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EFFECTIVENES OF ESSENTIAL OILS IN CONTROL OF *VERTICILLIUM DAHLIAE IN VITRO*

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SUMMARY

Volatile phase of essential oils of *Mentha piperita*, *Petroselinum crispum*, *Eucaliptus citriodora*, *Pinus sylvestris*, *Rosmarinus officinalis*, *Pimpinella anisum* and *Origanum vulgare* were tested for their effectiveness in control of *Verticillium dahliae*, *in vitro*. Percentage of inhibition of radial growth was calculated four days after exposure of mycelia to volatiles of essential oils. Also MIC and MFC were determined as well seven i.e. fourteen days after exposure. Highest value of inhibition expressed essential oil of *O. vulgare* with MIC and MFC applied at 0,04µl/ml of air, followed by essential oils of *P. anisum* (MIC – 0,1µl/ml of air, MFC – 0,3µl/ml of air). Essential oil of *E. citiodora* expressed MIC and MFC applied at 0,6µl/ml of air, while essential oil of *M. piperita* expressed only MIC when applied at 0,3µl/ml of air. Essential oils of *P. sylvestris, R. officinalis* and *P. crispum* din not express MIC or MFC even when applied at highest rate.

Key words: essential oils, control, *Verticillium dahliae*, tomato

INTRODUCTION

Tomato (*Lycopersicon esculentum* Mill.) is one of the most important vegetable crop worldwide. Due to antioxidant properties of its main components it is known as health stimulating fruit and it's used fresh as well as processed (Glogovac et al., 2010; Radzevičius et al., 2009). Tomato production is threatened by numerous fungal pathogens, every year (Đorđević et al., 2012a). *Verticillium dahliae* is one of them. This is soil pathogen that can affect the quality and quantity of tomato production for fresh consumption or for seed production (Bhat and Subbarao, 1999; Garcia, 2011; Ivanović and Ivanović, 2001).

The most efficient control of this pathogens was with methyl-bromide. However, due to negative impact on the ozone layer, this substance has been banned for use (Guillino et al., 2002). Taking methyl-bromide out of picture there was not any substance efficacy enough to take its place (Bell, 2000; Ioannou, 2000; Ivanović and Ivanović, 2007). Giullino et al. (2002) researched the chlor-picrin as a substitute for methyl-bromide, since they have been applied together in order to intensify the impact of methyl-bromide, but they did not obtain the results with satisfactory efficiency on these and other soil pathogens.

Taking into consider the growing need to preserve ecosystems and a growing awareness of the possible harmful effects of chemicals, it was necessary to find a long-term solution without the harmful impact on the ecosystem, on one hand, and to protect this important manufacturing, on the other. Applying the biological control of these pathogens is the solution (De Veger et al., 1995; Fravel, 2005; Paulitz and Belanger, 2001; Postma et al., 2003; Ramezani, 2008). One of the possible ways of biological control is the appliance of essential oils of some aromatic and medicinal plants. Since the plants are the source of large number of substances with fungicide and fungistatic effect, they could be the mentioned solution (Aslan et al., 2010; Isman, 2000; Tanović et al., 2005; Wilson et al., 1997). Many researches explored the influence of essential oils plants on fungi, pathogens of plants and fungi important for food industry, proved that these plant compounds could be the solution (Aslam et al., 2010; Paarveen et al., 2010; Đorđević et al., 2011, 2012b, 2013a, 2013b ; Tanović et al., 2009; Veljić et al., 2009; Zhang et al., 2009).

The aim of this study was to examine the effect of essential oils on *Verticillium dahliae* tomato pathogen, i.e. to find the toxicity of seven essential oils *in vitro* and to find the minimal inhibitory concentration (MIC) and minimal fungicidal concentration (MFC), which would give us the necessary information so we could further research their effect *in vivo*.

MATERIAL AND METHODS

Pathogen

Tomato pathogen *Verticilium dahliae*. was isolated from tomato plants and identified as tomato pathogen by inoculation and re-isolation and belong to phytopathogen collection of Institute for Vegetable Crops, Smederevska Palanka, Serbia.

Antifungal effect of essential oils

Effect of seven essential oils of the following plants: *Mentha piperita*, *Petroselinum crispum*, *Eucaliptus citriodora*, *Pinus sylvestris*, *Rosmarinus officinalis*, *Pimpinella anisum* and *Origanum vulgare* to mentioned pathogens have been researched. The effect has been determined by exposure of pathogen to volatile phase of oils (Tanović *et al.*, 2009; Soylu *et al.*, 2006). Mycelial plug of the pathogen (5x5mm) was transmitted to the center of petri plate (R = 9 cm), after which the petri plate has been turned upside down. Oils were inflicted on a sterile paper disk (R = 0.5 cm), located at the centre of petri plates, and 0.04, 0.06, 0.1,0.15, 0.3 and 0.6 µl/ml of air in petri plate has been applied. In order to facilitate the contact of volatile phase of oils and pathogens, petri plates were kept upside down. The plates were covered with self-adhesive foil to prevent release of oil vapors out of the plates. Petri plates were kept at 23°C. Petri plates with a drop of sterile distilled water instead of oil, were used as control. After four days, the growth of colonies has been measured and on the basis of these values the degree of inhibition of colony growth has been calculated. Seven days after this trail the inhibitory effect of oil on the pathogen has been determined. The concentration of oil that completely inhibited the growth of the pathogen mycelium was considered fungistatic and the lowest such value was determined as the minimum inhibitory concentration (MIC). The petri plates in which an inhibitory effect was found, were ventilated in a sterile laminar flow of air over a period of 30 minutes in order to completely remove a volatile oil phase, and then the petri plates were kept for seven days in a thermostat on the same temperature as previusly. This is done to determine the lethal concentration of oil. The concentration of oil in which there is no appearance of growth of pathogens mycelium after 7 days after ventilation was taken as fungicidal. The lowest such concentration was the minimum fungicidal concentration (MFC).

Statistical analysis

All trials have been set twice with five replications of each oil concentration. Percentage of inhibition of mycelia growth was calculated using following formula:

$(\%) = (g_c - g_t / g_c) \times 100$, where g_c is growth of mycelium in control plates, g_t growth of mycelium in treated plates.

RESULTS

Antifungal activity of essential oils

The obtained results showed that oils had different level of inhibition of mycelia growth of researched pathogen in different concentrations. Highest percent of inhibition of mycelial growth of *Verticillium dahliae* expressed oils of *O. vulgare* and *P. anisum* with 100% inhibition in the lowest applied concentration of 0,04 µl/ml of air. Essential oil of *M. piperita* has completely inhibited the growth of mycelia of this pathogen when applied in concentration of 0,3 µl/ml of air, and oils of *R. officinalis* and *E. citriodora* had the same effect at concentration 0,6 µl/ml of air (Figure 1.).

Toxicity of essential oils

Measurements of growth of pathogen mycelia seven days after treatment showed that essential oils had different degree of toxicity to this pathogen (Figure 2). Essential oil of *O. vulgare* expressed MIC and MFC at lowest applied concentration 0,04 µl/ml of air. Essential oil of *P. anisum* expressed MIC applied at 0,1 µl/ml of air while MFC had when applied at 0,3 µl/ml of air. Essential oil of *M. piperita* expressed only MIC when applied at 0,3 µl/ml of air while MFC value was not determined even when applied at highest concentration rate. Meanwhile, essential oil of *E. citriodora* expressed MIC and MFC both when applied at 0,6 µl/ml of air. Essential oils of *R.officinalis*, *P. silvestris* and *P. crispum* did not expressed MIC or MFC even when applied at highest concentration rate.

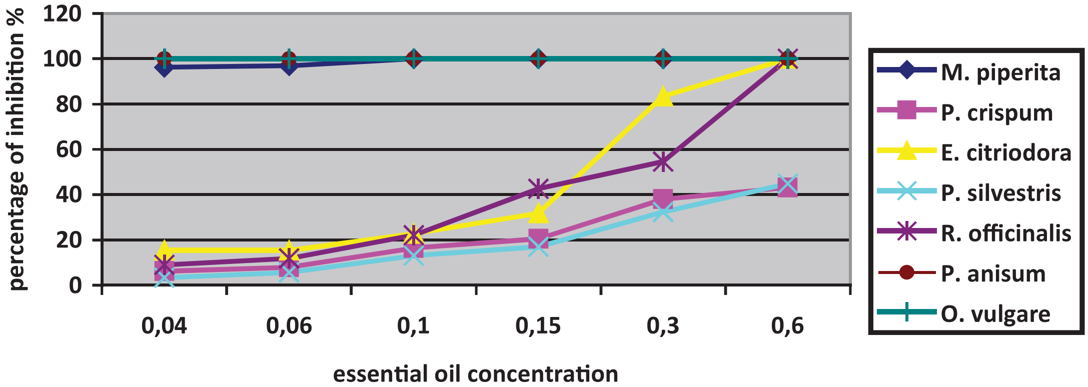

Figure 1. Effect of volatile phase of essential oils of growth on *Verticillium dahliae* after 4 day exposure *in vitro*.
Slika 1. Uticaj isparljivih faza etarskih ulja na porast *Verticillium dahliae* nakon 4 dana izlaganja *in vitro*

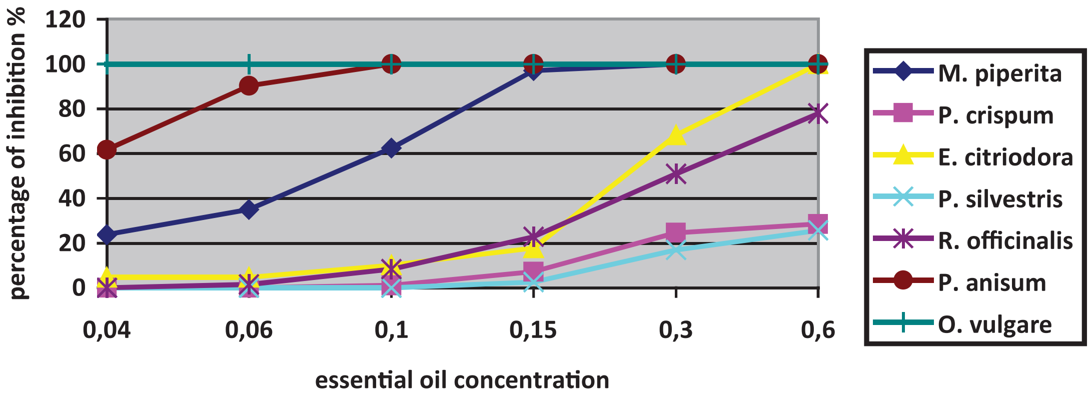

Figure 2. Effect of volatile phase of essential oils of growth on *Verticillium dahliae* after 7 days exposure *in vitro*.
Slika 2. Uticaj isparljivih faza etarskih ulja na porast *Verticillium dahliae* nakon 7 dana izlaganja *in vitro*

DISCUSSION

Antimicrobial activity of essential oils of aromatic and medicinal plants has been recognized for long time. They show strong inhibition effect to many different fungi. Strong antimicrobial effect of some essential oils has been previously reported (Feng and Zheng, 2007; Lee et al., 2007; Pradhanang et al., 2003; Soylu et al., 2006; Tanović et al., 2004, 2005, 2009; Veljić et al., 2009; Džamić et al., 2008a, 2008b). Comparison of results of different studies is difficult because of differences in plant extract composition and in methodologies of assessments of microbial activity (Arslan and Dervis, 2010). Results of this research showed that some of the tested oils can suppress growth of mycelia of *Verticillium dahliae*, *in vitro*. Essential oils of *O. vulgare* and *P. anisum* expressed highest antifungal activity against mentioned tomato pathogen. Volatile compounds of *O. vulgare* essential oil has been reported as strong inhibitor of mycelia growth of some soil-borne pathogens *Fusarium solani* var. *coeruleum*, *Fusarium oxysporum* f.sp. *lycopersici*, *Pythium ultimum*, *Rhizoctonia solani*, *Verticillium dahliae*, where pathogens were totally inhibited at minimum concentrations (Arslan and Dervis, 2010; Đorđević et al., 2013a; Daferera et al., 2003; Lee et al., 2007; Soylu et al., 2006). Essential oil of *P. anisum* was highly effective in control of *Fusarium oxysporum* f.sp. *lycopersici* with MIC 0,3 µl/ml of air, as well as *M. piperita* with MIC 0,3µl/ml of air (Đorđević et al., 2013a). Essential oil of *P.anisum* was reported to be fungicidal for *Botrytis cinerea* at 0,16 µl/ml of air, as well as the essential oil of *M. piperita* (Tanović et al., 2005). Research of Đorđević et al. (2013b) indicated fungicidal effect of essential oil of *P. anisum* on *B. cinerea* when applied at 0,1 µl/ml of air, while oil of *M. piperita* expressed both MIC and MFC applied at 0,15µl/ml of air. Essential oils of *O. vulgare* and *P. anisum* were highly effective in inhibition of *Alternaria alternata*, especially *O. vulgare* with MIC and MFC applied at 0,04µl/ml of air (Đorđević et al., 2012b).

There are other studies, as well, that indicated effectiveness of these and other essential oils to food-borne diseases as well (López et al., 2007; Đorđević et al., 2012a, 2012b).

Although there were studies that tried to identify mode of action of these essential oils it is still not completely understood (Adams et al., 1996; Chang et al., 2001; Ultee et al., 2002).

Due to the fact that the main compounds of the *O. vulgare* essential oil are carvacrol, thymol, c -terpinene and p-cymene, we can assume that these compounds are the main holders of antimicrobial activity and carriers of the mode of action of this oil(Daferera et al., 2003; Soylu et al., 2006).

The results of this research indicate that essential oils of *oreganum* and *anise* have potential to suppress this pathogen, *in vitro*. Therefore these oils could be used for control of *V. dahliae*, *in vivo* and the plant rich with these oils could be an important part of biological control strategy.

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EFIKASNOST ETERIČNIH ULJA U SUZBIJANJU *VERTICILLIUM DAHLIAE* PATOGENA PARADAJZA *IN VITRO*

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REZIME

Ispitiavana je efikasnost isparljive faze eteričnih ulja *Mentha piperita, Petroselinum crispum, Eucaliptus citriodora, Pinus sylvestris, Rosmarinus officinalis, Pimpinella anisum* i *Origanum vulgare* u inhibiciji porasta micelije patogena *Verticillium dahliae* u *in vitro* uslovima. Procenat inhibicije radijalnog porasta izračunavan je četiri dana od izlaganja micelije dejstvu para eteričnih ulja.. Takođe, izračunavana je i minimalna inhibitorna koncentracija (MIC) sedam dana nakon izlaganja kao i minimalna fungicidna koncentracija (MFC) četrnaest dana od izlaganja dejstvu para. Najveći procenat inhibicije pokazalo je ulje *O. vulgare* sa MIC i MFC primenjenim već u najnižoj koncentraciji 0,04µl/ml vazduha, za kojim sledi ulje *P. anisum* (MIC – 0,1µl/ml of air, MFC – 0,3µl/ml of air). Eterično ulje *E.citriodora* ostvarilo je MIC i MFC primenjeno u koncentraciji 0,6µl/ml vazduha, dok je eterično ulje *M. piperita* pokazalo samo MIC i to primenjeno u konc. 0,3µl/ml vazduha. Eterična ulja *P. sylvestris*, *R. officinalis* i *P. crispum* nisu pokazala zadovoljavajući nivo inhibicije a samim tim nisu imala ni MIC ni MFC vrednost.

Ključne reči: eterična ulja, kontrola, *Verticillium dahliae*, paradajz

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