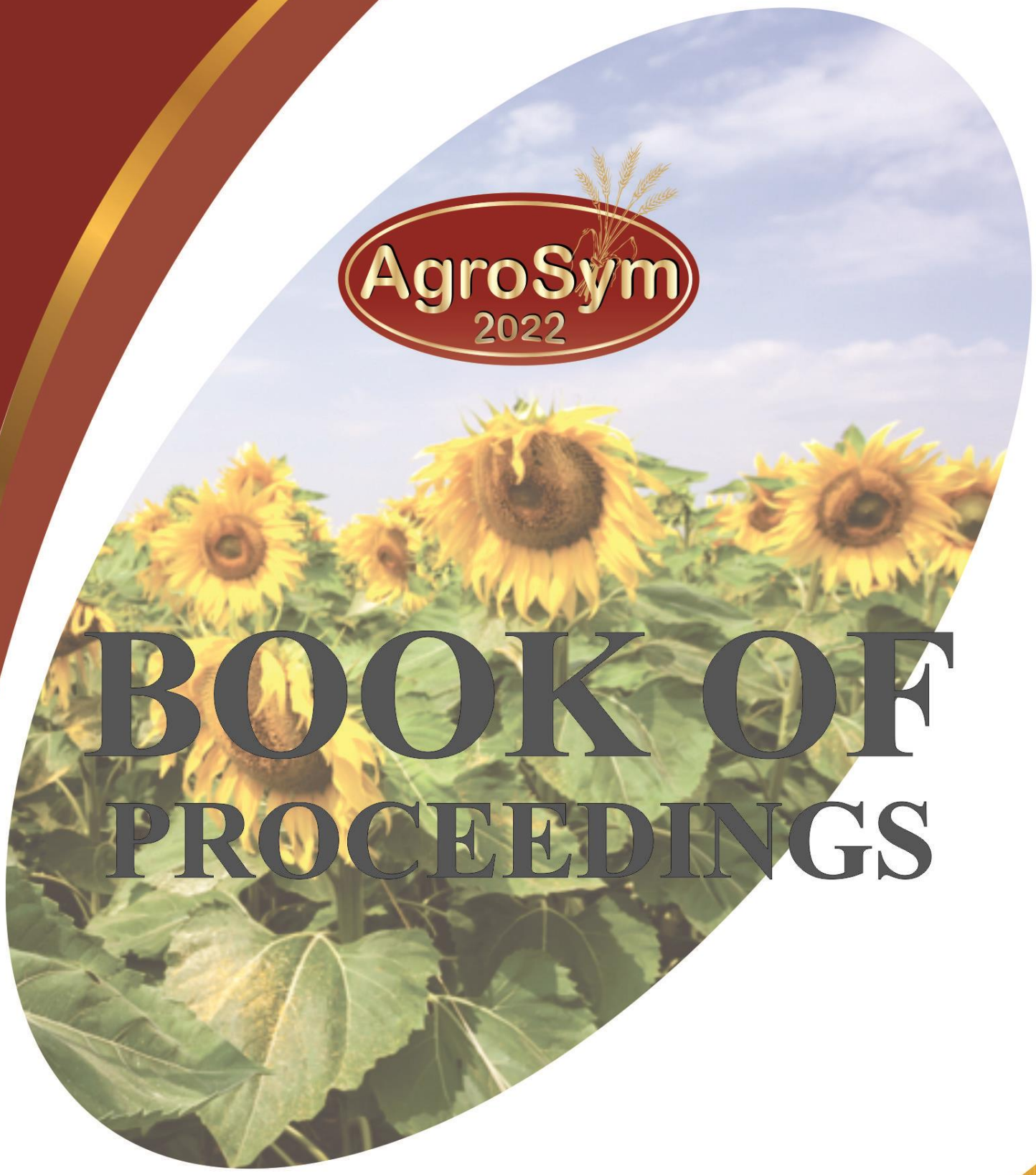

BOOK OF PROCEEDINGS

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ANTIFUNGAL ACTIVITY OF PLANT ESSENTIAL OILS TO THE FUSARIUM VERTICILLIOIDES ORIGINATED FROM GARLIC

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Abstract

Garlic (*Allium sativum* L.) is the second most cultivated *Allium* species worldwide. Many viral, fungal and bacterial diseases attack garlic plants and can be a limiting factor to garlic production. Fungal diseases control is mainly conducted by fungicides, which have detrimental impact on the environment. In this work, we explore the possibility of utilizing essential oils (EOs) as antifungal agents against a fungal isolate originated from symptomatic garlic cloves from Kraljevci locality, Srem District, Serbia, morphologically and molecularly identified as *Fusarium verticillioides.* EOs used in this study originated from several medicinal plants: Turkish pickling herb (*Echinophora tenuifolia*), oregano (*Origanum vulgare*), basil (*Ocimum basilicum*) and myrtle (*Myrtus communis*). Minimum inhibitory concentrations (MIC) were determined by microdilution method in 96 well microtiter plates. Microtiter plates were incubated for five days at 28°C. The experiment was repeated four times with trifloxystrobin as a positive control. The lowest concentrations without visible growth were defined as the minimal concentrations inhibiting fungal growth. Fungal spores were washed from the surface of potato dextrose agar (PDA) and spore suspension was adjusted to a concentration of approximately $5.0x10^4$ in a final volume of 100µl per well. The values of minimal inhibitory concentration (MIC) were carried out by Duncan's multiple range tests. An analysis of variance was performed on MIC data for four EOs applied on *F. verticillioides*. A significance was evaluated at $p<0.05$. STATISTICA v.7 (StatSoft, Inc.) was used for statistical analyses. The results of the antimicrobial activity of EOs using microdilution method showed a wide range of antifungal activity against *F. verticillioides*. The basil EO proved to be the most potent one (MIC-0,325±5,10µg/mL), followed by oregano (MIC-0,775±0,05µg/mL), myrtle (MIC-5,5±0,05µg/mL) and Turkish pickling herb (MIC-55±5,10µg/mL). The data obtained here suggest that the selected EOs can be applied as inhibitors to prevent growth of the phytopathogenic fungus *F. verticillioides*.

Keywords: *Garlic, Essential oil, Antagonistic, Minimum inhibitory concentration.*

Introduction

Garlic (*Allium sativum* L.) has been cultivated for more than five thousand years as people recognized garlic's physiological, nutritional and health benefits. Nowadays, garlic is the second most cultivated *Allium* species worldwide, as is in Serbia. Many phytopathogenic viruses, fungi and bacteria pose a threat to garlic cultivation, and can be a limiting factor to garlic production. Management of fungal diseases is mainly conducted by fungicides, which have detrimental impact on the environment.

Dry rot disease of garlic is a postharvest problem that can result in a severe attack of up to 30% of the garlic bulbs (Mondani et al., 2021). The disease symptoms are visible during the storage as necrotic spots on garlic bulbs, often with a developed mycelium.

Garlic protection from phytopathogenic fungi is difficult because there is a small selection of pesticides that are safe for human health. Garlic dry rot control is not fully established and only a few studies on the effectiveness of chemical treatments against *Fusarium* spp. in garlic are available (Mondani et al., 2021). Elshahawy et al. (2017) demonstrated *in vitro* assays, that Carbendazim had an inhibitory effect on fungal growth when followed by Metalaxyl + Mancozeb (8% + 64%) and thiophanate-methyl. Gálvez et al. (2017) investigated the ability of three commercial fungicides to control *F. proliferatum* in vitro with promising results, but field treatments failed to control garlic rot during the commercial stage. In our country, there are no registered chemical preparations for application in garlic against Fusarium Basal Rot (Collective of authors, 2020). Furthermore, a frequent use of pesticides have resulted in a development of fungal resistance and destruction of the environment. That is why an eco-friendly pesticide alternative is desirable to protect the quality and the yield of garlic crop.

Bacillus isolates and *Trichoderma* spp. are reported in literature as potential biocontrol agents against garlic dry rot. Over the years, essential oils have received renewed attention due to a wide spectrum of biological activities against several pests, especially microorganisms (Gayoso et al. 2005; Vitoratos et al., 2013; Mahilrajan et al., 2014). The antimicrobial activity of some selected species has already been demonstrated (Maksimović et al., 2005; Stević et al., 2014; Starović et al., 2016; 2017).

In this work we explore the possibility of utilizing plant essential oils as antifungal agents against *Fusarium verticillioides*.

Materials and methods

Isolation and identification of fungal species

During 2021, garlic plants showing symptoms of yellowing and rotting at early and intermediate stages of crop development, were observed. Rotted areas of the bulb progressed from the basal plate towards the neck of the bulb (Figure 1). Affected roots became dark brown to dark pink, while fungal growth was visible at the base of infected bulbs. The isolate used in this study originated from symptomatic garlic cloves from Kraljevci locality, Srem District, Serbia, and was morphologically and molecularly identified. Morphological identification of the isolate was made using the criteria of Gerlach and Nirenberg (1982) and Leslie and Summerell (2006). PCR was carried out for the purpose of identification of *Fusarium* species by amplifying the translation elongation factor-1a gene (tef-la) using the primer pairs EF1 and EF2 as described in O'Donnell et al. (1998).

Antifungal activity of EOs

Essential oils (EOs) extracted by hydro-distillation from several medicinal plants: Turkish pickling herb (*Echinopora tenuifolia*), oregano (*Origanum vulgare*), basil (*Ocimum basilicum*) and myrtle (*Myrtus communis)* - obtained by the kindness of the Professor M.M. Özcan from the Department of Food Engineering, Faculty of Agriculture, Selçuk University, 42079 Konya, Turkey, were used in antagonistic assays with a *Fusarium verticillioides* isolate originated from symptomatic garlic cloves from the locality Kraljevci (Serbia). A minimum inhibitory concentration (MIC) was determined by modified micro-dilution method in 96 well micro-sterile

0.75% saline containing 0.1% Tween 80 (vol/vol) (Balouiri et al., 2016). The fungal spore suspension was filtered and adjusted with sterile saline to a concentration of approximately $4{,}0x10^5$ spores per ml using a hemocytometer. In each well with 90 μL potato dextrose medium with appropriate dilutions of the EO, 10 μL of fungal inoculum was added. The experiment was repeated four times. Trifloxystrobin was used as a positive control. The MIC was defined as the lowest concentration of essential oils (EO) which completely inhibited the visible fungal growth.

Results and discussion

The morphological characteristics (Figure 1) of the pathogen correspond to the records for *F. verticillioides* described by Leslie and Summerell (2006). The amplification and sequencing of tef-la gene produced a sequence identifying garlic isolate used in this study as *F. verticillioides*.

FIGURE 4. Symptoms of *F. verticillioides* on garlic cloves (a, b); Colony morphology of *F. verticillioides* (c)

Garlic dry rot is a postharvest disease, but garlic infection starts in the field (Mondani et al., 2021, Stanković et al., 2007).

All tested oils showed some antifungal activity against the tested fungal pathogen. The results of the antimicrobial activity tests using microdilution method are summarized in Fig.2 and 3. The EOs showed a wide range of antifungal activity against *F. verticillioides*. The antifungal potential of oil tested can be presented as: *Ocimum basilicum* > *Origanum vulgare*> *Myrtus communis*> *Echinopora tenuifolia*. The oregano EOs proved to be the best inhibitor of the tested fungal isolate (MIC 0.033±51.96 μg/mL), followed by basil (MIC 0.075 ±0.05 μg/mL), then myrtle (MIC 5.500±0.50 μg/mL) and Turkish pickling herb MIC 55.000±5.19 μg/mL).

Echinophora tenuifolia		
Origanum vulgare		
Ocimum basilicum		
Myrtus communis		

Essential oils MIC

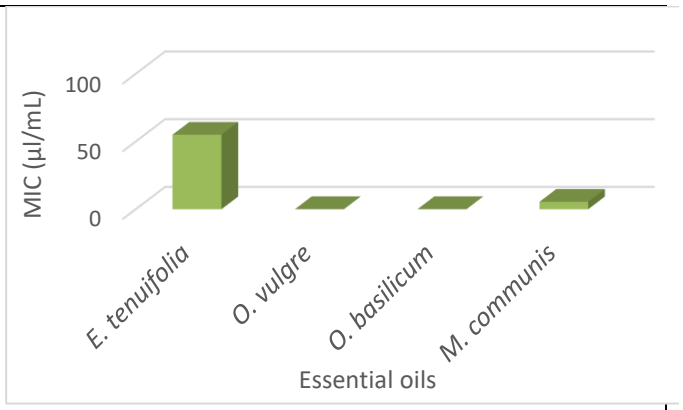

FIGURE. 2. Antifungal activity of essential oils expressed as minimal inhibitory concentrations (µg/mL)

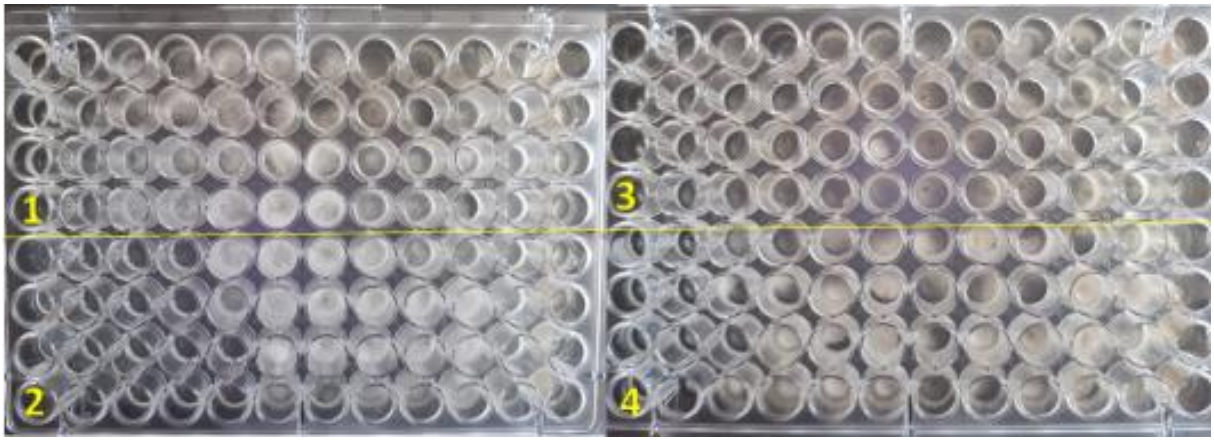

FIGURE 3. Micro-dilution method: antifungal effect of investigated essential oils (1) *Echinopora tenuifolia*; (2) *Origanum vulgare*; (3) *Ocimum basilicum*; (4) *Myrtus communis*

According to Stanković et al. (2007), garlic dry root causal agent in Serbia has been identified as *Fusarium proliferatum*. Here we provided the first report of the presence *F. verticillioides* on diseased bulbs with symptoms described as garlic dry root.

The resulted from our previous study (Starović et al., 2016; 2017) showed that that the oregano EO and basil from Turkey, manifest the high inhibitory effect against *Phomopsis theicola* (MIC5.5 µg/mL and 75.0 µg/mL respectively), while myrtle EOs showed very strong effect against *Fusarium* sp. especially *F. verticillioides* (MIC 3.25 µg/mL). Stević et al. (2014) demonstrated that oregano oil originating from Serbia could inhibit the growth of *Phomopsis* species at concentration of 70 µg/mL, while basil oil demonstrated a smaller effect (MIC 5950 µg/mL). Kocić-Tanackov et al. (2012) showed that the concentration of 25 µg/mL of oregano EOs inhibited the growth of *F. verticillioides* by 88%. The obtained results from this study indicate a very similar effect of selected EOs originating from Turkey and Serbia.

Conclusion

Based on our study, oregano and basil showed promising results in *F. verticilioides* control. Further studies could focus on the selection of essential oils and their application *in vitro*. These data can be a starting point for further experiments and development of biofungicides.

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