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Scientific paper

Development of a Thyme Essential Oil Formulation and Its Effect on *Monilinia fructigena*

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SUMMARY

Antifungal activity of thyme essential oil against *Monilinia fructigena* and development of an effective and stable oil formulation for agricultural use were studied in this paper. Bioactivity of the oil itself and its developed formulation, an emulsifiable concentrate (EC), was tested *in vitro* and *in vivo*. *In vitro* experiments, using a slightly modified agar overlay technique, showed that the initially emulsified thyme essential oil, as well as the developed formulation, significantly inhibited mycelial growth of *M. fructigena in vitro*. Experiments *in vivo*, performed on inoculated apple fruits, revealed that the formulation successfully decreased oil evaporation from the treated area and provided a significant level of *M. fructigena* suppression, 64.7-72.1% compared to the control. To our knowledge, an EC formulation of thyme essential oil for agricultural uses had never been developed before. The presented results are initial findings and evaluation of product activity should be continued in the field to determine its efficacy and activity spectrum, and to estimate the economic aspect of its use.

Keywords: Thyme; Formulations; Essential oils

INTRODUCTION

The development of modern fungicides, and the storage technologies improved during the 1960s and 1970s, have significantly extended the shelf life of fruit after harvest. However, postharvest losses caused by fungal diseases still vary from the estimated 5-20% in the United States to more than 50% in developing countries (Janisiewicz and Korsten, 2002). In addition, the use of fungicides is restricted due to consumers' demand for less chemical residues in fresh produce (Ragsdale and Sisler, 1994; Gullino

and Kuijpers, 1994). The application of substances of natural origin, such as essential oils, could be a desirable solution, safe both for human health and the environment (Janisiewicz and Korsten, 2002; Zhang and Zheng, 2005; Tasiwal et al., 2009). Essential oils have been well-known for their antimicrobial properties since the 1880s (Chamberlain, 1887). Some of them, such as the tea tree oil from *Melaleuca alternifolia* (Maiden & Betche) Cheel, have been effectively used in human medicine for years (Hammer et al., 2003). The inhibitory effect of tea tree oil on *Botrytis cinerea* and other postharvest fruit pathogens *in vitro* has also been documented (Tanović et al., 2005; Hrustić et al., 2012). However, its further examination in the field against *B. cinerea*, using the commercially available formulated product Timorex 66EC (Stockton Chemical Corporation, Israel), showed that grey mould control can be achieved only partially (13.3-55.9%), depending on disease pressure (Tanović et al., 2012).

In our previous investigation, thyme essential oil had exhibited a great potential as a crop protectant against some postharvest apple fruit pathogens (Tanović et al., 2010; Hrustić et al., 2011). Of the 56 tested oils, including tea tree oil which is already on the market, the volatile phase of thyme oil exibited the highest toxicity (Hrustić et al., 2012; Grahovac et al., 2012). Therefore, the aims of our present study were:

- to determine the effect of an emulsified thyme essential oil on a *Monilinia fructigena* isolate *in vitro* and *in vivo*
- to investigate the possibility of developing an effective thyme essential oil formulation with stable antifungal activity
- to test the bioactivity of such formulation using an isolate of *M. fructigena* as a model organism.

MATERIAL AND METHODS

Test organism

An isolate of *M. fructigena* was derived from a diseased apple fruit showing brown rot. Small fragments were aseptically excised from the border between healthy and diseased parenchymal apple tissue and placed onto the surface of potato-dextrose agar (PDA) in Petri plates. The obtained isolate was cultured on PDA at 24°C and stored at -80°C in 20% glycerol for long-term storage and at 4°C on PDA slants for short-term storage. Patogenicity of the isolate was confirmed by inoculation of a healthy apple fruit previously surface sterilized with 0.5% NaOCl. The obtained isolate was identified based on morphological characteristics of the colony and conidia using the synoptic key described by Lane (2002). Identification was confirmed by multiplex PCR, proposed by Côté et al. (2004), using the common reverse primer MO368-5 (5'-GCAAGGTGTCAAAACTTCCA-3), which is specific for *Monilinia* spp., and three species-specific forward primers: MO368-8R (5'-AGATCAAACATCGTCCATCT-3', for *M. fructigena* and *M. polystroma*), MO368-10R (5'-AAGATTGTCACCATGGTTGA-3', for *M. fructicola*) and Laxa-R2 (5'-TGCACATCATATCCCTCGAC- 3', for *M. laxa*). The template DNA for multiplex PCR was extracted from 7-day-old mycelium of the isolate grown on PDA medium, according to the method described by Harrington and Wingfiled (1995).

Inoculum preparation

Mycelial fragments (R=10 or 3 mm), cut from the edge of 10-day-old PDA culture of *M. fructigena,* were used in the experiments *in vitro* and *in vivo*, respectively.

Test substances

Commercially available thyme essential oil (*Thymus vulgaris* L.) was provided by Beolab Co., Belgrade, Serbia. Iprodione (Kidan 250 SC, Bayer CropScience, Germany) and tea tree essential oil (Timorex gold, Stockton Group, Israel) were used at label rates in all experiments.

Initial emulsion preparation

In order to test the contact activity of thyme essential oil against *M. fructigena in vitro* and *in vivo,* an initial thyme oil emulsion was prepared as follows: 100 µl of thyme essential oil was dissolved in 100 µl of 35% ethanol and detergent at 0.1% and emulsified in 10 ml of sterile distilled water. The effect of a blank preparation (a mixture of sterile distilled water and the same amounts of ethanol and detergent as in the initial emulsion) on mycelial growth was tested before the experiment.

Initial emulsion activity

An *in vitro* antagonistic assay was performed on 10 ml of solidified PDA medium in 90 mm Petri plates using a slightly modified agar overlay technique (Cooper, 1963). The tested substances (100 µl) were added into wells (10 mm), cut 1 cm from the edge of each Petri plate, while mycelial fragments (10 mm in diameter) of the tested isolate were placed 1 cm from the opposite end of each plate. The treatments included: initial thyme oil emulsion, two reference treatments (the commercial biofungicide based on tea tree essential oil and the conventional fungicide iprodione) and sterile distilled water as a negative control. The assessment was made seven days after treatment by measuring mycelial growth towards the well. The experiment was done in three replications and repeated once. Since all experimental conditions in the repeated experiment were the same, all obtained data were pulled together and subjected to the analysis of variance (ANOVA) and Duncan's multiple range test.

An *in vivo* antagonistic assay was performed on mature apple fruits (cv. Idared) of similar size. Each fruit was surface disinfected and wounded with a sterile nail (4-mm diameter and 3-mm depth). A 10 µl drop of each treatment (initial thyme oil emulsion, commercial tea tree oil, iprodione, and sterile distilled water) were added into each wound 15 minutes or 24 hours prior to inoculation, which was performed by placing a mycelial plug (3 mm) on each wound. Fruits inoculated with sterile PDA plugs were used as a negative control. The fruits were placed on two layers of moist paper towels on a wire rack in a plastic container at 24°C, 97% RH (relative humidity). The width and length of lesions on the inoculated fruits were measured seven days after inoculation. Each treatment was done in three replicates and the experiment was repeated once. The results from both experiments were pulled together and subjected to the analysis of variance (ANOVA) and Duncan's multiple range test.

Formulation development

The vegetable oil used for formulating thyme essential oil was purchased from a commercial source (Oleon, Belgium) and used without further purification. Surfactants of commercial quality (Rhodia, Italy and Ajinomoto OmniChem, Belgium) were used.

The emulsifiable concentrate (EC) of thyme essential oil was prepared by mixing the essential oil (10%) with nonionic emulsifiers (10%) and esterified rape seed oil (80%). A magnetic stirrer (IKA, RH basic 2) was used for homogenization (30 minutes). A blank formulation was prepared the same way as the EC formulation and contained vegetable oil instead of thyme essential oil.

Several important physical parameters of the formulation product were determined, using the following standard CIPAC methods: MT 3 for density, MT 75 for pH value, MT 47.2 for persistent foam, MT 36.1 for emulsion stability and re-emulsification (CIPAC, 1995).

Formulation activity

In vitro and *in vivo* activity of the developed formulation was determined the same way as it was described for the initial emulsion testing. Prior to the experiment, the effect of blank formulation on mycelial growth was tested.

RESULTS

Test organism

After 4-day incubation of apple tissue fragments on PDA medium at 24°C, a creamy to yellow colony with sparse sporulation and entire margin developed. Conidia were unicellular, hyaline, ellipsoid or ovoid, arranged in chains. Based on morphological characteristics of the colony and arrangement and shape of its conidia, the isolate was identified as *M. fructigena*. Identification was confirmed by the resulting 402 bp PCR product, which was proposed by Côté et al. (2004) as the product size specific to *M. fructigena*.

Initial emulsion activity

As the results presented in Figure 1 indicate, significant differences in mycelial growth *in vitro* were found ($p<0.01$) as a consequence of the activity of different treatments. Duncan's multiple range test showed that the initial thyme oil emulsion exhibited significantly stronger mycelial growth inhibition than tea tree oil, which was used for comparison. Both treatments were, however, less effective than the fungicide iprodion applied at label rate. The blank preparation did not cause any inhibitory effect on mycelial growth, and its effect was therefore excluded from the analysis.

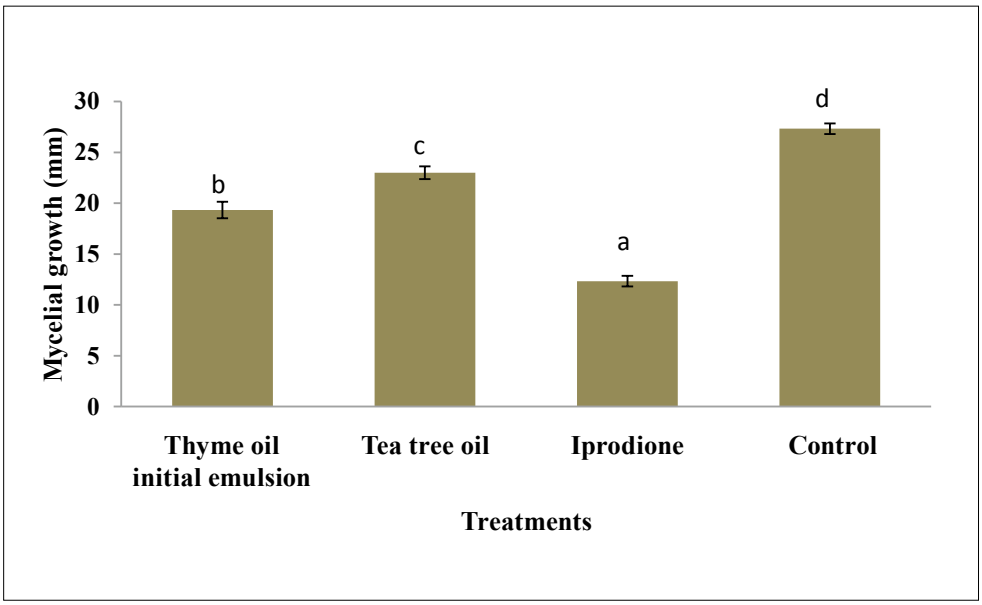

Figure 1. The effects of initial thyme oil emulsion and reference treatments on mycelial growth (Mean ± SD) of *M. fructigena in vitro*. Bars with the same letters are not significantly different (Duncan's test, $p > 0.01$).

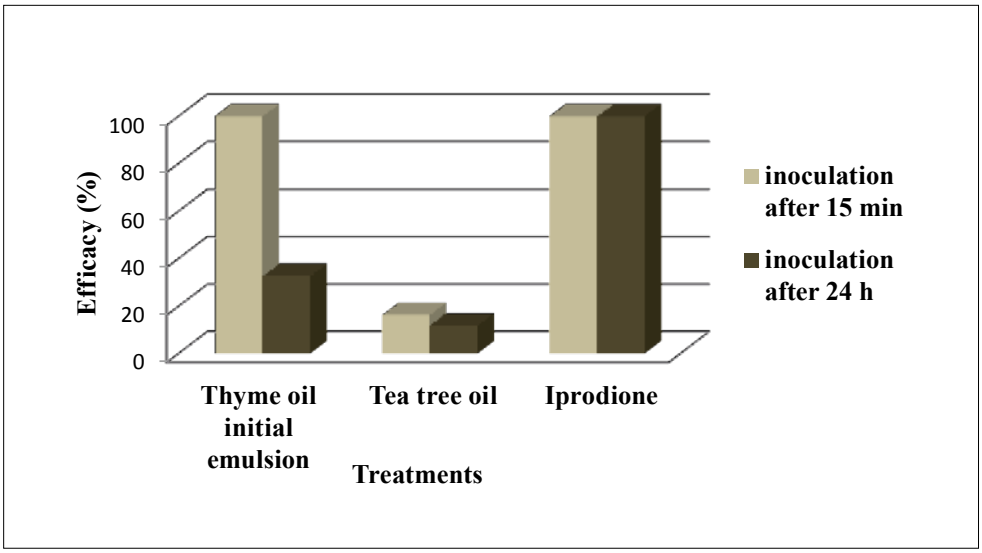

Figure 2. The effects of initial thyme oil emulsion and reference treatments in supression of brown rot divelopment on apple fruits inoculated with *M. fructigena* 15 minutes or 24 hours after treatment

In the experiments *in vivo*, the initial thyme oil emulsion suppressed entirely the development of disease on inoculated apple fruits (Figure 2). However, the effect of the initial emulsion decreased from 100%, achieved when inoculation was done 15 minutes after treatment, to 32.7% when inoculation was delayed for 24 hours.

Formulation characteristics

Starting from thyme essential oil as an active substance, an emulsifiable concentrate (EC) was developed. Physical properties of the formulation are shown in Table 1.

Table 1. Physical properties of the developed formulation based on thyme essential oil

Density (g/cm^3)		0.900
pH value (1%)		3.24
Persistent foam (cm^3)		28
Stability of Emulsion and Reemulsification (cm^3)	0.5 h	0/0
	1 h	0/0
	2 h	0/0
	24 h	0/0
	REE	0/0

Formulation activity

In our *in vitro* experiment, the developed thyme oil formulation demonstrated strong mycelial growth inhibition (52.4%), compared to the control (Figure 3). However, there was a significant difference between the developed formulation and the fungicide iprodione ($p < 0.01$). The blank formulation did not cause any inhibitory effect on mycelial growth, and its effect was therefore excluded from further analysis.

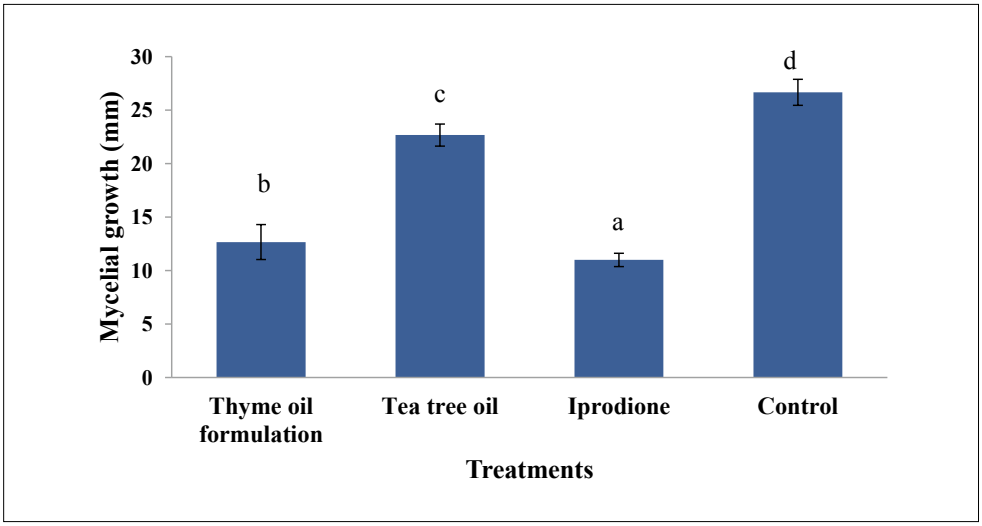

Figure 3. The effects of the thyme oil formulation and reference treatments on mycelial growth (Mean ± SD) of *M. fructigena in vitro*. Bars with the same letters are not significantly different (Duncan's test, $p > 0.01$).

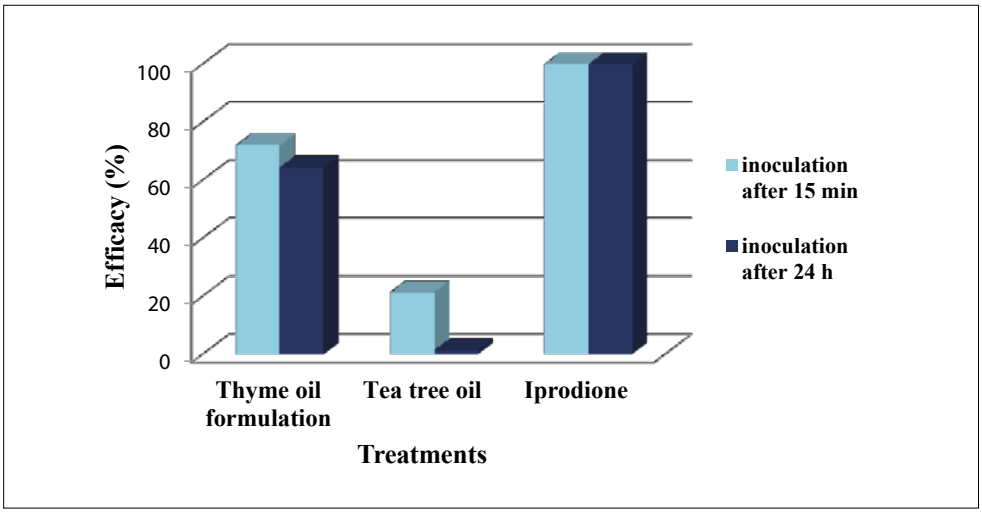

Figure 4. Efficacy of the developed thyme oil formulation and reference treatments in suppression of brown rot development on apple fruits inoculated with *M. fructigena* 15 minutes or 24 hours after treatment

In the experiment *in vivo*, the formulated product achieved 72.1% efficacy when inoculation was performed 15 minutes after treatment. When inoculation was delayed for 24 hours, the decrease in efficacy of 27.9% was not statistically significant (Figure 4). It indicates that evaporation of the essential oil from treated surface was successfully reduced by the developed formulation. It was also observed that components of the formulated product, as well as the product itself, did not cause any phytotoxic effects.

DISCUSSION

The results of this study confirmed a reported strong inhibitory potential of thyme essential oil against *M. fructigena* (Hrustić et al., 2011). A modified agar overlay technique was used to test its contact activity since such effect had been previously determined for the oil vapor phase. It is assumed that testing and evaluation of the antimicrobial activity of essential oils is often very difficult because of their volatility, water (in)solubility, and complexity (Janssen et al., 1987). A number of oils have been tested against fungi using different techniques (Daferera et al., 2003; Tanović et al., 2004; Tanović et al., 2009; Bakkali et al., 2008; Ćosić et al., 2010; Arslan and Dervis, 2010). Common assay techniques can be classified based on whether or not they require a homogeneous dispersion in water (Janssen et al., 1987). The agar overlay technique, which does not require homogeneous dispersion of oil in water, uses reservoirs (holes or cylinders) or discs as the source of vapor. Techniques that do require homogeneous dispersion in water, known as dilution techniques, are mostly used to determine the minimum inhibitory concentration (MIC) or minimum lethal concentration (MLC) of an investigated oil (Janssen et al., 1987). According to Morris et al. (1979), the results obtained using these two groups of techniques are not necessarily comparable. In addition, simultaneous use of different techniques can yield different results depending on the chemical properties of the oil tested (Pellecuer et al., 1980). Therefore, the results presented in different articles after testing essential oils for antimicrobial activity are very difficult to compare (Janssen et al., 1987). Differences in microbial growth, the method of exposure of microorganisms to a plant oil, the solubility of that oil or its components, the use of an emulsifier and its amount, and other elements may account for differences in the results obtained *in vitro*. In order to confirm the validity of *in vitro* results, *in vivo* studies may be required (Hammer et al., 1999).

To determine the full potential of thyme essential oil as a crop protectant, we created an initial oil emulsion allowing the diffusion of oil towards the tested isolate. Under such conditions, we observed a significant inhibition of mycelial growth of the *M. fructigena* isolate *in vitro*, confirming previous promising reports for this oil as an antifungal substance. The initial oil emulsion caused 100% disease prevention when inoculation of apple fruits was performed 15 minutes after treatment. However, inoculation that was delayed for 24 hours resulted in a significant efficacy decrease (32.7%), indicating possible oil evaporation from the treated surface. It is generally accepted that the most important limitation for application of essential oils as crop protectants is their relatively quick volatilization (Dayan et al., 2009). This problem can be resolved in different ways during the formulation process (Moretti et al., 2002). One of possible ways is mixing the essential oil with (a) carrier oil phase(s) to suppress essential oil volatilization. Vegetable oils are a good choice for carrier oil phase as they are fully biodegradable and therefore environmentally friendly. Even more appropriate are esterified derivates of vegetable oils that are less viscous and consequently more convenient for formulations. The addition of surfactants is necessary because of a poor water solubility of essential oils. Surfactants are also necessary for assisting in the spreading and penetration of the formulated product (Wang and Liu, 2007). Taking into account the physicochemical characteristics of thyme essential oil, a simple way of volatilization control by carrier oil phase was used in our study. The modified carrier oil phase in our thyme essential oil formulation is a renewable source and a value-adding quality to the product. Stable efficacy of the formulated product in case of delayed inoculation confirmed our assumption that the formulation would be able to decrease oil evaporation from treated area.

An additional important problem in practical application of essential oils in agriculture is a lack of persistent efficacy in the field (Chen at al., 2013). This problem could also be resolved by improved formulation. Newly-developed essential oil formulations should ensure stability during biopesticide production, processing and storage, improve convenience for users, protect the biopesticide from environmental conditions and increase biopesticide activity against target organisms. (Chen at al., 2013, Isman, 2000). In conclusion, further experiments are needed to evaluate the product's activity under field conditions and economic aspects of its use. To our knowledge, an EC formulation of thyme essential oil had never been developed before. Therefore, the results of our *in vitro* and *in vivo* experiments

are initial findings and product testing should be continued to determine its effect under field conditions as well as its spectrum of activity.

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REFERENCES

Arslan, M., & Dervis, S. (2010). Antifungal activity of essential oils against three vegetative-compatibility groups of Verticillium dahliae. *World Journal of Microbiology and Biotechnology,* 26(10), 1813-1821. doi:10.1007/s11274-010-0362-2

Bakkali, F., Averbeck, S., Averbeck, D., & Idaomar, M. (2008). Biological effects of essential oils: a review. *Food and Chemical Toxicology,* 46(2), 446-75. pmid:17996351. doi:10.1016/j.fct.2007.09.106

Chamberlain, M. (1887). Les essences au point de vue de leurs pro-89-96, prietes antiseptiques. *Anneles Institut Pasteur I,* 153-154.

Chen, K.N., Chen, C.Y., Lin, Y.C., & Chen, M.J. (2013). Formulation of a Novel Antagonistic Bacterium Based Biopesticide for Fungal Disease Control Using Microencapsulation Techniques. *Journal of Agricultural Science,* 5(3), 153.

CIPAC. (1995). CIPAC Handbook - Volume F. Physico-Chemical Methods for Technical and Formulated Pesticides. In W. Dobrat & A. Martin (Eds.), *Collaborative International Pesticides*. Harpenden Herts, England: Analytical Council Limited.

Cooper, K.E. (1963). The theory of antibiotic inhibition zones. In F. Kavanagh (Ed.), *Analytical microbiology*. (pp. 1-86). New York, USA: Academic Press.

Côté, M., Tardif, M., & Meldrum, A.J. (2004). Identification of Monilinia fructigena, M. fructicola, M. laxa, and Monilia polystroma on Inoculated and Naturally Infected Fruit Using Multiplex PCR. *Plant Disease,* 88(11), 1219-1225. doi:10.1094/PDIS.2004.88.11.1219

Ćosić, J., Vrandečić, K., Poštić, J., Jurković, D., & Ravlić, M. (2010). Antifugalno djelovanje eteričnih ulja na porast fitopatogenih gljiva in vitro. *Poljoprivreda,* 16(2), 25-28.

Daferera, D.J., Ziogas, B.N., & Polissiou, M.G. (2003). The effectiveness of plant essential oils on the growth of Botrytis einerea, Fusarium sp. and Clavibacter michiganensis subsp. michiganensis. *Crop Protection,* 22(1), 39-44. doi:10.1016/S0261-2194(02)00095-9

Dayan, F.E., Cantrell, C.L., & Duke, S.O. (2009). Natural products in crop protection. *Bioorganic & Medicinal Chemistry,* 17(12), 4022-34. pmid:19216080

Grahovac, M., Hrustić, J., Tanović, B., Inđić, D., Vuković, S., Mihajlović, M., & Gvozdenac, S. (2012). In vitro effects of essential oils on Colletotrichum spp. *Agriculture and Forestry (Podgorica),* 57(4), 7-15.

Gullino, M.L., & Kuijpers, L.A. (1994). Social and political implications of managing plant diseases with restricted fungicides in Europe. *Annual review of phytopathology ,* 32(2), 559-81. pmid:19877842

Hammer, K.A., Carson, C.F., & Riley, T.V. (1999). Antimicrobial activity of essential oils and other plant extracts. *Journal of Applied Microbiology,* 86(6), 985-990. pmid:10438227. doi:10.1046/j.1365-2672.1999.00780.x

Hammer, K.A., Carson, C.F., & Riley, T.V. (2003). In vitro activity of Melaleuca alternifolia (tea tree) oil against dermatophytes and other filamentous fungi. *Journal of Antimicrobial Chemotherapy,* 50(2), 195-9. pmid:12161399. doi:10.1093/jac/dkf112

Harrington, T.C., & Wingfield, B.D. (1995). A PCR-based identification method for species of Armillaria. *Mycologia,* 87, 280-288.

Hrustić, J., Tanović, B., Grahovac, M., Mihajlović, M., Delibašić, G., & Vukša, P. (2011). In vitro i in vivo efekat etarskog ulja timijana na Monilinia fructigena. In: Zbornik rezimea radova XI savetovanja o zaštiti bilja, Zlatibor. 36-37.

Hrustić, J., Tanović, B., Mihajlović, M., Grahovac, M., & Delibašić, G. (2012). Effects of essential oils on Monilinia spp. in vitro. In: Proceedings Annual Mediterranean Group of Pesticide Research (MGPR) Meeting and International Conference on Food and Health Safety: Moving Towards a Sustainable Agriculture, Belgrade, Serbia. 85.

Isman, B.M. (2000). Plant essential oils for pest and disease management. *Crop Protection,* 19(8-10), 603-608. doi:10.1016/S0261-2194(00)00079-X

Janisiewicz, W.J., & Korsten, L. (2002). Biological control of postharvest diseases of fruits. *Annual review of phytopathology,* 40, 411-441. pmid:12147766

Janssen, A.M., Scheffer, J.J., & Baerheim, S.A. (1987). Antimicrobial activity of essential oils: A 1976-1986 literature review. Aspects of the test methods. *Planta Med,* 53(5), 395-8. pmid:3324126. doi:10.1055/s-2006-962755

Lane, C.R. (2002). A synoptic key for differentiation of Monilinia fructicola, M. fructigena and M. laxa, based on examination of cultural characters. *EPPO Bulletin,* 32(3), 489-493. doi:10.1046/j.1365-2338.2002.00595.x

Moretti, M.D., Sanna-Passino, G., Demotis, S., & Bazzoni, E. (2002). Essential oil formulations Useful as a new tool for insect pest control. *AAPS PharmSciTech,* 3(2), 1-11.

Morris, J.A., Khettry, A., & Seitz, E.W. (1979). Antimicrobial activity of aroma chemicals and essential oils. *Journal of the American Oil Chemists' Society,* 56(5), 595-603. pmid:479491. doi:10.1007/BF02660245

Pellecuer, J., Jacob, M., Simeon de Buochberg, M. Dusart, G. Attisto, M. Barthez, M. Gourgas, L. Pascal, B. & Tomei, B (1980). Essais d'utilisation d'huiles essentielles de plantes aromatiques mediterraneennes en odontologie conservatrice [pouvoir antibacterien]. *Plantes Medicinales et Phytotherapie,* 14, 83-98.

Ragsdale, N.N., & Sisler, H.D. (1994). Social and political implications of managing plant diseases with decreased availability of fungicides in the United States. *Annual Review of Phytopathology,* 32(1), 545-57. pmid:19877846

Tanović, B., Hrustić, J., & Delibasić, G. (2010). Toxicity of volatile phase of essential oils from aromatic and medicinal plants to apple fruit pathogens in vitro. In: Book of abstracts of 28th International Horticultural Congress, Lisboa, Portugal. 83.

Tanovic, B., Hrustic, J., Grahovac, M., Mihajlovic, M., Delibasic, G., Kostic, M., & Indic, D. (2012). Effectiveness of fungicides and an essential-oil-based product in the control of grey mould disease in raspberry. *Bulgarian Journal of Agricultural Science,* 18, 689-695.

Tanović, B., Milijašević, S., Obradović, A., Todorović, B., Rekanović, E., & Milikić, S. (2004). In vitro efekti etarskih ulja iz začinskih i lekovitih biljaka na patogene koji se prenose zemljištem. *Pesticidi i fitomedicina,* 19(4), 233-240.

Tanović, B., Milijašević, S., Todorović, B., Potočnik, I., & Rekanović, E. (2005). Toksičnost etarskih ulja za Botrytis cinerea Pers. in vitro. *Pesticidi i fitomedicina,* 20(2), 109-114.

Tanović, B., Potočnik, I., Delibašić, G., Ristić, M., Kostić, M., & Marković, M. (2009). In vitro effect of essential oils from aromatic and medicinal plants on mushroom pathogens: Verticillium fungicola var. fungicola, Mycogone perniciosa, and Cladobotryum sp. *Archives of Biological Sciences,* 61(2), 231-237. doi:10.2298/ABS0902231T

Tasiwal, V., Benagi, V.I., Hegde, Y.R., Kamanna, B.C., & Naik, K.R. (2009). In vitro evaluation of botanicals, bioagents and fungicides against anthracnose of papaya caused by Colletotrichum gloeosporioides (Penz.) Penz. and Sacc. Karnataka. *Journal of Agricultural Sciences,* 22(4), 803-806.

Wang, C., & Liu, Z. (2007). Foliar uptake of pesticides: Present status and future challenge. *Pesticide Biochemistry and Physiology,* 87(1), 1-8. doi:10.1016/j.pestbp.2006.04.004

Zhang, H., Zheng, X., & Xi, Y. (2005). Biological control of postharvest blue mold of oranges by Cryptococcus laurentii (Kufferath) Skinner. *BioControl,* 50(2), 331-342. doi:10.1007/s10526-004-0452-x

Razvoj formulacije na bazi etarskog ulja timijana i njen uticaj na *Monilinia fructigena*

REZIME

U radu je proučavana antifungalna aktivnost etarskog ulja timijana na *Monilinia fructigena*, kao i mogućnost razvoja efikasne i stabilne formulacije ulja za upotrebu u poljoprivredi. Bioaktivnost etarskog ulja kao i razvijene formulacije – koncentrata za emulziju (EC) testirana je u ogledima *in vitro* i *in vivo*. Ogledi *in vitro*, izvedeni po delimično modifikovanoj metodi testiranja antimikrobne aktivnosti na površini agara, pokazali su da i inicijalno emulgovano ulje i razvijena formulacija značajno inhibiraju porast micelije izolata *M. fructigena*. U ogledima *in vivo* na inokulisanim plodovima jabuke pokazano je da je procesom formulisanja značajno smanjena isparljivost ulja sa tretirane površine i postignuta inhibicija razvoja mrke truleži ploda 64,7-72,1% u poređenju sa kontrolom. Koliko nam je poznato, u ovom radu je po prvi put razvijena EC formulacija etarskog ulja timijana za upotrebu u poljoprivredi. Dobijeni rezultati predstavljaju početak istraživanja koje treba dopuniti rezultatima ispitivanja efekata razvijene formulacije u uslovima praktične primene, proučavanja spektra njenog delovanja, kao i ekonomske isplativosti njene primene.

Ključne reči: Timijan; formulacije; etarska ulja