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CHEMICAL COMPOSITION AND ANTIFUNGAL ACTIVITY OF *SALVIA OFFICINALIS* (L.), *S. CRYPTANTHA* (MONTBRET ET AUCHER EX BENTH.), *S. TOMENTOSA* (MILL.) PLANT ESSENTIAL OILS AND EXTRACTS

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ABSTRACT

The essential oils from *Salvia officinalis* L., *S. cryptantha* (Montbret et Aucher ex Benth.) and *S. tomentosa* (Mill.) were extracted with hydrodistillation method and determined by using GC/MS analysis. This study investigated the *in vitro* effectiveness of essential oils and extracts of *S. officinalis*, *S. cryptantha* and *S. tomentosa* (collected from Tokat province) against eight fungal plant pathogens. Sterile PDA was prepared and then cooled to 40°C, after which the plant essential oils and/or extract were added. PDA without extract was used as negative control, while PDA with a Propineb-containing fungicide was used as positive control. According to the GC/MS analysis, the principal components of *S. cryptantha, S. tomentosa* and *S. officinalis* essential oils were determined as eucalyptol (27.64%), Camphor (29.87%), α-pinene (11.91%); β-thujene (40-69%), borneol (1.79-10.90%), camphor (0.40-7.25%); 3-thujonene (31.95%), camphor (28.53%), eucalyptol (7.35%), respectively. Based on the current results, the plant essential oils and extracts were determined to have negative effects on plant pathogens fungi. These effects changed depending on the extract, the type of sage the essential oil/extract that was obtained, the dose of essential oil or extract, and the fungus species.

KEYWORDS:
Antifungal effect, Essential oil, Sage, Salvia

INTRODUCTION

In recent years, there has been a growing interest in plant essential oils and extracts, owing to the natural compounds they contain. The biological activity exhibited by these compounds has increased the economic importance of these essential oils and extracts, and led to their increasing use in natural therapies and alternative medicine. Pesticides used against plant pathogens which are a significant cause of economic losses in agriculture have been well known to be harmful for human health and the environment. Knowledge of these substances' harmful effects has inspired studies attempting to determine the effectiveness of these biologically active essential oils and extract, which may present an alternative to conventional pesticides.

There are 236 genus and 7133 species of plants currently recognized as belonging to the Lamiaceae family, which has a wide geographic distribution around the world [1]. The *Salvia* L. genus (Lamiaceae), which is known in Turkey as (sage), is an important medicinal plant that is widely cultivated around the world. Owing to their rich content of essential oils, *Salvia* species are aromatic plants that find widespread use not only as spices and traditional remedies, but also in the perfume and cosmetic industry. In Turkey, *Salvia* has been commonly consumed among the population as a bloating remedy, cold remedy, stomach relaxant, diuretic, and tonic drink [2-4]. The latest studies report as many as 95 species of *Salvia* within the country [5]. *Salvia* occupies an important place in Turkey's flora, and is characterized by a high level of endemism (51%) [6-7].

Plants of the Lamiaceae family, including the *Salvia* genus, are especially rich in terpenoid compounds; they also contain flavonoids, essential oils, phenolic compounds, and, to a lesser extent, quinonoids [8-11]. Owing to their content of terpenoids and phenolic compounds, oils and extracts from these plants have high biological activity. Biological activity in *Salvia* species has been extensively studied by many researchers. Studies in the literature reported that the *Salvia* species had antibacterial [12], antifeedant [13], antioxidant [14], cytotoxic [15], antiviral [16], antifungal [17, 18], and antimicrobial effects [19, 20]. Studies have also been performed on the antimicrobial effects of *S. cryptantha*, which is an endemic species.

TABLE 1
Plant pathogen fungi used experiments

Fungi	Plant that fungus was isolated
Sclerotinia sclerotiorum (Lib.) de Bary	Potato (*Solanum tuberosum* L.)
Alternaria solani (Ell. And G. Martin)	Tomato (*Solanum lycopersicum* L.)
Ascochyta rabiei (Pass) Labr.	Chickpea (*Cicer arietinum* L.)
Botrytis cinerea Pers.	Strawberry *(Fragaria* L.)
Rhizoctonia solani Kühn	Potato (*Solanum tuberosum* L.)
Penicillum italicum Wehmer	Lemon (*Citrus limon*)
Aspergillus niger van Tieghem	Onion (*Allium cepa* L.)
Monilia laxa Aderh. and Ruhl. (Honey)	Apple (*Malus domestica* Borkh., 1803)

The current study was conducted to investigate the antifungal potential of extracts and essential oils obtained from *S. tomentos* and *S. cryptantha*, which are naturally distributed in the Tokat province of Turkey, and *S. officinalis* which is a cultivated plant.

MATERIALS AND METHODS

Preparation of Plant Materials. The plant materials used in the experiments were collected from the provincial center and districts of Tokat in 2012, during the plants' vegetative period, and were left to dry under shade.

Extraction of essential oils. Essential oils of plants were obtained with hydro-distillation method using a Schilcher apparatus. Pure water was added to weighed plant samples (1:10 w/v) and boiled for 2 hours. Obtained essential oils were maintained until used in the experiment [21].

The Determination of Essential Oil Contents of Plants (GC-MS). A 20 mg of essential oil was dissolved in 1.2 mL of acetone and it was ready for analysis. Analysis was conducted with BPX5 (0.25 mm ID, 0.25 m of film survivability) 30 m of capillary column and with a Perkin Elmer Clarus500 GC-MS. Injection volume was determined to be 2 µL and injection port temperature to be 250 °C. As the carrier gas, 50:1 split ratio of helium was used with 1 ml min flow rate. Furnace program was started with the temperature of 50 °C with 5 °C / min heating rate and heated up to 100 °C and it was kept at this temperature for 2 minutes and then it heated up to 220 °C with 3 °C/ min heating rate and waited for 2 minutes at this temperature. Total program duration was set to 30 minutes. MS parameters; ionization; EI ionizing energy were set to 70 eV and ion source temperature was set to 250 °C. Elucidation of the components was conducted with the comparison of the retention time of the existing standard components in the column with the retention time of sample components, the co-injection or retention index values (RI) given in the literature and the comparison of the specific volume spectrums of components in digital setting and data in available MS libraries (NIST, Wiley, and Pfleger). The relative ratio of each component was calculated by dividing the peak area to total peak area and multiplying the value with a hundred in Turbomass ver 5.4.2 software.

Preparation of water, methanol and ethanol extracts of plant samples. (1) Preparation of Water Extracts. Dried herbal materials were powdered by grinding with a plant grinding mill. 400 gr of ground plant material were put in a glass container containing 1000 ml of pure water and shook for 24 hours at 120 rpm in an orbital shaker and then solid residues were removed using filtering paper. Solid residues were completely removed using centrifuge for 15 minutes at 5000 rpm.

(2) Preparation of Methanol and Ethanol Extracts. Two 100 g plant materials were put in 1 L. erlenmayers separately and, then, methanol and ethanol were added as 600 ml of each. Mixtures were shaken for 24 hours at room temperature at 120 rpm in an orbital shaker. The extract was, then, filtered using paper filter. Methanol and ethanol were removed by evaporating at 32- 40 °C. The remaining extract was used to prepare a stock solution with pure water [22].

Fungus Cultures. The plant pathogenic fungi (Table 1) in this study were obtained from stock cultures kept at the Phytopathology Laboratory of Gaziosmanpaşa University Agricultural Faculty's Plant Protection Department. The *Ascochyta rabiei* culture was obtained from the Biology Department of Gaziantep University, and the *Botrytis cinerea* culture was obtained from the Ankara Plant Protection Central Research Institute. Fungus cultures were used after being allowed to incubate at 25±2 °C for seven days in 60 mm Petri dishes containing 10 ml PDA.

***In vitro* antifungal experiments of extracts and essential oils**. PDAs were autoclaved and chilled to 40 °C. Essential oils were mixed with melted sterile PDA to have a last concentration of 0, 100, 500, 1000, 2000 ppm and different plant extracts obtained (water, ethanol, methanol) were mixed with melted sterile PDA to have final con-

centrations of 1%, 3%, 7%, 10% and 20%. PDA was poured into 60 mm petri dishes (as 10 mm). Mycelium discs obtained from the 7-day fungus culture were put in petri dishes (5 mm in diameter). After inoculation, fungus culture was left for incubation at 28 °C for 7 days. Fungal development was recorded after 7 days [23]. Blocking in the development was calculated using the following formula [24]. A Propineb-containing fungicide was used as the positive control. The experiment was performed with four recurrences and replicated twice.

I=100×(DC -DT)/DC

I: Inhibition percentage compared to the control (Mycelium growth)

DC: Mycelium development in the control

DT: Mycelium development in essential oil applications

Antifungal Effects of Plant Essential Oil and Extracts in *In vivo*. In this study investigated the *in vivo* antifungal effect of essential oils and plant extracts from *S. cryptantha*, *S. tomentosa*, *S. officinalis* (water, methanol and ethanol extracts) on the *Botrytis cinerea*, *Monilia laxa*, *Aspergillus niger* and *Penicillum* sp. pathogens. In this context, apples were used for *Botrytis cinerea* and *Monilia laxa*, while lemons were used for *Aspergillus niger* and *Penicillum* sp. For each fruit, 8 mm mycelium disks were inoculated onto 8 mm scratches on the fruits' surface. Ambient humidity was raised following inoculation. Six fruits were used for each application. To prevent exposure to water, the fruits were placed in a container with plastic pipettes. During the experiments, 150 µl of essential oil at a 2000 ppm concentration was applied to the paper disks at the center of the containers. Plant extracts (in water, methanol and ethanol) were administered at a dose of 20%. A carbendazim-containing fungicide was used as the positive control. Following each application, the containers were closed and stored at +20°C. After five days, the radius of the rotting area that developed on the fruit's surface was measured starting from the inoculum's edge. All experiments were performed twice [25].

Statistical Analysis. The analysis of variance (ANOVA) was used to determine the significance levels of differences between experiment treatments, and the averages were compared using the DUNCAN test. Statistical analyses were conducted byusing the SPSS software.

RESULTS AND DISCUSSION

The results of the chemical composition of volatile oil *S. officinalis*, *S. cryptantha* and *S. tomentosa* are given in Table 2. The compositions of essential oils of the species are distinct similarities in species. Four *S. tomentosa* population essential oil consisted of 41 compounds, and 98.12-99.14% of the oil's composition was identified. The major compounds of this essential oil were determined as β-thujene (40-69%), borneol (1.79-10.90%) and camphor (0.40-7.25%). Previous studies reported contents of 1,8-cineol (17%), β-caryophyllene (11%), cyclofenchene (10%) and δ-cardine (6%) in *S. tomentosa* essential oils [26], and oil *S. tomentosa* contained β-pinene (39.7%), α-pinene (10.9%) and camphor (9.7%) as major components [27]. While essential *oil* main components *of S. tomentosa* collected from Thassos in Greece are determined as α-pinene, 1,8-cineole, cis-thujone, borneol, main components of essential oils in Tharace were determined as α-pinene, β-pinene, camphor, camphene[28].In similar studies; α-pinene, camphor and borneol [29]; β-pinene [30]; 1,8-cineole, camphor, camphene and β-pinene [31] α-pinene, germacrene D, β-pinene, α-humulene, veridiflorol ve limonene [32] have been reported as significant components of *S. tomentosa*. In the *S. officinalis* essential oil was identified 31 components including basic components 3-thujone (31.95%), camphor (28.53%), 1,8-cineole (7.35%) and camphene (4.57%) (Table 2).

Previous studies reported α- thujone, 1,8-cineole, camphor, β- thujone, borneol, viridiflorol, α-humulene, manool, bornyl acetate, *cis*-thujone, β-caryophyllene, *trans*-thujone as main components of *S. officinalis* oil [33; 34; 35; 36; 37; 38; 14]. In the essential oil of *S. cryptantha* beingan endemic species was identified 32 components predominantly eucalyptol (27.64%), camphor (29.87%), α-pinene (11.91%) and borneol (6.57%) (Table 2). The studies carried out on *S. cryptantha* were to less than other species. In the previous studies, it was reported that camphor (19.1%), 1,8-cineole (16.4%), borneol (11.9%), viridiflorol (11.5%) were determined in *S. cryptantha* grown Kayseri [39] while valence (31.80%), 1,8-cineole (%17.43), camphor (13.73%), Isoberneol (10.79%) were determined as major components in the oil of *S. cryptantha* in Konya [40].

***In vitro* antifungal effects of plant extracts and essential oils.** By infecting cultivated plants, plant pathogenic fungi can cause significant yield losses in agricultural production. Thus, managing these plant pathogens has been a great importance. The environmental problems associated with synthetic fungicides that have been extensively used for combatting plant pathogenic fungi, as well as the growing resistance of fungi to these agents, have engendered the need for new control strategies. For this reason, there has been increasing research in recent years on the effectiveness of various biological agents against pathogenic fungi. In this context, a range of studies investigating the effects of extracts and essential oils from plants of different families (and extracted with different

solvents) on plant pathogenic fungi have been initiated, many of which are still ongoing. In the present study, it was investigated and evaluated the effect of plant extracts and essential oils from *S. cryptantha*, *S. tomentosa*, *S. officinalis* on major plant pathogenic fungi.

The results from our *in vitro* trials have demonstrated that extracts and essential oils from the *Salvia* species were effective to varying extents against the eight plant pathogenic fungi. *S. officinalis* extracts and essential oils showed greater effects against fungi compared to the controls. However, it was observed that this effect varied depending on the extracts, essential oils, and the species of fungus. Water extract from *S. officinalis* was found to be more effective compared to other extracts and essential oils. *S. officinalis* water extract inhibited the mycelium growth of *A. solani*, *R. solani*, *M. laxa,* and *B. cinerea* pathogens by 100%. Methanol and ethanol extracts and essential oil of *S. officinalis* did not affect the mycelium growth of *S. sclerotiorum* (Table 3). It has been reported that while *S. officinalis* essential oil has varying degrees of effectiveness against *Candida*, *Aspergillus* and *Dermatophyte* samples, *Candida* and *Aspergillus*

TABLE 2
Chemical composition of *Salvia* species.

		S. tomentosa				*S. cryptantha*	*S. officinalis*
RT	**Essential oil**	P1	**P2**	**P3**	**P4**		
6.23	**α-Pinene**	6.55	4.22	5.66	3.12	11.91	3.37
6.64	**Camphene**	1.60	2.98	1.10	0.78	6.32	4.57
7.19	**α-Phallendrene**	0.39	1.07	1.92	1.49	0.59	tr
7.34	**β-Thujene**	54.11	40.69	69.12	49.12	4.01	1.36
7.54	**β-Myrcene**	tr	1.64	1.08	2.99	0.91	0.66
8.35	**α-Terpinen**	0.21	tr	tr	tr	-	0.16
8.62	**β-Cymene**	0.14	0.91	0.25	0.19	-	0.20
8.68	**Limonene**	1.15	tr	0.87	0.52	2.13	1.58
8.88	**Eucalyptol**	1.31	0.66	1.55	0.97	27.64	7.35
9.48	**γ-Terpinene**	3.18	1.56	0.41	0.31	0.37	0.35
10.24	**Terpinolen**	-	-	-	-	0.07	0.26
10.77	**β-Pinene**	0.16	0.35	1.65	1.99	0.17	0.35
11.13	**α-Thujone**	tr	tr	tr	tr	0.14	tr
11.18	**3-Thujanone**	tr	tr	tr	tr	tr	31.95
11.51	**Thujone**	tr	tr	tr	tr	tr	5.22
12.44	**Sabinyl acetate**	tr	1.11	0.54	2.42	0.07	-
12.77	**Camphor**	3.69	7.25	4.45	0.40	29.87	28.53
13.24	**cis-Sabinol**	-	-	-	-	0.10	0.18
13.77	**Borneol**	3.98	10.90	1.79	3.20	6.57	3.13
13.95	**4-Terpineol**	0.49	0.66	0.49	5.91	0.88	0.35
14.63	**α-Terpieol**	4.27	1.67	1.85	6.52	0.47	0.25
16.38	**Fenchene**	0.13	0.22	0.52	0.40	tr	-
18.00	**Bornyl acetate**	0.75	7.01	0.61	1.13	0.50	2.25
18.40	**Estregole**	tr	tr	tr	tr	-	0.55
20.30	**α-Cubebene**	0.37	tr	0.10	0.18	-	-
21.26	**Zingiberene**	0.23	tr	tr	tr	-	-
21.49	**Copaene**	0.49	0.18	0.23	1.07	0.11	tr
21.84	**β-Bourbonene**	0.78	tr	0.27	0.50	0.37	-
22.01	**δ-Cadinene**	tr	tr	tr	0.29	-	-
22.83	**Dehydro Aromadendrene**	0.28	tr	tr	tr	-	-
23.36	**Isocaryophyllene**	4.26	1.98	0.91	1.19	1.03	1.26
23.84	**β-Cubebene**	0.65	tr	tr	1.12	-	2.05
24.21	**Isoledene**	0.36	tr	tr	tr	1.41	tr
24.87	**α-Caryophyllene**	2.86	1.23	0.44	0.74	-	tr
25.67	**γ-Muurolene**	2.44	tr	tr	tr	0.11	-
25.91	**α-Amorphene**	tr	0.34	0.29	0.34	0.45	-
26.50	**Eremophilene**	0.43	tr	tr	tr	-	-
26.62	**γ-Cadinene**	0.44	tr	0.43	0.41	-	-
26.92	**Palustrol**	0.29	tr	0.52	0.26	-	-
27.35	**Germacrene D**	-	-	-	-	0.22	-
29.96	**Spathulenol**	0.74	1.07	0.81	0.65	tr	tr
30.13	**Caryophyllene oxide**	0.17	6.91	0.37	2.46	0.52	0.13
30.62	**γ-Gurjunene**	2.53	1.16	0.58	2.74	1.15	3.65
31.24	**unidentified oxygenated sesquiterpene**	tr	2.75	0.33	4.71	tr	0.16
32.97	**unidentified oxygenated sesquiterpene**	-	-	-	-	1.19	-
	TOTAL	**99.45**	**98.50**	**99.14**	**98.12**	**99.26**	**99.85**

TABLE 3
Effect of *S. officinalis* on plant pathogenic fungi mycelium growth (mm)

Application	Doses	As	Rs	Asn	Pi	Ss	Ml	Bc	Ar
Water extracts	**Control**	**60.00a***	**60.00a**	**57.92a**	**45.02a**	**60.00a**	**45.04a**	**47.44a**	**21.50a**
	Fungicide	0.00f	0.00c	12.73d	0.00e	0.00d	18.26d	0.00d	0.00d
	1%	52.33b	35.57b	46.25b	36.38b	60.00a	40.77b	37.72b	15.17b
	3%	47.36c	38.28b	49.87ab	32.58b	60.00a	36.82c	20.04c	19.00a
	7%	29.16d	0.00c	34.51c	21.57c	60.00a	16.54d	3.16d	5.90c
	10%	21.96e	0.00c	31.48c	14.26d	43.17b	0.00e	0.00d	6.55c
	20%	0.00f	0.00c	26.01c	16.56d	40.52c	0.00e	0.00d	5.38c
Methanol extracts	**Control**	**60.00a**	**60.00a**	**57.92a**	**45.02a**	**60.00a**	**45.04a**	**47.44a**	**21.50a**
	Fungicide	0.00e	0.00e	12.73d	0.00c	0.00b	18.26c	0.00d	0.00e
	1%	26.27b	47.17b	47.55b	25.22b	60.00a	17.94c	25.92b	16.06b
	3%	21.09c	45.64b	40.72b	25.68b	60.00a	17.99c	22.02b	15.13bc
	7%	20.84c	45.09b	41.84b	22.67b	60.00a	21.92b	19.76bc	13.87bc
	10%	19.89cd	40.07c	37.54bc	22.64b	60.00a	18.15c	19.92bc	13.46c
	20%	19.31d	32.39d	28.87c	22.48b	60.00a	18.20c	13.20c	10.75d
Ethanol extracts	**Control**	**60.00a**	**60.00a**	**57.92a**	**45.02a**	**60.00a**	**45.04a**	**47.44a**	**21.50a**
	Fungicide	0.00e	0.00e	12.73d	0.00d	0.00b	18.26c	0.00d	0.00d
	1%	18.80c	42.98b	40.07b	25.30b	60.00a	15.15d	31.59b	13.83b
	3%	17.21d	42.72b	26.08c	21.53bc	60.00a	15.46d	23.88bc	13.24b
	7%	19.35bc	38.272c	33.25bc	21.04bc	60.00a	19.00bc	23.54bc	13.61b
	10%	20.39b	36.2363c	29.30c	19.33bc	60.00a	19.89b	21.16c	13.39b
	20%	17.49d	30.9400d	29.53c	17.20c	57.85a	18.20c	16.95c	10.19c
Essential oils	**Control**	**60.00a**	**60.00a**	**57.92a**	**45.02a**	**60.00a**	**45.04a**	**47.44a**	**21.50a**
	Fungicide	0.00e	0.00e	12.73d	0.00c	0.00b	18.26e	0.00c	0.00d
	100ppm	57.67a	34.12b	46.27b	35.34b	60.00a	24.48b	43.54a	18.34b
	500ppm	51.39b	17.03c	47.39b	35.44b	60.00a	22.51c	40.49ab	16.77bc
	1000ppm	43.66c	15.99c	46.57b	28.41b	60.00a	20.64d	30.64b	16.44bc
	2000ppm	19.38d	9.14d	32.32c	28.12b	60.00a	18.20e	29.96b	15.01c

As: *Alternaria solani*, Rs: *Rhizoctonia solani*, Asn: *Aspergillus niger*, Pi: *Penicllum italicum*, Ss: *Sclerotinia sclerotiorum*, Ml: *Monilia laxa*, Bc: *Botrytis cinerea*, Ar: *Ascochyta rabiei*
*Means in the same column by the same letter are not significantly different to the test of ANOVA (a = 0.05).

are relatively more resistant compared to dermatophytes [18]. A 1:40 dilution of acid extracts from *S. sclarea* and *S. officinalis* were reported to inhibit *A. solani* growth, while extracts in water or saline solution were less effective on *A. solani* [41]. In contrast, *S. officinalis* water extracts were observed to fully inhibit *A. solani* mycelium growth in our study. This difference might have stemmed from dissimilarities between two studies regarding methodology and applied doses. *S. officinalis* extracts were reported to reduce *Plasmopara vitocola* growth by 63%under field conditions [42].

S. tomentosa plant extract and essential oils exhibited comparatively less effects on plant pathogens than *S. officinalis*. At an application dose of 20%, *S. tomentosa* water extract inhibited *B. cinerea* mycelium growth by 100%, and *R. solani* mycelium growth by 83.72%. As with *S. officinalis*, water extracts of *S. tomentosa* did not work against *S. sclerotiorum*. A1000 ppm *S. tomentosa* essential oil was able to inhibit *A. rabiei* pathogen mycelium growth by 100%. *S. tomentosa* methanol and ethanol extracts and essential oils inhibited plant pathogenic fungi growth to different degrees (Table 4).

There have been limited studies investigating the antifungal potential of *S. tomentosa* against plant pathogenic fungi. On the other hand, various researchers have reported *S. tomentosa* plant extracts as having a variety of antibacterial, insecticidal, and antioxidant effects. For example, *S. tomentosa* essential oils were demonstrated to have antibacterial effects against gram-positive and gram-negative bacteria [26], while *S. tomentosa* extracts and essential oils were found to exhibit various degrees of antimicrobial effects against microorganisms [27], as well as insecticidal effects against *Acanthoscelides obtectus* and *Tribolium castaneum* [43]. These different studies are indicative of a wide variety of biological activity for *S. tomentosa* essential oils and extracts. In this context, the findings of the present study showed that *S. tomentosa* essential oils and extracts were also effective against various plant pathogenic fungi. This diversity of biological activity and broad-range effectiveness stems largely from the phenolics, diterpenoids, and flavonoids – active compounds of known effectiveness – found in *S. tomentosa* [44-46].

S. cryptantha plant extracts and essential oils had various degrees of effectiveness against plant pathogenic fungi. This effectiveness varied depending on plant extracts and essential oils used, and the dose of application. At its highest application dose (20%), *S. cryptantha* water extract inhibited *R. solani* and *S. sclerotiorum* mycelium growth by 100% and 98.63%, respectively. *S. cyptantha* methanol extract demonstrated the highest level of effectiveness against the *A. solani* pathogen, while exhibiting the least effectiveness against the *S. sclerotiorum* pathogen with an inhibition rate of 9.4%. Ethanol extracts had similar levels of effectiveness as the methanol extract. Although *S. cryptantha* essential oil had a limited effectiveness against *S. sclerotiorum*, it was had more significant effects on other pathogens. At a dose of 2000 ppm, *S. cryptantha* essential oil showed the highest effectiveness against *M. laxa,* with 59.72% growth inhibition (Table 5).

These results indicated *S. cryptantha* had biological activity and antifungal potential. Studies showed that *S. cryptantha* essential oils and methanol extracts had antimicrobial and antioxidant activity [47, 48], and that *S. cryptantha* exhibited contact toxicity against *Sitophilus granarius* L. and *S. oryzae* L, which are significant storage pathogens [49].

Our study results showed that essential oils and extracts from *S. tomentosa*, *S. officinalis* and *S. cryptantha* had selective antifungal activity against eight important plant pathogenic fungi (*S. sclerotiorum*, *A. solani*, *A. rabiei*, *R. solani*, *B. cinerea*, *P. italicum*, *A. niger*, and *M. laxa*). The believe that these results stem from the compounds found in *Salvia* species extracts and essential oils, such as camphor, eucalyptol(1,8 cineole), and α-pinene, whose biological activity have been demonstrated by different researchers [50-53].

***In vivo* antifungal activities of plant extracts and essential oils.** In the *in vivo* experiments, the highest plant extract and essential oil doses used in *in vitro* experiments were applied against the plant pathogens *M. laxa*, *B. cinerea*, *A. niger,* and *P. italicum*. In general, the effectiveness exhibited by the plant extracts and essential oils was lower under *in vivo* conditions compared to *in vitro* results. However, the application of these plant extracts and essential oils slowed down pathogen growth compared to control. *S. officinalis* essential oil had the

TABLE 4
Effect of *S. tomentosa* on mycelium growth (mm) of plant pathogenic fungi

Application	Doses	As	Rs	Asn	Pi	Ss	Ml	Bc	Ar
	Control	**60.00a***	**60.00a**	**57.92ab**	**45.02a**	**60.00a**	**45.04b**	**47.44a**	**21.50a**
Water extracts	**Fungicide**	0.00e	0.00e	12.73d	0.00c	0.00b	18.26e	0.00d	0.00d
	1%	58.85a	60.00a	60.00a	52.95a	60.00a	53.38a	41.38ab	16.57b
	3%	52.19b	56.51a	55.58ab	50.36a	60.00a	46.49b	34.18b	14.68b
	7%	30.85c	34.19b	53.03ab	26.79b	60.00a	24.94d	10.37c	8.11c
	10%	26.47d	27.26c	51.11b	27.38b	60.00a	29.72c	10.39c	8.78c
	20%	27.24d	9.77d	40.24c	29.76b	60.00a	23.70d	.0000d	8.88c
	Control	**60.00a**	**60.00a**	**57.92a**	**45.02a**	**60.00a**	**45.04a**	**47.4450a**	**21.50a**
Methanol extracts	**Fungicide**	0.00e	0.00e	12.73d	0.00d	0.00c	18.26d	0.00d	0.00d
	1%	29.84b	60.00a	51.08ab	32.05bc	60.00a	22.29b	32.34b	14.13b
	3%	24.85c	53.14b	44.18bc	33.88b	60.00a	20.15c	25.21bc	13.49b
	7%	25.20c	52.71bc	40.58bc	28.03bc	60.00a	19.81cd	20.36c	13.90b
	10%	24.99c	51.45c	35.11c	30.88bc	59.01a	19.53cd	20.71c	14.31b
	20%	22.75d	47.26d	32.44c	24.33c	49.98b	19.37cd	19.24c	10.66c
	Control	**60.00a**	**60.00a**	**57.92a**	**45.02a**	**60.00a**	**45.04a**	**47.44a**	**21.50a**
Ethanol extracts	**Fungicide**	0.00e	0.00f	12.73e	0.00c	0.00d	18.26b	0.00d	0.00d
	1%	22.87b	52.60b	44.97b	27.93b	60.00a	18.13b	35.02b	13.06b
	3%	18.72c	50.30c	42.39bc	30.01b	58.68a	17.49b	21.76c	9.60c
	7%	19.52c	49.68c	40.40bc	24.33b	54.64b	17.84b	23.12c	10.51c
	10%	19.43c	47.95d	32.62cd	20.92b	53.43b	17.80b	20.73c	10.35c
	20%	16.96d	36.59e	27.64d	24.08b	48.21c	14.82c	14.91c	9.20c
	Control	**60.00a**	**60.00a**	**57.92a**	**45.02a**	**60.00a**	**45.04a**	**47.44a**	**21.50a**
Essential oils	**Fungicide**	0.00f	0.00d	12.73b	0.00d	0.00b	18.26cd	0.00d	0.00c
	100ppm	56.16b	60.00a	60.00a	48.19ab	60.00a	33.08b	29.82b	16.85b
	500ppm	51.92c	55.37b	57.95a	39.72bc	60.00a	31.33b	25.50bc	15.54b
	1000ppm	15.63d	53.03c	57.25a	34.46c	60.00a	20.37c	23.07bc	0.00c
	2000ppm	10.56e	52.00c	54.46a	32.23c	60.00a	15.74d	21.38c	0.00c

As: *Alternaria solani*, Rs: *Rhizoctonia solani*, Asn: *Aspergillus niger*, Pi: *Penicllum italicum*, Ss: *Sclerotinia sclerotiorum*, Ml: *Monilia laxa*, Bc: *Botrytis cinerea*, Ar: *Ascochyta rabiei*

*Means in the same column by the same letter are not significantly different to the test of ANOVA (a = 0.05).

TABLE 5
Effect of *S. cryptantha* on plant pathogenic fungi mycelium growth (mm)

Application	Doses	As	Rs	Asn	Pi	Ss	Ml	Bc	Ar
Water extracts	**Control**	**60.00a***	**60.00a**	**57.92a**	**45.02a**	**60.00a**	**45.04a**	**47.44a**	**21.50a**
	Fungicide	0.00e	0.00e	12.73e	0.00d	0.00c	18.26e	0.00c	0.00e
	1%	49.61b	60.00a	45.27bc	37.07b	60.00a	31.65b	40.78a	15.15b
	3%	30.22c	31.09b	48.42b	36.49b	60.00a	22.47cde	26.57b	12.80cd
	7%	28.45c	22.43c	40.92bcd	26.23c	60.00a	25.03c	30.35b	16.49bc
	10%	21.02d	5.95d	37.21cd	28.12c	49.32b	22.89cd	23.50b	12.26d
	20%	20.51d	0.00e	35.60d	25.05c	3.797c	20.33de	22.87b	10.98d
Methanol extracts	**Control**	**60.00a**	**60.00a**	**57.92a**	**45.02a**	**60.00a**	**45.04a**	**47.44a**	**21.50a**
	Fungicide	0.00e	0.00e	12.73d	0.00c	0.00d	18.26d	0.00e	0.00e
	1%	24.61b	38.93d	40.96b	17.49b	60.00a	21.98b	29.37b	14.42b
	3%	19.86c	42.30c	32.07bc	17.93b	60.00a	20.46bc	24.19c	13.16bc
	7%	19.47c	48.01b	31.22bc	16.69b	57.91b	18.88cd	23.18cd	11.52cd
	10%	18.32d	47.30b	31.41bc	15.72b	54.69c	18.66d	23.10cd	11.41cd
	20%	18.10d	40.05d	26.72c	15.82b	54.34c	18.70d	18.44d	9.77d
Ethanol extracts	**Control**	**60.00a**	**60.00a**	**57.92a**	**45.02a**	**60.00a**	**45.04a**	**47.44a**	**21.50a**
	Fungicide	0.00h	0.00g	12.73d	0.00e	0.00c	18.26d	0.00e	0.00c
	1%	33.95b	51.03b	35.84b	34.88b	60.00a	25.14b	36.05b	20.31a
	3%	29.56c	47.22c	37.69b	26.32c	60.00a	24.25b	28.40c	17.84b
	7%	20.53e	38.71e	24.69cd	15.21d	60.00a	20.21c	21.29d	17.30b
	10%	21.83d	41.03d	19.83cd	15.70d	58.07ab	20.27c	20.88d	17.34b
	20%	17.63g	34.63f	21.47cd	12.52d	56.59b	17.55d	17.95d	17.17b
Essential oils	**Control**	**60.00a**	**60.00a**	**57.92a**	**45.02a**	**60.00a**	**45.04a**	**47.44a**	**21.50a**
	Fungicide	0.00d	0.00e	12.73c	0.00d	0.00c	18.26c	0.00e	0.00c
	100ppm	47.96b	60.00a	45.98b	43.35a	60.00a	28.63b	29.03bc	16.82b
	500ppm	38.26c	53.93b	44.51b	29.29b	60.00a	19.17c	31.34b	15.38b
	1000ppm	37.38c	48.39c	43.28b	19.07c	60.00a	19.25c	23.71cd	15.60b
	2000ppm	33.69c	32.38d	41.70b	13.47c	57.00b	18.14c	21.99d	14.92b

As: *Alternaria solani*, Rs: *Rhizoctonia solani*, Asn: *Aspergillus niger*, Pi: *Penicllum italicum*, Ss: *Sclerotinia sclerotiorum*, Ml: *Monilia laxa*, Bc: *Botrytis cinerea*, Ar: *Ascochyta rabiei*

*Means in the same column by the same letter are not significantly different to the test of ANOVA (a = 0.05).

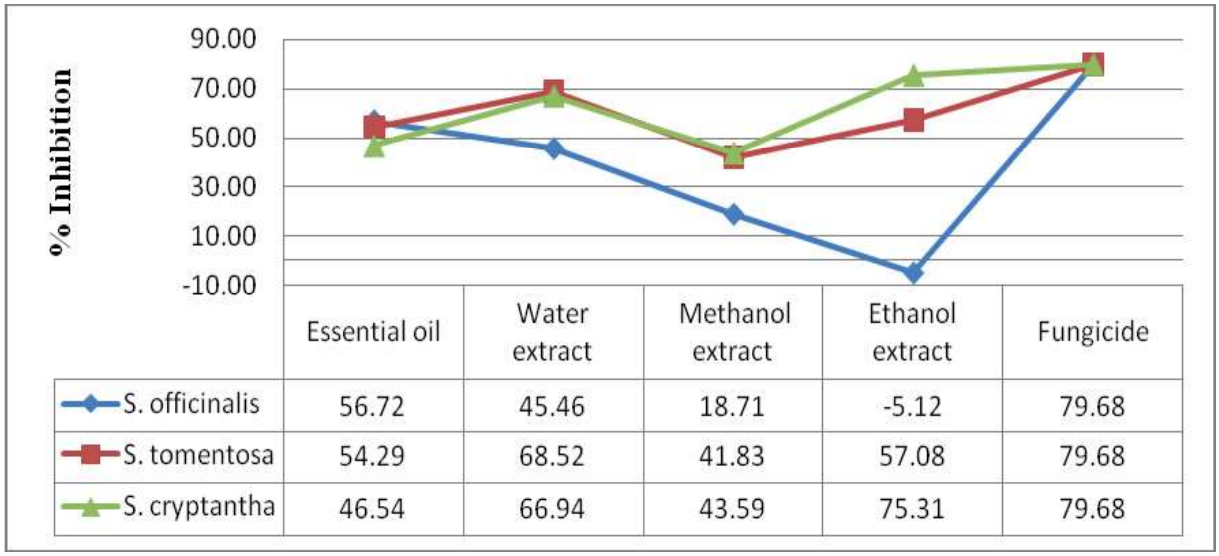
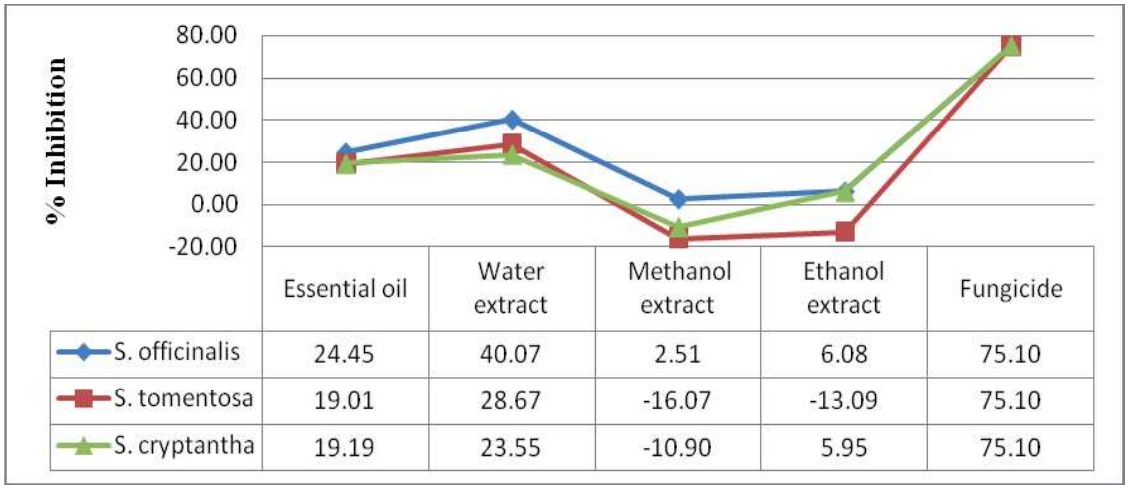


FIGURE 1
The effects of *Salvia* species essential oils and extracts on *B.cinerea* mycelium growth.



	Essential oil	Water extract	Methanol extract	Ethanol extract	Fungicide
S. officinalis	24.45	40.07	2.51	6.08	75.10
S. tomentosa	19.01	28.67	-16.07	-13.09	75.10
S. cryptantha	19.19	23.55	-10.90	5.95	75.10

FIGURE 2
The effects of *Salvia* species essential oils and extracts on *M. laxa* mycelium growth

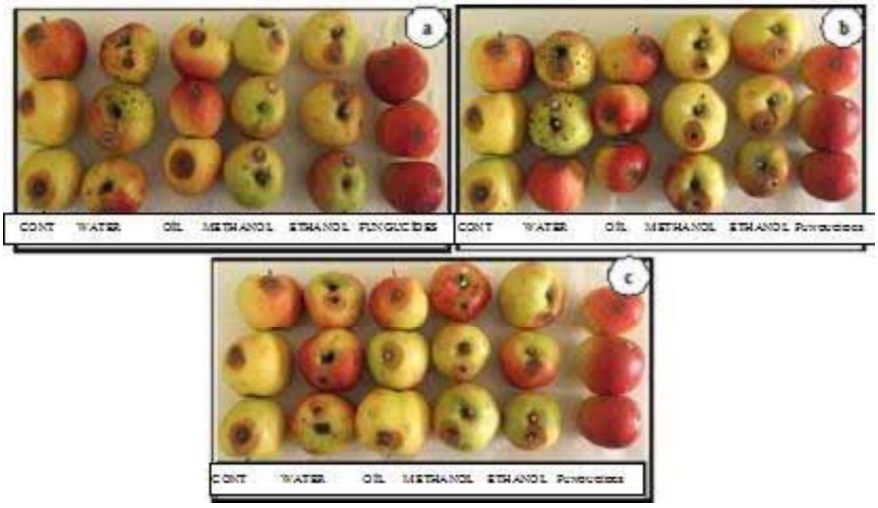
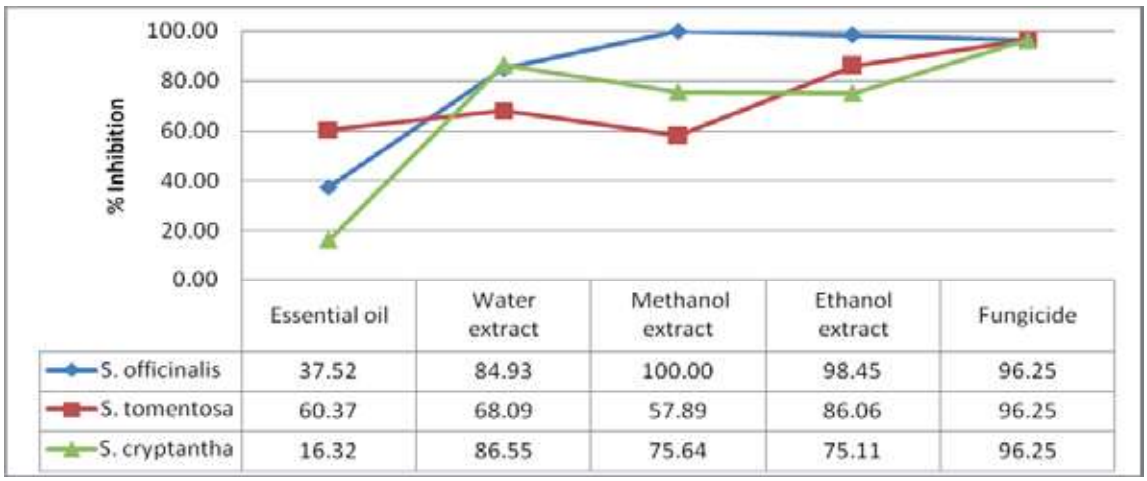


FIGURE 3
The effects of *S. officinalis* (a), *S. tomentosa* (b) and *S. cryptantha* (c) extracts and essential oils against *M. laxa* pathogen



	Essential oil	Water extract	Methanol extract	Ethanol extract	Fungicide
S. officinalis	37.52	84.93	100.00	98.45	96.25
S. tomentosa	60.37	68.09	57.89	86.06	96.25
S. cryptantha	16.32	86.55	75.64	75.11	96.25

FIGURE 4
The effects of *Salvia* species essential oils and extracts on *A. niger* mycelium growth.

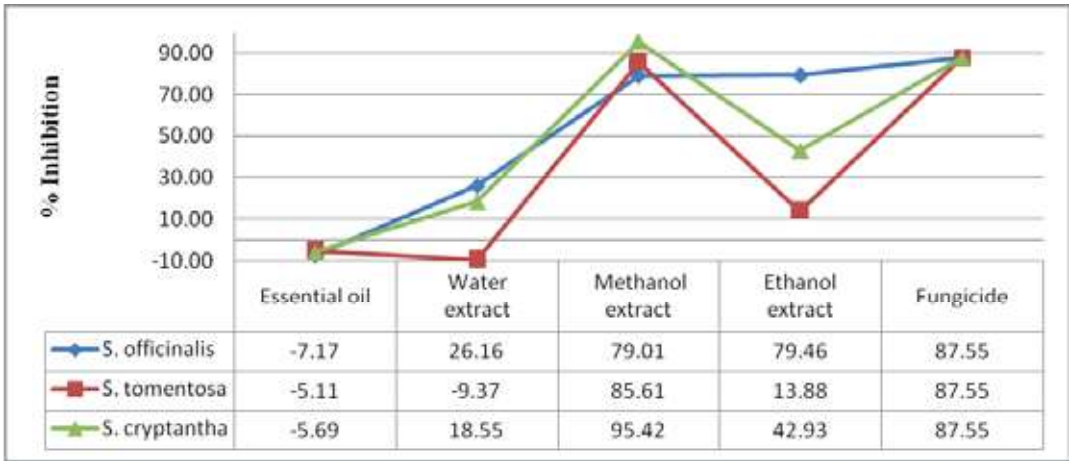


FIGURE 5
The effects of *Salvia* species essential oils and extracts on *P. italicum* mycelium growth.

highest effect on *M. laxa*, *B. cinerea* and *P. italicum* growth, while *S. tomentosa* essential oil had the highest effect on *A. niger* growth (Figure 1, Figure 2, Figure 3, Figure 4, Figure 5).

The effects of the *Salvia* species extracts on pathogens varied depending on extracts used, the dose of application, and the pathogen being targeted. Under *in vivo* conditions, the *S. cryptantha* ethanol extract had the highest effect on *B. cinerea* growth, inhibiting it by 75.31%, while the *S. tomentosa* water extract had the second highest effect, inhibiting growth by 68.52%. The highest antifungal effect on *M. laxa* was observed with the *S. officinalis* water extract, which inhibited growth by 40.07%. In addition, the *S. officinalis* methanol extract had the highest effect on *A. niger* mycelium growth by inhibiting it 100%, while *S. cryptantha* methanol showed the highest effect against *P. italicum* mycelium growth, inhibiting it by 95.42%. [54] described that although *S. officinalis* essential oils are effective against the *S. sclerotiorum* pathogen in *in vitro* applications, the same level of effectiveness is not observed during *in vivo* applications. It has reported that while *Azadirachta indica* extracts inhibit *Pyricularia orzae* radial growth by 68.7 to 83.6% under *in vitro* conditions (i.e. agar environment), the level of inhibition decreases to 55.2 to 67.9% under *in vivo* conditions [55].

In the *in vivo* application, *M. laxa* had the highest tolerance against plant extracts and essential oils, and was followed, in decreasing order of resistance, by *P. italicum*, *B. cinerea* and *A. niger*. The highest effect on the pathogens was observed by plant water extracts, followed in decreasing order by methanol extracts, and ethanol extracts and essential oils. The differences in effectiveness were due to the selectivity of the plant extracts and essential oils, and the differences between the pathogens' growth styles. Differences in chemical composition between the various extracts and essential oils are also likely to have contributed to these differences [56-62].

CONCLUSION

In conclusion, we demonstrated that plant extracts and essential oils from the endemic species *S. cryptantha* and *S. tomentosa* and cultivated *S. officinalis* species possessed antifungal activity both under *in vitro* and *in vivo* conditions, with the level of effectiveness varying depending on dose, extract, and plant pathogen. In light of the problems associated with the cost, adverse environmental effects, residues in food, and increasing pathogen resistance of synthetic fungicides, which are widely used against plant pathogenic fungi, the results of the present study and other studies investigating alternative antifungal agents are of particular importance for managing pathogenic fungi.

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