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Antifungal activity of *Salvia officinalis* subsp. *lavandulifolia* and *Salvia officinalis* subsp. *major* aqueous extracts against *Botrytis cinerea*

A *Salvia officinalis* subsp. *lavandulifolia* és a *Salvia officinalis* subsp. *major* vizes kivonatainak gombaellenes aktivitása a *Botrytis cinerea* fertőzés ellen

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ABSTRACT

The use of pesticides dominates agriculture today, but unfortunately poses several threats to the environment and humans, through bioaccumulation and biomagnification. This research tested the efficacies of *Salvia officinalis* subsp. *lavandulifolia* and *Salvia officinalis* subsp. *major* aqueous extracts against *Botrytis cinerea* in order to curb chemical control and subsequent reduction in their threats. The leaves of *Salvia officinalis* subsp. *lavandulifolia* and *Salvia officinalis* subsp. *major* were collected in July and September 2019 and shed-dried. Then 90 ml hot distilled water was added to10 g of the crumbled leaves from each sample, left for 24 hours and the filtrate was used to prepare two different media concentrations: CC1 containing 99 ml PDA (i.e. Potato Dextrose Agar) and 11 ml extract (or ion-exchange water as control 1); and CC2 containing 88 ml PDA and 22 ml extract (or ion-exchange water as control 2). The 10 ml from each mixture (CC1, CC2 and the controls) was used as the growth media in 5 replications. Each Petri dish was centrally inoculated with 9 mm^2 *B. cinerea* mycelia, and the area of colony was taken. All the extracts effectively inhibited the growth of *Botrytis cinerea*, with Som2 showing the highest inhibition. In *Salvia officinalis* subsp. *lavandulifolia*, Sol1 (July harvested) showed higher inhibition (53.90%) compared to September harvested Sol2 (40.78%) in CC2 at 3 days post inoculation (PI), while in *Salvia officinalis* subsp. *major*, Som2 (September harvested) showed higher inhibition (15.01%) compared to July harvested Som1 (0.04%) in CC1 at 7 days PI. Generally, CC2 showed higher inhibition compared to CC1.

Keywords: pesticide, mycelia, harvest, poisoned food technique, inoculation, inhibition

ÖSSZEFOGLALÁS

A mai mezőgazdaságra jellemző a növényvédő szerek használata, de ez a bioakkumuláció és a biomagnifikáció révén sajnos számos veszélyt jelent a környezetre és az emberekre. Kutatásunkban a *Salvia officinalis* subsp. *lavandulifolia* és a *Salvia officinalis* subsp. *major* genotípusok vizes kivonatainak a hatását vizsgáltuk a *Botrytis cinerea* kórokozó ellen a kémiai védekezés és az esetleges kockázatok csökkentése érdekében. A *Salvia officinalis* subsp. *lavandulifolia* és a *Salvia officinalis* subsp. *major* leveleit 2019 júliusában és szeptemberében gyűjtöttük össze és szárítottuk meg. Az egyes minták 10 g morzsolt leveléhez 90 ml forró desztillált vizet adunk, majd 24 órán át hagyjuk 90 ml forró desztillált vízben, és a szűrletből két különböző fertőzött táptalaj-koncentrációt készítettünk: a CC1 jelű 99 ml PDA-t (burgonya-dextróz-agar) és 11 ml kivonatot tartalmaz (vagy ioncserélt vizet kontrollként); míg a CC2 88 ml PDA-t és 22 ml kivonatot tartalmaz (vagy ioncserélő vizet, mint 2. kontroll). Mindegyik keverékből (CC1, CC2 és a kontrollok) 10-10 ml-t használtunk tenyésztő tápközegként 5 ismétlésben. Minden Petri-csésze közepébe beoltottunk 9 mm^2 területű *Botrytis cinerea* micéliumot és a telep területét vizsgáltuk. Valamennyi kivonat hatásos volt a *Botrytis cinerea* szaporodása ellen, közülük a Som2 mutatta a legnagyobb gátló hatást. A *Salvia officinalis* subsp. *lavandulifolia* Sol1 (júliusban betakarítva) magasabb gátlást mutatott

(53,90%), mint a szeptemberi betakarított Sol2 (40,78%) CC2-ben az oltás utáni 3. napon (PI), míg a *Salvia officinalis* subsp. *major*, Som2 (szeptemberi betakarítással) nagyobb gátlású hatású volt (15,01%), mint a júliusban betakarított Som1 (0,04%) CC1-ben a 7. napon a PI-ben. Általában a CC2 esetében nagyobb gátló hatást figyeltünk meg a CC1-hez képest.

Kulcsszavak: peszticid, micélium, betakarít, fertőzött táptalaj, oltás, gátlás

INTRODUCTION

The use of pesticides and other agrochemicals became an integral part of the agricultural system in the last century, leading to a substantial increase in crop yields and food production (Alexandratos and Bruinsma, 2012). This use of chemicals gains much importance as it is still being in practice today, but unfortunately poses several threats to the environment and humans as well through biomagnification and bioaccumulation (Stephen et al., 2016). Pesticides are one of the few toxic substances released deliberately into the environment to kill living organisms such as weeds (herbicides), insects (insecticides), fungi (fungicides), and rodents (rodenticides) (Matthews, 2006).

The grey mould pathogen *Botrytis cinerea* is a major threat to crop productions in a large variety of field and greenhouse cultures (Matthias et al., 2007) and in several economically important horticultural and floral crops (González et al., 2016). The phytopathogenic fungus causes grey mould on over 230 hosts and the common damages include onion rot neck rot, grey mould of seedlings and strawberries, noble rot and grey mould in grapes, calyx end rot in apples and blight mould, soft rot in fruits among others (Vallejo et al., 2002). Considering the importance of this pathogen and its significant damage to agricultural products, its control management is therefore necessary in order to contain their destructive effect.

Research now focuses on large number of diverse bioactive molecules produced by plants (Suffredini, 2004). These are the so-called secondary metabolites that can protect the plants against many complications by acting as antifungal, antibacterial as well as antioxidants (Evans et al., 1986). Several studies have demonstrated the different biological activities of aromatic and medicinal plants, focusing mainly on antifungal, antibacterial, antioxidant and insecticidal activities (El-Ouadi, 2015), and proven to have inhibitory action on the growth and toxinogenesis of several bacteria and fungi responsible for food infections (Tzortzakis 2006; Faostat 2004). With this important breakthrough, the use of these harmful chemicals used in plant protection can be substituted with ecologically friendly ones.

Salvia officinalis is commonly known for its tremendous activities, ranging from medicinal to antimicrobial. It is used for the treatment of varied disorders including seizure, rheumatism, inflammation, dizziness, ulcers, gout, tremor, paralysis, hyperglycaemia, and diarrhoea (Zargari, 1990; Garcia et al., 2016). *Salvia officinalis* extract was reported to be highly protective against *Plasmopara viticola* in grape vines (Dagostin et al., 2010), the essential oil having strong bactericidal and fungicidal activity (Bouaziz et al., 2009) such as against *Botrytis cinerea* (Carmela et al., 1996), as well as strong antioxidant activity (Mekhaldi et al., 2014). This research is aimed at testing the efficacies of *Salvia officinalis* subsp. *lavandulifolia* and *Salvia officinalis* subsp. *major* aqueous extracts against *Botrytis cinerea*.

MATERIALS AND METHODS

Preparation of herbal extracts

The leaves of *Salvia officinalis* subsp. *lavandulifolia* and *Salvia officinalis* subsp. *major* were collected from the Department of Horticulture, University of Pannonia, Georgikon Faculty, at two different harvesting seasons (July and September 2019), shed-dried and crumbled. The 10 g of the crumbled leaves were put in a glass cylinder and 90 ml boiling distilled water was added on to the leaves and stirred using a sterile loop. The mouth of the cylinder was then covered with aluminium foil for 24 hours to prevent volatile constituents from escaping.

The extracts were filtered, and the filtrates kept in glass cylinders for future use.

Media preparation

Potato Dextrose Agar (PDA) was prepared according to the manufacturer with little modification. The 47 g PDA (39 g + 20%) was dissolved in 1000 ml distilled water (in a universal bottle), autoclaved at 121 °C for 15 mins, cooled to 50 oC, and finally mixed with the herb extracts. The commercially prepared PDA contains 20 g dextrose, 15 g agar and 4 g potatoes starch.

Preparation of B. cinerea cultures

Grapevine berries infected with *B. cinerea* were collected and kept in a wet chamber. Suspension was prepared from separated conidia and pipetted on to a Petri dish with PDA medium. The sample was spread over the surface using a sterile plastic spreader. After about 30 hours the germinating conidia were transferred on to a clear PDA plate. After a 3-day incubation period, the *B. cinerea* cultures were ready to use.

Poisoned food technique and controls preparation

The poisoned media were prepared from homogenous mixture of the herbal extracts (or ion-exchange water) and the PDA in two different combinations as outlined below:

- Concentration 1: 99 ml PDA and 11 ml herbal extract
- Concentration 2: 88 ml PDA and 22 ml herbal extract
- Control 1: 99 ml PDA and 11 ml ion-exchange water
- Control 2: 88 ml PDA and 22 ml ion-exchange water

The final mixtures of the above combinations from each harvesting time as well as the controls were used to prepare solid growth media in Petri dishes, each containing 20 ml of each separate concentration mixture. Each treatment mixture was replicated five times.

Inoculation with B. cinerea

The three-day old cultures of *B. cinerea* (containing only the hyaline mycelia) were used to inoculate the media already supplemented with herbal extracts. The 9 mm^2 portions were cut from the *B. cinerea* pure cultures using sterile needle and placed at the centre of each Petri dish containing certain experimental treatment. The Petri dishes were sealed using parafilm and incubated for 7 days at room temperature.

Measurement of B. cinerea growth

The diameters of the fungal colonies in the culture media were measured in two perpendicular directions and the average taken and recorded. The mean diameters were taken from each culture at three and seven days after inoculation and used to compute the area of the fungal growth in each case.

Statistical analysis

Two-Way ANOVA was used to compare the means of the area of the fungal colonies between the two plant extracts (*Salvia officinalis* subsp. *lavandulifolia* and *Salvia officinalis* subsp. *major*) and the controls in the two harvesting times (July and September), between the two concentrations and between harvesting times within each plant extract.Duncan Multiple range was used to separate the means where significant.

RESULTS AND DISCUSSION

The results of the experiment showed significant difference ($P<0.01$) in the mean area of *B. cinerea* colonies between the test extracts and the control, as well as significant difference ($P<0.01$) between concentration 1 (2754.58 cm^2) and concentration 2 (1945.41 cm^2) at 3 days Post Incubation (PI). The mean area of fungal colonies was significantly different ($P<0.05$) between the test extracts including the control, and also between CC1 (5465.60 cm^2) and CC2 (5154.34 cm^2) at 7 days PI ($P<0.05$). The abbreviations used in the text are clarified in Table 1.

The comparison of mean area of colonies and % inhibition at 3 days Post inoculation (PI) for the extracts and the control is presented in Table 2. The result for CC1 shows that all the extracts tested (Sol1, Sol2, Som1 and Som2) significantly inhibited the growth of the pathogen

(*B. cinerea*) compared to the control group, stressing their fungistatic potential. Among the four extracts, Som2 (2412.14 cm^2; 33.23% inhibition) had significantly lower colony growth compared to Sol2 (2682.04 cm^2; 25.76% inhibition) but was not significantly different to Sol1 (2475.12 cm^2; 31.49% inhibition), and Som1 (2590.89 cm^2; 28.28% inhibition).

Table 1. Abbreviations used in the text

Acronym	Full meaning
Sol1	*Salvia officinalis* subsp. *lavandulifolia* at first harvesting time (July)
Sol2	*Salvia officinalis* subsp. *lavandulifolia* at second harvesting time (September)
Som1	*Salvia officinalis* subsp. *major* at first harvesting time (July)
Som2	*Salvia officinalis* subsp. *major* at second harvesting time (September)
CC1	Concentration 1 (99 ml PDA and 11 ml extract or water)
CC2	Concentration 2 (88 ml PDA and 22 ml extract or water)
PI	Post Inoculation

The result for CC2 shows that the control (3281.85 cm^2) has a significantly higher area of colony compared to all the tested extracts. Among the extracts however, the area of the colony in Som1 (1655.58 cm^2; 49.55% inhibition) was not significantly different from that of all the other extracts (Sol1, Sol2 and Som2). Sol1 (1513.05 cm^2; 53.90% inhibition) and Som2 (1333.16 cm^2; 59.34% inhibition) had the lowest area of colonies with no significantly different inhibitory effect between them, but with significantly lower colony area compared to Sol2 (1943.39 cm^2; 40.78% inhibition).

The antifungal activity of these extracts to the growth of the *B. cinerea* is not surprising as several other plant extracts such as garlic extracts in apple (both curative and protective), *Allium hirtifolium* (leaves and inflorescence), *Hypericum perporatum* and *Gossypium hirsutum* (leaves), *Dodonaea vivosa* (inflorescence), essential oils of *Solidago canadensis* and tea tree among others, were found to be inhibitory to the pathogen as well (Bahraminejad et al., 2015; Daniel et al., 2014; Li et al., 2017; Liu et al., 2012; Pedro et al., 2016). A 10% dilution of crude extracts of *Adenocalyma alleaceum*, *Tulbaghia violacea*, *Allium* spp. and *Capsicum* spp. were reported to 100% inhibit the germination of *B. cinerea* spores (Wilson et al. 1997). The promising activities of Salvia species are also important factors. Geuenich et al. (2008) and Edris et al. (2007) reported that the aqueous extract of *Salvia officinalis* possesses an antioxidant, antiviral, antibacterial and fungicidal effects. The significant growth inhibition recorded for the *Salvia* species can be linked to the phenolic and flavonoid contents, as the main components (rosmarinic acid, caffeic acid, and Luteolin-7-O-glycoside) were proven to possess cytotoxic and antimicrobial activity (Badiee et al., 2012; Hu and Kitts, 2003; Matejczyk et al., 2018; Oliveira et al., 2013; Xavier et al., 2009).

Significant difference was observed in the colony area for *Salvia officinalis* subsp. *lavandulifolia* in terms of the harvesting times only at the CC2. This variation is attributable to the fact that the compositions of essential oils and extracts obtained from the same plant depend on the time of harvest and the specific plant organ (Perry et al. 1999). The reason that CC1 does not show significant effect as CC2 may be that the difference in the major bioactive constituents responsible for antifungal activity is not sufficient to cause a significant reduction in the growth of the pathogen. In both CC1 and CC2, a significant difference in colony area was only observed between Sol2 and Som2. This is a clear indication of a significant change in the bioactive compounds in the species at that moment of harvest leading to a significant variation in the activity.

In general, *Salvia officinalis* subsp. *major* had a better fungicidal potential compared to *Salvia officinalis* subsp. *lavandulifolia* at 3 days PI. Although it was reported by Jirovetz et al. (2007) that S. *lavandulifolia* essential oil possess the strongest antifungal activity against *Candida albicans* compared to other *Salvia* species, only the ethanolic extracts contain the specific components that define the chromatograph profile of the essential oil (Dob et al., 2007).

Table 2. Comparison of mean colony area and % inhibition for the extracts and control at the two different concentrations at 3 days PI

Extract	CC1	% Inhibition (Relative to control)	CC2	% Inhibition (Relative to control)
Control	3612.71^{c} ± 67.03	0	3281.85^{c} ± 120.36	0
Sol1	2475.12a^{b} ± 67.03	31.49	1513.05^{a} ± 120.36	53.90
Sol2	2682.04^{b} ± 67.03	25.76	1943.39^{b} ± 120.36	40.78
Som1	2590.89^{ab} ± 67.03	28.28	1655.58^{ab} ± 120.36	49.55
Som2	2412.14^{a} ± 67.03	33.23	1333.16^{a} ± 120.36	59.34

Values are presented as means ± SE. Means with the same superscripts across columns are not significantly different (two-way ANOVA, Duncan's post hoc; P<0.05)

Because each *Salvia* sp. has marked morphological and genetic variations that reflect their geographical origins, and that these variations provide each extract with a specific fingerprint composition with distinct organoleptic properties (Santos and Fernandes, 2001; Maksimovic et al., 2007) as well as the fact that the extractable compounds depend on the solvent used, the result of this finding may account for the difference in the solvent used for the extraction as hydrophilic phenols and rosmarinic acid that play a crucial role in antimicrobial activity are very low in the essential oils compared to the aqueous extracts (Durling et al., 2007). Thus, the inhibition in the essential oils may not be directly linked to these hydrophilic compounds, but perhaps their interaction with other compounds in the essential oil as reported by Pierozan et al. (2009). It may also be due to the difference in the feeding behavior of the *B. cinerea* (necrotrophic) and *Candida albicans* (biotrophic).

The comparison of the mean colony area for the two different concentrations (CC1 and CC2) for each of the test extracts as well as the control at 3 days PI is presented in Table 3 and Figure 1. The mean colony area at CC2 was significantly lower (P<0.01) than that of the CC1 in all four test extracts, but the variation was only significant in the case of the control (P<0.05). The mean colony area at CC1 ranged from 2412.14 cm^2 for Som2 to 3612.71 cm^2 for the control, while the mean colony area at CC2 ranged from 1333.16 cm^2 for Som2 to 3281.85 cm^2 for the control. The variation between the concentrations was at its minimal in the control group and at maximum in Som2.

The area of the colony was found to be significantly different in all the test extracts when compared to the control and in regards to the two concentrations. This is because antimicrobial and antifungal activities of the extracts are dose dependent (Jirovetz et al., 2007). Agar dilution may probably be the reason for the significant difference in the concentrations with respect to the control group, as addition of more water reduces the agar enrichment in the media.

The comparison of mean colony area and % inhibition at 7 days PI for the extracts and the control is presented in

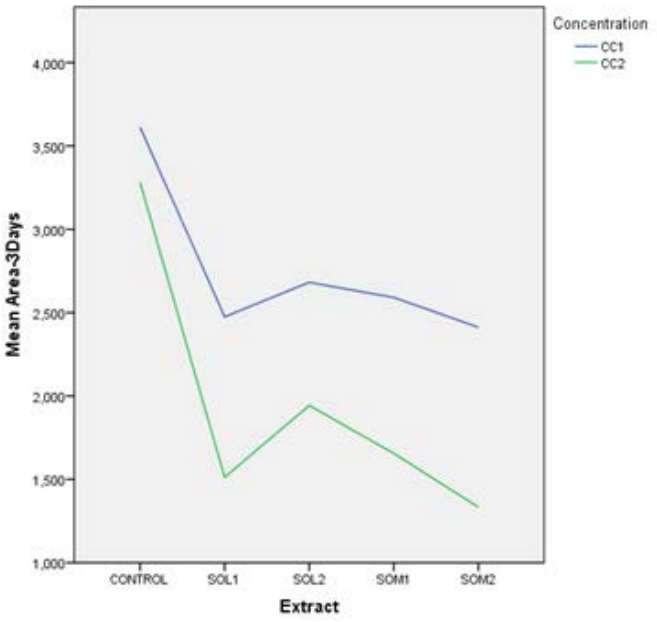

Figure 1. Marginal means of the concentrations against the test extracts and control at 3 days PI

Table 3. Comparison of concentrations for the mean colony area for each extract and control at 3 days PI

	Control	Sol1	Sol2	Som1	Som2
CC 1	$3612.71^{b} \pm 97.41$	$2475.12^{b} \pm 97.41$	$2682.04^{b} \pm 97.41$	$2590.89^{b} \pm 97.41$	$2412.14^{b} \pm 97.41$
CC 2	$3281.85^{a} \pm 97.41$	$1513.05^{a} \pm 97.41$	$1943.39^{a} \pm 97.41$	$1655.58^{a} \pm 97.41$	$1333.16^{a} \pm 97.41$

Values are presented as means ± SE. Means with the same superscripts across columns are not significantly different (two-way ANOVA, Duncan's post hoc; P<0.05)

Table 4. The result for CC1 shows that the area of colony in Som2 (4824.48 cm^2; 15.01% inhibition) was significantly smaller than all other treatments i.e. Control (5676.79 cm^2; 0% inhibition), Sol1 (5676.79 cm^2; 0% inhibition), Sol2 (5676.79 cm^2; 0% inhibition) and Som1 (5473.13 cm^2; 0.04% inhibition). However, the area of the colony did not differ significantly between the control, Sol1, Sol2 and Som1. With respect to CC2, the mean colony area across all the treatment groups (Control, 5676.79 cm^2; Sol1, 4889.62 cm^2; Sol2, 5108.64 cm^2; Som1, 5263.07 cm^2) was not significantly different.

The results for the colony area after 7 days showed that the earlier observed differences between the extracts themselves and the control became insignificant, except for the CC1 of Som2 where the colony area appeared to be significant compared to all other extracts (P<0.05). This may be since the phytochemical components responsible for the inhibition contained in the concentrations used are not enough to continually inhibit the pathogen growth, or the phytochemicals gradually undergo reduction in activity, resulting in their loss of effectivity. Also due to the complex nature and myriad forms of resistance strategies of *B. cinerea* (Fillinger and Walker, 2016), resistance may be developed against the extracts between the 3 to 7 days period. This is in conformity with the report by Wilson et al. (1997) where he found out that the essential oils in *S. officinalis* and S. *lavandulifolia* were inhibitory to the growth of the spores of *B. cinerea* only at concentrations ≥50%, while the inhibition was effective at 25% concentration for only 20 hours, but ineffective after 40 hours of treatment. Increased concentration of herbal extracts from CC1 to CC2 might have prevented sclerotia development in Som1 and Sol2, while in all other treatments including the control many sclerotia developed in the culture.

The comparison of the mean colony area indicating fungal growth at the two different concentrations (CC1 and CC2) for each of the test extracts as well as the control at 7 days PI is presented in Table 5 and Figure 2. The variation in the mean colony area at the CC1 and CC2 in each of the Control, Sol2, Som1 and Som2 was not significant.

Table 4. Comparison of mean colony area and % inhibition for the extracts and control at the two different concentrations at 7 days PI

Extract	CC1	% Inhibition (Relative to control)	CC2	% Inhibition (Relative to control)
Control	$5676.79^{b} \pm 123.73$	0	$5676.79^{a} \pm 317.52$	0
Sol1	$5676.79^{b} \pm 123.73$	0	$4889.62^{a} \pm 317.52$	13.87
Sol2	$5676.79^{b} \pm 123.73$	0	$5108.64^{a} \pm 317.52$	10.01
Som1	$5473.13^{b} \pm 123.73$	0.04	$5263.07^{a} \pm 317.52$	7.29
Som2	$4824.48^{a} \pm 123.73$	15.01	$4833.60^{a} \pm 317.52$	14.85

Values are presented as means ± SE. Means with the same superscripts across columns are not significantly different (two-way ANOVA, Duncan's post hoc; P<0.05)

Table 5. Comparison of concentrations for the mean colony area for each extract and control at 7 days PI

	Control	Sol1	Sol2	Som1	Som2
CC 1	5676.79[a] ± 240.96	5676.79[a] ± 240.96	5676.79[a] ± 240.96	5473.13[a] ± 240.96	4824.48[a] ± 240.96
CC 2	5676.79[a] ± 240.96	4889.62[b] ± 240.96	5108.64[a] ± 240.96	5263.07[a] ± 240.96	4833.60[a] ± 240.96

Values are presented as means ± SE. Means with the same superscripts across columns are not significantly different (two-way ANOVA, Duncan's post hoc; P<0.05)

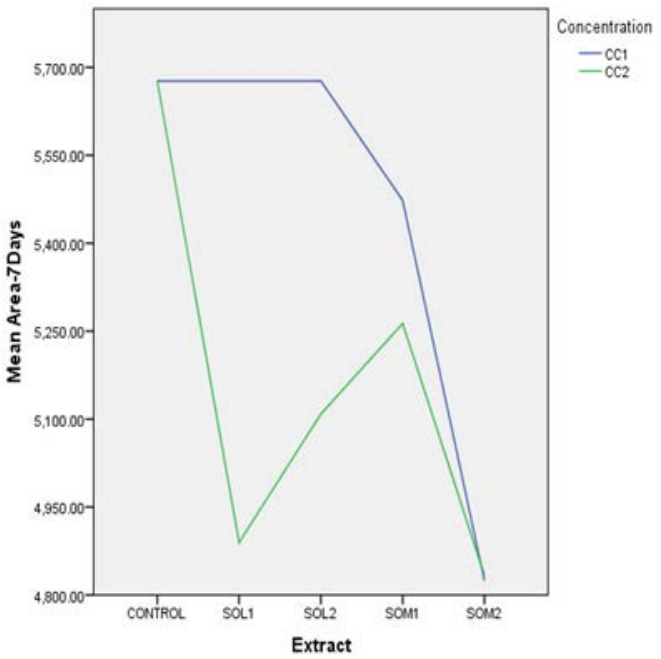

Figure 2. Marginal means of the concentrations against the test extracts and control at 7 days PI

However, the mean colony area at CC2 was significantly smaller than at CC1 in Sol1. The mean colony area at CC1 ranged from 4824.48 cm^2 for Som2 to 5676.79 cm^2 for the Control, Sol1 and Sol2, while the mean area at CC2 ranged from 4833.60 cm^2 for Som2 to 5676.79 cm^2 for the control. The variation between the concentrations was at its minimal in the Control group (zero) and maximum in Sol1.

Only Sol1 showed a significant difference in the colony area between the CC1 and CC2 at the 7 days PI. This implies that the slight increase in the concentration is sufficient to cause significant fungistatic activity in the Sol1 and not sufficiently enough to cause significant variations in the other extracts. It may be possible that further increase in the concentration may lead to further significant growth reduction or even death of the fungal colonies, especially in the case of Sol1.

CONCLUSIONS

The research has established that both *Salvia officinalis* subsp. *lavandulifolia* and *Salvia officinalis* subsp. *major* aqueous extracts were effective against the growth of *Botrytis cinerea*. However, in general, there is significant difference in their growth inhibition, with Som2 showing the highest inhibition of the pathogen growth (up to 59.34%). The growth inhibition of the extracts against the *B. cinerea* was found to be dose dependent in all the extracts tested at 3 days PI, however, this was maintained only in Sol1 at 7 days PI. Harvesting times influenced the effectiveness of the extracts for both *Salvia officinalis* subsp. *lavandulifolia* and *Salvia officinalis* subsp. *major* aqueous extracts but in a different manner. In *Salvia officinalis* subsp. *lavandulifolia*, the difference existed only with respect to CC2 at 3 days PI where Sol1 (harvested in July) showed higher inhibition than Sol2 (harvested in September). In contrast, the difference existed only with respect to CC1 at 7 days PI in *Salvia officinalis* subsp. *major* where Som2 (harvested in September) showed higher inhibition than Som1 (harvested in July).

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REFERENCES

Alexandratos, N., Bruinsma, J. (2012) World agriculture towards 2030/2050, the 2012 revision. ESA Working paper No. 12-03. Rome: FAO.

Badiee, P., Nasirzadeh, A. R., Motaffaf, M. (2012) Comparison of *Salvia officinalis* L. Essential oil and antifungal agents against Candida species. Journal of Pharmaceutical Technology and Drug Research, 1, 7. DOI: https://doi.org/10.7243/2050-120X-1-7

Bahraminejad, S., Amiri, R., Abbasi, S. (2015) Anti-fungal properties of 43 plant species against Alternaria solani and *Botrytis cinerea*. Archives of Phytopathology and Plant Protection, 48 (4), 336-344. DOI: https://doi.org/10.1080/03235408.2014.888236

Bouaziz, M., Yangui, T., Sayadi, S., Dhouib, A. (2009) Disinfectant properties of essential oils from *Salvia officinalis* L. cultivated in Tunisia. Food and Chemical Toxicology, 47, 2755–2760. DOI: https://doi.org/10.1016/j.fct.2009.08.005

Carmela, C., Mario, D. L. M., Alessandra, T. P. (1996) Activity of the Oil of *Salvia officinalis* L. Against *Botrytis cinerea*. Journal of Essential Oil Research, 8 (4), 399-404.

Dagostin, S., Formolo, T., Giovannini, O., Pertot, I., Schmitt, A. (2010) *Salvia officinalis* extract can protect grapevine against Plasmopara viticola. Plant Disease, 94, 575-580. DOI: https://doi.org/10.1094/PDIS-94-5-0575

Daniel, C. K., Lennox, C. L., Vries, F. A. (2014) In vivo application of garlic extracts in combination with clove oil to prevent postharvest decay caused by *Botrytis cinerea*, Penicillium expansum and Neofabraea alba on apples. Postharvest Biology and Technology, 99, 88–92. DOI: https://doi.org/10.1016/j.postharvbio.2014.08.006

Dob, T., Berramdane, T., Dahmane, D., Benabdelkader, T., Chelghoum, C. (2007) Chemical composition of the essential oil of *Salvia officinalis* from Algeria. Chemistry of Natural Compounds, 43, 491-494. DOI: https://doi.org/10.1007/s10600-007-0173-3

Durling, N. E., Catchpole, O. J., Grey, J. B., Webby, R. F., Mitchell, K. A., Yeap Foo, L., Perry, N. B. (2007) Extraction of phenolics and essential oil from dried sage (*Salvia officinalis*) using ethanol–water mixtures. Food Chemistry, 101, 1417-1424. DOI: https://doi.org/10.1016/j.foodchem.2006.03.050

Edris, A. E., Jirovetz, L., Buchbauer, G., Denkova, Z., Stoyanova, A., Slavchev, A. (2007) Chemical Composition, Antimicrobial Activities and Olfactoric Evaluation of a *Salvia officinalis* L. (Sage) Essential Oil from Egypt. Journal of Essential Oil Research, 19 (2),186-189. DOI: https://doi.org/10.1080/10412905.2007.9699256

El Ouadi, Y., Manssouri, M., Bouyanzer, A., Majidi, L., Lahhit, N., Bendaif, H., Costa, J., Chetouani, A., Elmsellem, H., Hammouti, B. (2015) Essential Oil Composition and Antifungal Activity of *Salvia officinalis* Originating from North-East Morocco, Against Postharvest Phytopathogenic Fungi in Apples. Der Pharma Chemica, 7 (9), 95-102. [Online] Available at: Available online at http://derpharmachemica.com/archive.html [Accessed 20 August 2019].

Evans, J. S., Pattison, E., Morris, P. (1986) Antimicrobial Agents from Plant Cell Culture. In: Morris, P., Scraggs, A., Stanford, A., Fowler, M., eds. Secondary Metabolites in Plant Cell Culture. London: Cambridge University. p. 12.

Fillinger, S., Walker, A.S. (2016) Chemical Control and Resistance Management of Botrytis Diseases. In: Fillinger, S., Elad, Y., eds. Botrytis – the Fungus, the Pathogen and its Management in Agricultural Systems. Switzerland: Springer International Publishing. pp.189-216. DOI: http://dx.doi.org/10.1007/978-3-319-23371-0_10

FAO (2004) FAOSTAT Database. Food and Agricultural Organization of the United Nations. (Online) Available at: http://faostat.fao.org/default.aspx?alias=faostat&lang=es (Accessed 25 July 2019).

Garcia, C. C. S., Menti, C., Lambert, A., Barcellos, T., Moura, S., Calloni, C., Branco, C. S., Salvador, M., Roesch-Ely, M., Henriques, J. A. P. (2016) Pharmacological perspectives from Brazilian *Salvia officinalis* (Lamiaceae): antioxidant, and antitumor in mammalian cells. Anais da Academia Brasileira de Ciências, 88 (1), 281-292. DOI: https://doi.org/10.1590/0001-3765201520150344

Geuenich, S., Goffinet, C., Venzk, S., Nolkemper, S., Baumann, I., Plinkert, P., Reichling, J., Keppler, O. T. (2008) Aqueous extracts from peppermint, sage and lemon balm leaves display potent anti-HIV-1 activity by increasing the virion density. Journal of Retrovirology, 5, 27. DOI: https://doi.org/10.1186/1742-4690-5-27

González, C., Brito, N., Sharon, A. (2016) Infection Process and Fungal Virulence Factors. In: Fillinger, S., Elad, Y., eds. Botrytis – the Fungus, the Pathogen and its Management in Agricultural Systems. Switzerland: Springer International Publishing, pp. 229-246. DOI: http://dx.doi.org/10.1007/978-3-319-23371-0_12

Hu, C., Kitts, D. D. (2003) Antioxidant, prooxidant, and cytotoxic activities of solvent-fractionated dandelion (*Taraxacum officinale*) flower extracts in vitro. Journal of Agricultural and Food Chemistry, 51 (1), 301-310. DOI: https://doi.org/10.1021/jf0258858

Jirovetz, L., Wlcek, K., Buchbauer, G., Gochev, V., Girova, T., Stoyanova, A., Schmidt, E., Geissler, M. (2007) Antifungal activities of essential oils of Salvia *lavandulifolia*, *Salvia officinalis* and *Salvia sclarea* against various pathogenic Candida species. Journal of Essential Oil Bearing Plants, 10 (5), 430-439. DOI: https://doi.org/10.1080/0972060X.2007.10643576

Li, Y., Shao, X., Xu, J., Wei, Y., Xu, F., Wang, H. (2017) Tea tree oil exhibits antifungal activity against *Botrytis cinerea* by affecting mitochondria. Food Chemistry, 34, 62-67. DOI: http://dx.doi.org/10.1016/j.foodchem.2017.04.172

Liu, H., Deng, Z., Wu, T., Liu, R., Loewen, S., Tsao, R. (2012) Microwave-assisted extraction of phenolics with maximal antioxidant activities in tomatoes. Food Chemistry, 130, 928–936. DOI: https://doi.org/10.1016/j.foodchem.2011.08.019

Maksimovic, M., Vidic, D., Milos, M., Solic, M.E., Abadzic, S., Siljak-Yakovlev, S. (2007) Effect of the environmental conditions on essential oil profile in two Dinaric Salvia species: S. brachyodon Vandas and *S. officinalis* L. Biochemical Systematics and Ecology, 35 (8), 473-478. DOI: https://doi.org/10.1016/j.bse.2007.02.005

Matejczyk, M., Świsłocka, R., Golonko, A., Lewandowski, W., Hawrylik, E. (2018) Cytotoxic, genotoxic and antimicrobial activity of caffeic and rosmarinic acids and their lithium, sodium and potassium salts as potential anticancer compounds. Advances in Medical Sciences, 63 (1), 14-21. DOI: https://doi.org/10.1016/j.advms.2017.07.003

Matthews, G. A. (2006) Pesticides- Health, Safety and the Environment. Oxford: Blackwell Publishing.

Matthias, H., Michaela, L., Matthias, K., Astrid, S., Andreas, M., Oliver, R., Gunther, D. (2007) Genetics and Biology of *Botrytis cinerea*. 14th International Botrytis Symposium. Cape Town, South Africa, 21-26th October 2007, African Sun Media Pty (Ltd.), pp. 17-18.

Mekhaldi, A., Bouznad, A., Djibaoui, R., Hamoum, H. (2014) Phytochemical Study and Biological Activity of Sage (*Salvia officinalis* L.). International Journal of Bioengineering and Life Sciences, 8 (11), 1253-1257.

Oliveira, K. B., Palu, E., Weffort-Santos, A. M., Oliveira, B. H. (2013) Influence of rosmarinic acid and *Salvia officinalis* extracts on melanogenesis of B16F10 cells. Revista Brasileira de Farmacognosia, 23 (2), 249-258. DOI: https://doi.org/10.1590/S0102-695X2012005000135

Pedro, A., Carlos, L. C., Julio, A., Guillermo, S., Juan, A. C., Jose, B., Mario S., Olov, S., Rudy, R., Mario, A. (2016) Antifungal activities of extracts produced by liquid fermentations of Chilean Stereum species against *Botrytis cinerea* (grey mould agent). Crop Protection, 89, 95-100. DOI: https://doi.org/10.1016/j.cropro.2016.07.014

Perry, N. B., Anderson, R. E., Brennan, N. J., Douglas, M. H., Heaney, A.J., Mcgimpsey, J. A., Smallfield, B. M. (1999) Essential oils from Dalmatian sage (*Salvia officinalis* L.): variations among individuals, plant parts, seasons, and sites. Journal of Agricultural and Food Chemistry, 47, 2048-2054. DOI: https://doi.org/10.1021/jf981170m

Pierozan, M. K., Pauletti, G. F., Rota, L., Santos, A. C., Lerin, L. A., Di Luccio, M., Mossi, A. J., Atti-Serafini, L., Cansian, R. L., Oliveira, J. V. (2009) Chemical characterization and antimicrobial activity of essential oils of *Salvia* L. species. Creative Information Journal, 29 (4), 764-770. DOI: https://doi.org/10.1590/S0101-20612009000400010

Santos-Gomes, P. C., Fernandes-Ferreira, M. (2001) Organ- and season dependent variation in the essential oil composition of *Salvia officinalis* L. cultivated at two different sites. Journal of Agricultural and Food Chemistry, 49, 2908-2916. DOI: https://doi.org/10.1021/jf001102b

Stephen, M., David, S., Katherine, J., Simon S., Gavin, R., Vincent P. (2016) Potentially Toxic Concentrations of Synthetic Pyrethroids Associated with Low Density Residential Land Use. Frontiers in Environmental Science, 4(75), 1-13. DOI: https://doi.org/10.3389/fenvs.2016.00075

Suffredini, I. B., Sader, H. S., Gonçalves, A. G., Reis, A. O., Gales, A. C., Varella, A. D., Younes, R. N. (2004) Screening of Antibacterial Extracts from Plants Native to the Brazilian Amazon Rain Forest and Atlantic Forest. Brazilian Journal of Medical and Biological Research, 37, 379–384. DOI: https://doi.org/10.1590/S0100-879X2004000300015

Tzortzakis, N. G. (2006) Maintaining Postharvest Quality of Fresh Produce with Volatile Compounds. Innovative Food Science and Emerging Technologies, 8, 111-116. DOI: https://doi.org/10.1016/j.ifset.2006.08.001

Vallejo, I., Carb, M., Fernandez Acero, F. J., Rebordinos, L., Cantoral, J. M. (2002) Evolution of the Resistance of *Botrytis cinerea* Isolates to Benzimidazole and Dicarboximide in Southwestern Spain, Phytopathology, 92, 82.

Wilson, C. L., Solar, J. M., El Ghaouth, A., Wisniewski, M. E. (1997) Rapid evaluation of plant extracts and essential oil for antifungal activity against *Botrytis cinerea*. Plant Disease, 81, 204-210. DOI: https://doi.org/10.1094/PDIS.1997.81.2.204

Xavier, C. P., Lima, C. F., Fernandes-Ferreira, M., Pereira-Wilson, C. (2009) Salvia fruticosa, *Salvia officinalis*, and Rosmarinic Acid Induce Apoptosis and Inhibit Proliferation of Human Colorectal Cell Lines: the role in MAPK/ERK pathway. Nutrition and Cancer, 61 (4), 564-571. DOI: https://doi.org/10.1080/01635580802710733

Zargari, A. (1990) Medicinal Plants. (4th ed). Tehran: Tehran University Press.