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Blažo LALEVIĆ[1*], Teofil GAVRIĆ[2], Amina STAMBOLIĆ[2], Amer SUNULAHPAŠIĆ[3], Vedrana KOMLEN[4], Lejla ČENGIĆ[2], Saud HAMIDOVIĆ[2]

ANTIFUNGAL POTENTIAL OF PEPPERMINT, BASIL AND SAGE ESSENTIAL OILS

SUMMARY

Synthetic fungicides are the most effective protection against plant pathogens, but, their uncontrolled and long-term use can lead to many harmful effects: environmental degradation, human health problems and pathogen resistance. The biological compounds contained in essential oils have no harmful effects on humans or the environment and can therefore be an alternative to synthetic fungicides. Essential oils are products of plant metabolism and often show antifungal, antiviral, antibacterial and insecticidal effects. The aim of this study was to investigate the antimicrobial potential of essential oils from peppermint, basil and sage essential oils obtained by hydrodistillation, on *Fusarium* sp. and *Aspergillus* sp. The experiment was carried out on potato dextrose agar. After inoculation of the agar with fungal mycelia, paper discs impregnated with 10 µl of oils were placed on the agar surface. In the control, the impregnation was carried out with distilled water. The inhibition zones were measured after 3, 6 and 9 days. The results showed that peppermint oil had the highest antimicrobial potential compared with other oils. Sage essential oil showed the lowest antifungal suppression. A negligible zone of inhibition was observed in the control. A statistically significant influence betwwen the oils and the incubation period was found in this study. Our results confirm the potential use of peppermint essential oil for the suppression of *Fusarium* sp. and *Aspergillus* sp. growth.

Keywords: antifungal potential, *Aspergillus*, *Fusarium*, inhibition zone, plant essential oils.

[1] Blažo Lalević[*] (corresponding author email: blazol@agrif.bg.ac.rs), University of Belgrade, Faculty of Agriculture, Nemanjina 6, Belgrade-Zemun, SERBIA
[2] Teofil Gavrić, Amina Stambolić, Lejla Čengić, Saud Hamidović, University of Sarajevo, Faculty of agriculture and food science, Zmaja od Bosne 8, Sarajevo, BOSNIA AND HERZEGOVINA;
[3] Amer Sunulahpašić, Ministry of agriculture, water management and forestry, Travnik, BOSNIA AND HERZEGOVINA
[4] Vedrana Komlen, The University "Džemal Bijedić", Agromediterranean Faculty, University Campus, Mostar, BOSNIA AND HERZEGOVINA
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INTRODUCTION

Agricultural plant production is often associated with phytopathogen infestation (Köhl *et al*., 2019) and yield losses (Ampt *et al*., 2019). Chatterjee *et al*. (2016) found that the occurrence of plant disease leads to an annual loss of 10-15 percent of major crops worldwide. Peng *et al*. (2021) point out that more than two-thirds of plant diseases are associated with the occurrence of pathogenic fungi. Therefore, plant diseases need to be controlled using different techniques (Tsegaye *et al*., 2018), which requires redesigning agroecosystems to improve plant resistance to pathogens (McDonald and Stukenbrock, 2016). Pesticides show high efficiency in suppressing the occurence of *Fusarium* (Arie, 2019) and *Aspergillus* (Geetha *et al*., 2016); species of these fungal genera are frequently detected on various plant materials (Kalman *et al*., 2020; Perrone *et al*., 2007). However, Aktar *et al*. (2009) demonstrated that the use of pesticides is associated with higher plant productivity, avoidance of yield losses and control of disease vectors. On the other hand, the extensive use of pesticides led to significant degradation of the agroecosystem and human health (Salameh *et al*., 2006; Zheng *et al*., 2016). Therefore, several alternative methods to replace pesticides have been proposed (Nile *et al*., 2019).

The pesticidal properties of many plant species and their products are well-known and may represent formulations with potential applications against pathogens (Stevenson *et al*., 2017). Nxumalo *et al*. (2021) described the possibility of medicinal plant extracts as an alternative technology for synthetic chemicals. Several *in vitro* studies have been conducted to determine the potential of various plant extracts in suppressing growth of fungal genera such as *Candida*, *Aspergillus*, *Penicillium*, etc. (Azaz *et al*., 2004; Chuang *et al*., 2007; Hammer *et al*., 1999; Omran and Esmailzadeh, 2009).

The objective of this paper is to evaluate the antifungal potential of the essential oils of peppermint, basil and sage against *Fusarium* sp. and *Aspergillus* sp. in *in vitro* experiment.

MATERIAL AND METHODS

Fusarium sp. and *Aspergillus* sp. used in this research belong to the collection of microorganisms Department of Microbiology at the Faculty of agriculture and food science in Sarajevo (Bosnia and Herzegovina). These fungal isolates were previously cultivated and stored at 4 °C on the nutrient medium potato dextrose agar (PDA).

The shoots of peppermint, basil and sage were used for the extraction of plant material. Extracts were obtained through hydrodistillation (30 g of plant material and 400 ml of distilled water) using Neo Clevenger-type apparatus. The antifungal properties of plant extracts were determined using a disc diffusion assay. Potato dextrose agar (Himedia, India), previously sterilized at 120 °C for 20 min, was inoculated with pure cultures of *Fusarium* sp. and *Aspergillus* sp. Fungal mycelia were placed at four places on the agar surface, about 5 mm from the edge of the Petri dishes. In the center of the agar plate, one sterile filter paper disc (about

5 mm in diameter) containing 10 μl of the plant extract was placed. In control, distilled water was used for the impregnation of the disc. All experiments were performed in triplicate. Incubation of Petri dishes was performed at 22 °C for 9 days. All experiments were performed in 4 replications. The zone of inhibition (expressed in centimeters) was measured on the 3, 6 and 9 day of incubation.

For the determination of the statistical significance of obtained results, SPSS software (version 22, SPSS Inc., Chicago, IL) was used. $P<0.05$ was chosen as a parameter of statistical significance between the treatments.

RESULTS AND DISCUSSION

Our results showed that all tested essential oils had an inhibitory effect on the growth of *Fusarium* sp. (Table 1). The degree of inhibition depended on the essential oil and the incubation period.

All tested oils showed significantly higher values of the inhibition zone compared to the control (Table 1). After three, six and nine days of incubation, peppermint essential oil showed the statistically highest effect. The diameter of the inhibition zone with peppermint essential oil was 1.58, 0.68 and 0.23 cm, respectively. Sage essential oil showed a larger diameter of the inhibition zone after three days, while after six and nine days, the effect of basil essential oil was more pronounced compared with sage. In all treatments, the diameter of the inhibition zone statistic decreased significantly with increasing of incubation time for peppermint and sage essential oils. In the control, no zone of inhibition was observed after 6 and 9 days.

Table 1. Diameter of inhibition zone (cm) of *Fusarium* sp.

D	Essential oils											
	peppermint			basil			sage			control		
	Time of incubation (days)											
	3	6	9	3	6	9	3	6	9	3	6	9
1	1.6	0.7	0.2	0.8	0.4	0.2	0.8	0.3	0.0	0.3	0.0	0.0
2	1.5	0.6	0.2	0.6	0.2	0.0	0.8	0.3	0.0	0.3	0.0	0.0
3	1.3	0.5	0.2	0.5	0.2	0.0	0.8	0.3	0.1	0.2	0.0	0.0
4	1.9	0.9	0.3	0.8	0.3	0.1	0.7	0.2	0.0	0.2	0.0	0.0
A	1.58[a]	0.68[a]	0.23[c]	0.68[a]	0.28[c]	0.75[c]	0.78[a]	0.28[b]	0.03[c]	0.25[a]	0.0[b]	0.0[b]
T	0.83[a]			0.41[b]			0.36[c]			0.08[d]		

Legend: D – discs; A – average; T – total average

Legend: D – discs; A – average; T – total average

The essential oils of peppermint, basil and sage had an inhibitory effect on the growth of *Aspergillus* sp. (Table 2). Significantly lower values for the diameter of the inhibition zone diameter were observed for all essential oils during incubation. As in the experiment with *Fusarium* mycelia, peppermint essential oil showed the highest zone of inhibition compared with other treatments (0.63, 0.35 and 0.15 cm after 3, 6, and 9 days of incubation, respectively). Basil essential oil showed a stronger effect than sage essential oil, especially after three and six days. At the end of the incubation period, the same values were found for the diameter of inhibition zone. In the control, a negligible zone of inhibition was observed after three days; further incubation revealed no zone of inhibition.

Table 2. Diameter of inhibition zone (cm) of *Aspergillus* sp.

D	Essential oils									Control		
	peppermint			basil			sage					
	Time of incubation (days)											
	3	6	9	3	6	9	3	6	9	3	6	9
1	0.7	0.4	0.2	0.6	0.3	0.1	0.4	0.2	0.1	0.1	0.0	0.0
2	0.7	0.4	0.2	0.5	0.2	0.0	0.3	0.1	0.0	0.0	0.0	0.0
3	0.5	0.3	0.1	0.4	0.2	0.0	0.4	0.2	0.0	0.0	0.0	0.0
4	0.6	0.3	0.1	0.5	0.2	0.0	0.2	0.1	0.0	0.0	0.0	0.0
A	0.63[a]	0.35[b]	0.15[c]	0.5[a]	0.23[b]	0.03[c]	0.33[a]	0.15[b]	0.03[c]	0.03[a]	0.0[b]	0.0[b]
T	0.38[a]			0.25[b]			0.17[c]			0.01[d]		

Legend: D – discs; A – average; T – total average

Essential oils from medicinal and aromatic plants have antimicrobial properties and can be used to combat plant pathogens. Essential oils from the *Lamiaceae* family are known as antifungal agents (Santra and Banarjee, 2020). Plants from the *Lamiaceae* family are distributed worldwide and represent a cost-effective source for the extraction of essential oils that can be used in agriculture (Feng *et al*., 2011; Mamgain *et al*., 2013). Essential oils from *Lamiaceae* are often used to control fungal pathogens in crop production, such as *Fusarium*, or fungi responsible for food spoilage, such as *Aspergillus* (Couladis *et al*., 2004; Soliman and Badeaa, 2002).

Peppermint essential oil was recommended as the best inhibitor of *Fusarium* growth compared with other natural products (Kumar *et al*., 2016). Helal *et al*. (2006) found that the application of peppermint essential oil was effective against 11 fungi, including *Aspergillus* species. Our results are consistent with the report of Guynot *et al*. (2003) who found that peppermint essential oil inhibited the growth of *Aspergillus flavus* and *A. niger*.

Kocić-Tanaskov *et al*. (2011) reported the inhibition of *Fusarium* species growth using different concentrations of basil essential oil. The highest inhibitory effect of basil oil against *Fusarium* growth was described by Hashem *et al*. (2010). Our results are in agreement with those of Pandey and Dubey (1994), who described moderate to poor antifungal activity of basil essential oil.

Our results showed that the lowest inhibition zone of fungal growth was achieved with sage essential oil. Ferdes *et al*. (2017) described the low effect of sage essential oil on the growth of *A. niger* and *Fusarium oxysporum*. Sage essential oil showed no significant suppression of the growth of *Aspergillus flavus* (Foltinova *et al*., 2017). However, the fungotoxic effect of sage essential oil was observed at higher concentrations of this product (Daferera *et al*., 2003).

CONCLUSION

This study confirms that the presented essential oils can be used to control the growth of *Fusarium* and *Aspergillus* mycelia. The highest inhibition zone was observed with the use of peppermint and the lowest with the use of sage essential oil. Further studies will focus on the inhibition of other fungal species and a detailed content analysis of these natural plant products.

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