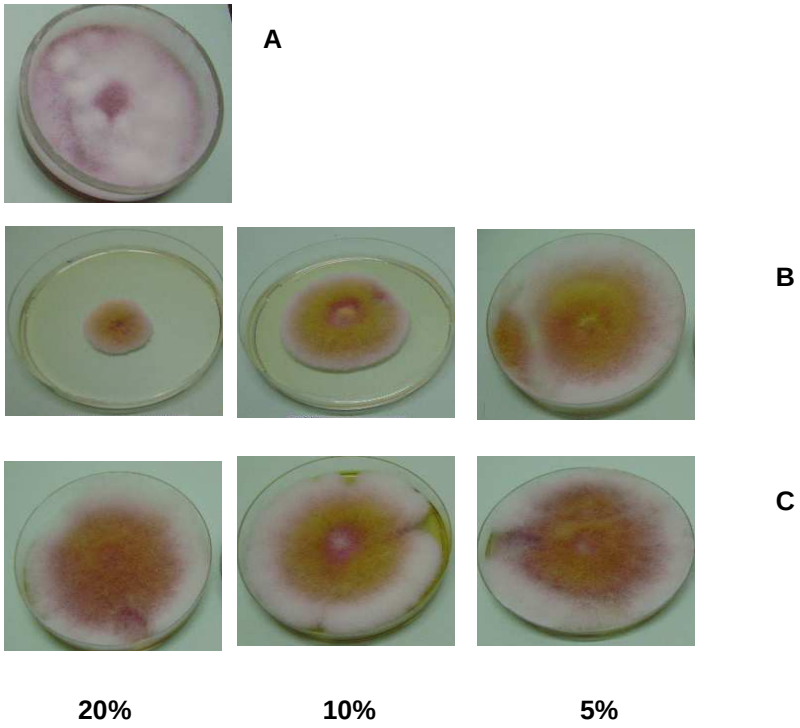

Fig. 1. *In vitro* biological activity of some plant extracts against development of *Fusarium oxysporum* (Fo 18) from blackcurrant. A – control; B – *Allium sativum* extract; C – *Achillea millefolium* extract

Table 3. Biological action of plant extracts on mycelial growth of *Fusarium oxysporum*

Plant extract	EC50 values for mycelial growth (%)	
	EC 50	EC90
Achillea millefolium	>20	>20
Allium sativum	10	21.2
Artemisia dracunculus 'Sativa'	20	41.2
Hyssopus officinalis	15.3	27.5
Mentha sp.	14.5	29.6
Rosmarinus officinalis	16.7	27.6
Satureja hortensis	12.8	22.3
Tagetes patula	35.2	114.1
Valeriana officinalis	14.8	23.5

sativum, at 20% concentration followed by *Satureja hortensis* and *Valeriana officinalis* – 71.4% efficacy (fig. 1). At the same concentration, the extracts of *Mentha* sp., *Rosmarinus officinalis*, *Hyssopus officinalis* and *Artemisia dracunculus* '*Sativa'* inhibited the mycelial growth with an average between 50% and 62.8%. *Tagetes patula* extract recorded a reduced efficacy (42.8%).

At 10% concentration, the most effective extract in inhibiting the mycelial growth was *Satureja hortensis* (64.3% inhibition of radial growth), followed by *Hyssopus officinalis* (57.1%) and *Allium sativum* (50%). The other tested extracts exhibited lower efficacies, between 42.8%, for *Mentha* sp. and 35.7% for *Artemisia dracunculus* 'sativa', *Rosmarinus officinalis*, *Valeriana officinalis*, and *Tagetes patula.*

Of all the tested extracts, *Achillea millefollium* did not inhibit the mycelial growth of Fo 18 isolate, no fungicidal effect being observed at all three concentrations tested (fig. 1). No effect on mycelial growth was recorded for *Rosmarinus officinalis*, *Satureja hortensis*, *Allium sativum* and *Hyssopus officinalis* extracts, tested at 5% concentration.

The level of sensitivity of *F. oxysporum* was expressed as EC50 and EC90 concentrations (tab. 3). *Fusarium oxysporum* Fo 18 isolate appeared to be the most sensitive to *Allium sativum* (EC50 value 10; EC90 value 21.2) followed by *Satureja hortensis* (EC50 value 20; EC90 value 22.3), and *Valeriana officinalis* (EC50 value 14.8; EC90 value 23.5).

DISCUSSION

Many researches have been carried out to find alternative to fungicides and environmentally safe methods to control plant diseases [Agbenin et al. 2004, Yucel et al. 2007, Dubey 2011]. Thus, plant extracts have been tested against *Fusarium oxysporum*: *F. oxysporum* f. sp. *lycopersici* [Hassanein et al. 2010, Dwivedi and Enespa 2012, Darmadi et al. 2015, Ramaiah et al. 2015, Singh et al. 2015, Chougule and Andoji 2016, Rawal et al. 2016, Siddiqui et al. 2016, Nafar 2017], *F. oxysporum* f. sp. *capsici* [Suprapta and Khalimi 2012, Shafique et al. 2015], *F. oxysporum* f. sp. *lentis* [Belabid et al. 2010, Garkoti et al. 2013], *F. oxysporum* f. sp. *phaseoli* [Obongoya et al. 2010], *F. oxysporum* f. sp. *ciceri* [Kamdi et al. 2012 , Minz et al. 2012, Shukla and Dwivedi 2012], *F. solani* f. sp. *melongenae* [Siva et al. 2008, Dwivedi and Enespa 2012, Dwivedi et al. 2015], *Fusarium oxysporum* f. sp. *gladioli* [Riaz et al. 2008, Raj and Kumar 2009, Chohan et al. 2011, Pârvu and Pârvu 2011, Kadam et al. 2014, Jan et al. 2015], *F. oxysporum* f. sp. *albedinis* [Boulenonuar et al. 2009], *F. oxysporum* f. sp. *tulipae* [Pârvu and Pârvu 2011], *F. oxysporum* f. sp. *cepae* [Javaid and Akhtar 2015], *F. oxysporum* f. sp. *sesami* [Syed et al. 2015], *F. oxysporum* f. sp. *cubense* [Oladipo et al. 2015], *F. oxysporum* f. sp. *zingiberi* [Vivek et al. 2013], *F. oxysporum* [Sharma and Kumar 2009, Chaudhuri and Guha 2010, Manmohan and Govindaiah 2012, Dissanayake and Jayasinghe 2013, Dissanayake 2014, Abdel-Ghany et al. 2015, Martin et al. 2016]. Referring to blackcurrant, there are a few literature on this subject.

***Allium sativum* extract**. Our results highlight the highest inhibitory effect of *A. sativum* extract on mycelial growth of *Fusarium oxysporum*, at 20% concentration. Many other studies reported the fungitoxic effect of *Allium* species extracts. Thus, aqueous extracts of *Allium cepa*, *A. sativum*, *A. senescens* ssp. *montanum* have been shown to be effective in inhibiting *F. solani* in peanut [Ahmed et al. 2012], and potato [Shresta and Tiwari 2009], *F. solani* f. sp. *melongenae* [Bowers and Locke 2000]; *F. oxysporum* f. sp. *gladioli* [Riaz et al. 2008, 2009, Chohan et al. 2011, Pârvu and Pârvu 2011], *F. oxysporum* f. sp. *tulipae* [Pârvu et al. 2011a], *F. oxysporum* [Aba Alkhail 2005]. Also, benzene and methanol extracts of *Allium sativum* bulbs showed a strong effect in inhibiting mycelial growth of *F. oxysporum* f. sp. *ciceris* [Sahayaraj et al. 2006]. The fungicidal activity of *A. fistulosum* it was hyghlighted by ultrastructural changes in *F. oxysporum* f. sp. *tulipae* treated hyphae, which have affected their viability [Pârvu et al. 2010a]. Garlic extract has the ability to affect a wide range of soilborne fungal pathogens [Sealy et al. 2007]. It is well known that the antifungal and antibacterial properties of alliaceous plants is due to the sulfur content and other phenolic compounds [Rivlin

2001, Griffith et al. 2002, Pârvu et al. 2010b, Pârvu et al. 2011b]. *Allium sativum* extract inhibited also the mycelial growth of another two important blackcurrant fungal pathogens, *Botrytis cinerea* and *Alternaria alternata* [Şesan et al. 2015, 2016].

***Achillea millefolium* extract**. No efficacy was recorded for *A. millefolium* extract on the mycelial growth of *F. oxysporum* in blackcurrant. This extract had only a low inhibitory activity on *B. cinerea* and *A. alternata* in blackcurrant [Şesan et al. 2015, 2016].

***Mentha* spp. extract.** A moderate activity of *Mentha* sp. extract was observed in our study. Other studies reported the remarkable antifungal activity of different species of mint like *M. arvensis*, *M. piperita*, and *M. spicata* against *F. oxysporum* [Bowers and Locke 2000, Nosrati et al. 2011, Singh and Kumar 2011, Kazemi et al. 2012, Hadi et al. 2013]. The same *Mentha* spp. extract had a good *in vitro* efficacy in our previous work on *Alternaria alternata* [Şesan et al. 2016], and a moderate one on *Botrytis cinerea* isolated from blackcurrant [Şesan et al. 2015].

***Hyssopus officinalis* extract**. On mycelial growth of *F. oxysporum* isolate, this extract had recorded a moderate efficacy, at 20% and 10% concentrations. On the other hand, total inhibition of mycelial growth of *B. cinerea* in blackcurrant, was reported for *H. officinalis* extract, even at 5% concentration [Şesan et al. 2015]. On *A. alternata* isolated from blackcurrant, this extract had only a moderate inhibitory *in vitro* activity and only at 20% concentration [Şesan et al. 2016].

***Tagetes patula* extract**. Although in our study, this extract had a low efficacy (42.8% at 20% concentration), other studies reported a significantly inhibition of *Fusarium* mycelial growth by different *Tagetes* species, as *T. erectus* on *F. oxysporum* f. sp. *gladioli*, *F. monilifome*, *F. oxysporum* f. sp. *capsici*, *F. oxysporum* f. sp. *chrysanthemi* [Riaz et al. 2008, Yasmin et al. 2008, Begum et al. 2010, Chohan et al. 2011, Singh and Kumar 2011] and *T. minuta* on *F. oxysporum* [Obongoya et al. 2010]. It is considered that the fungicidal properties of *Tagetes* species it is due to the presence of thiophene in the plant tissues [Gomez-Rodriguez et al. 2003, Romagnoli et al. 2005]. *Tagetes patula* extract had a good *in vitro* efficacy on mycelial growth of *Botrytis cinerea* and *Alternaria alternata* pathogens, isolated from blackcurrant [Şesan et al. 2015, 2016].

***Valeriana officinalis* extract**. Our study showed a good *in vitro* efficacy of *V. officinalis* extract, at 20% concentration on *F. oxysporum* radial growth. This extracts had been reported with a very high *in vitro* and *in vivo* efficacy against *A. alternata* [Şesan et al. 2016] and with no activity against *B. cinerea* in blackcurrant [Şesan et al. 2015].

***Satureja hortensis* extract**. The highly effective fungicidal properties of *Satureja hortensis* extract was reported on *Fusarium* species [Sadeghi-Nejad et al. 2010]. In our study, this extract exhibited a good efficacy, at 20% concentration. The same concentration lead to 100% *in vitro* and *in vivo* efficacy in inhibiting the mycelial growth of *B. cinerea* and *A. alternata* in blackcurrant [Şesan et al. 2015, 2016].

***Rosmarinus officinalis* extract**. A moderate *in vitro* efficacy was recorded for *R. officinalis* extract on mycelial growth of *F. oxysporum*. This extract had a low efficacy against *B. cinerea* in blackcurrant [Şesan et al. 2015].

***Artemisia dracunculus* 'sativa' extract**. Our results highlight a lack of efficacy of *Artemisia dracunculus* 'sativa' extract in mycelial growth inhibition of *A. alternata* isolated from blackcurrant.

CONCLUSIONS

Our results are the first ones in Romania on plant extracts efficacy in controlling *Fusarium* disease in blackcurrant. The fungitoxic effects of some tested phytoextracts indicate the potentials of selected plant species as a source of natural fungicides.

Antifungal activity was confirmed by *Allium sativum*, *Satureja hortensis* and *Valeriana officinalis* extracts, which were the most effective on *F. oxysporum* mycelial growth. This could be an important step towards the possibilities of using natural plant products to manage *F. oxysporum*.

Research is ongoing to assess the possible effects on beneficial soil organisms. Further green house and field experiments are needed to investigate the *in vivo* effects of these extracts for the management of *Fusa-*

rium disease in blackcurrant. If the soilborne *Fusarium* population can be reduced and the disease development achieved, then these plant extracts have potential as environmentally safe alternatives and as components in a sustainable/integrated disease management.

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