

Allelopathic and Antifungal potentials of endemic *Salvia absconditiflora* Greuter & Burdet collected from different locations in Turkey

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ABSTRACT

We determined the antifungal and bioherbicidal properties of essential oils of *Salvia absconditiflora*. Its plant samples were collected from 10-locations in Kırşehir province, Turkey and dried in shade. Essential oils of dried plant samples were extracted by hydro-distillation method using a Schilcher device and the compounds identified in *S. absconditiflora* essential oils by GC-MS analysis were: Camphor (10.52-58.64%), Bicyclo [2.2.1] Heptane-2-One, 1.7.7 (21.94-30.16%) and viridiflorol (3.42-25.2%). *S. absconditiflora* essential oil (a dose of 10 µl/petri dishes) inhibited the mycelium growth of *Sclerotinia sclerotiorum* and *Alternaria solani* pathogens by 9.3 and 54.40 %, respectively. At dose of 20 µl/petri dish, the essential oil completely inhibited the mycelium growth of both pathogens. The *S. absconditiflora* essential oil at 20 µl/petri dish. was 100 % phytotoxic to seed germination and seedling growth of *Lepidum sativum* and *Amaranthus retroflexus*. Thus *S. absconditiflora* essential oil can be used as an alternative to synthetic fungicide and herbicide to control the plant pathogenic fungi and weeds, respectively.

Keywords: Allelopathy, *Amaranthus retroflexus*, antifungal, chemical compounds, essential oils, fungi, fungicide, GCMS, herbicide, w *Lepidum sativum*, seed germination, seedling growth, weeds

INTRODUCTION

The Lamiaceae (Labiatae) family, has 236 genera and 7133 species, hence, distributed Worldwide in tropical and temperate regions (11,22). Turkey is one of the important gene centre of Lamiaceae family [45 genus, 558 species and 742 taxa are found and the endemism rate is 42.2% (5,31)]. *Salvia* genus (Lamiaceae family) has 96 species and subspecies in Turkey and the endemic rate is 51% (14,15). *Salvia* genera are important aromatic plants, rich in essential oils used for various purposes (54). These oils are rich in terpenoid compounds, flavonoids, phenolic compounds and quinoids (4,7,15,16,49). *Salvia absconditiflora* Greuter & Burdet (Synonym : *Salvia cryptantha* Montbret & Aucher ex Benth) is an endemic specie in the C group (Figure 1) (15) in rocky areas, calcareous hills and high-altitudes (700-2500 m). This genus grows in Terrestrial Central Anatolia, Turkey and is medicinal plant [antitumor (35), antioxidant and wound healing (43)] and this plant has biological activity(27,52). It is perennial plant (10 to 30 cm in height) (24,35).

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This study aimed to determine the chemical composition of essential oils from *Salvia absconditiflora* plant and studied their used in biological activity against plant pathogens and weeds.

MATERIALS AND METHODS

I. Plant Samples: *Salvia absconditiflora* plant samples (shoot + steam + leaf) were collected from 10-sites during the flowering stage in 2017-2018 Kırşehir (North: 39° 08' 27.50" East: 34°07'01.15", altitude:1088 m), Total mean rainfall : 173.4 mm, mean temperature : 14.8 °C (Table 1). The collected plants shoot, stem and leaves were dried in shade at 25°C and stored for the experiments.

Table 1. *Salvia absconditiflora* populations collected from Kırşehir province

| Site Number | Locations | Coordinates | | Altitude (m) |
|-------------|----------------------------|----------------|--------------|--------------|
| | | North | East | |
| 1 | Çiçekdağ entry | 39° 29' 8.27" | 34°19'49.3" | 1108 |
| 2 | Centry/Campus | 39° 08' 27.50" | 34°07'01.15" | 1088 |
| 3 | Village Akçakent/ Hamzabey | 39° 36' 32.01" | 34°4'37.95" | 1263 |
| 4 | Kaman exit | 39° 18' 25.97" | 33°43'2.22" | 1249 |
| 5 | Mucur exit | 39° 03' 03.73" | 34°29'29.09" | 1143 |
| 6 | Akçakent entry | 39° 32' 17.5" | 34°0'8.77" | 1021 |
| 7 | Akpınar | 39° 27' 13.02" | 33°58'32.63" | 1059 |
| 8 | Çiçekdağ exit | 39° 34' 3.24" | 34°23'7.16" | 1237 |
| 9 | Kaman-Sarıyahşi | 39° 06' 24.12" | 33°54'56.01" | 906 |
| 10 | Mucur entry | 39° 03' 50.14" | 34°20'14.84" | 1115 |



Figure 1. *Salvia absconditiflora* plant Greuter & Burdet

II. Extraction of essential oils: Essential oils of plant samples were extracted by hydro-distillation method using Clevenger-type apparatus. Distilled water was added to the weighed dry plant samples (1:10 w/v) and distilled for 3 h. The extracted essential oils were stored until the start of experiments (45).

III. GC and GC-MS analysis: GC analyses of *Salvia absconditiflora* essential oils were done by Agilent brand 7890A model GC. Oil was diluted in acetone (1:10) and injected in a BPX90 column (100m x 0.25mm x 0.25µm) separate. The carrier gas was helium at 5 psi inlet pressure. Injector and detector (FID) temperatures were 120 and 254 °C, respectively. The column temperature was programmed from 60 to 120 °C at 5°C/min with the initial and final temperatures held for 3 and 16 min. Diluted samples of 1.0 µL were injected in the split (1:5) mode. Total analysis time was 43 min. Quantitative data were obtained electronically from FID area percent data without the use of correction factors.

MS results were compared and identified with the computer mass libraries Wiley and NIST. The relative peak area percentages of compounds were calculated based on the FID data.

IV. Bioassay for herbicidal effects: In petri dishes (9 cm dia), 25-seeds of *Lepidium sativum* and *Amaranthus retroflexus* were sown equidistance with 2 layers of filter paper, moistened with 5 ml distilled water per petri dish. Essential oils were used as gaseous form vapour due to their low water solubility. The essential oils [0 (control), 0.5, 1.0, 5.0, 10 and 20 µl/petri dish] were dripped using a micropipette on drying paper glued to the covers of the petri dishes, the petri dishes were immediately closed and wrapped tightly with parafilm (34). Petri dishes were incubated [24± 1 °C and 12 h light-12 h dark] 4 weeks (26,29,39). The treatments were replicated 4-times in completely randomised Design. Germination rate, radicle and shoot lengths were determined after 28th days.

V. Bioassay for fungicidal effects: Plant pathogenic fungi *Sclerotinia sclerotiorum* and *Alternaria solani* were obtained from the stock cultures in Phyto-clinical laboratories of our Department. Young fungus cultures of these stock cultures, grown at 25 ±2 °C in 90 mm petri dishes containing 20 ml potato dextrose agar (PDA) were used in the experiment. The prepared PDAs were autoclaved and cooled to 40 °C and transferred to petri dishes (60 mm dia). Sterile filter paper (5 mm dia) was adhered to the covers of PDA transferred petri dishes. The mycelium from 7-days old fungus cultures were transferred into petri dishes. The essential oils at 0.5, 1.0, 1.5, 2.0, 5.0, 7.0, 10 and 20 µl/petri dish doses were dripped on the filter papers stuck to the covers of petri dishes using micropipettes. Fungus cultures were incubated for 7 days at 25 ± 2 °C, thereafter inoculation and fungal growth was recorded (20). The treatments were replicated 4-times in completely randomised Design. The inhibition in growth compared control was calculated as under (36).

$$I=100 \times (DC -DT)/DC$$

Where, I: Inhibition (%) compared to control (Mycelium growth), DC: Mycelium growth in control, DT: Mycelium growth in plant extract treatments.

VI. Soil Physico-chemical Properties: Soil samples collected from 0-20 cm depth were analyzed for soil texture, water saturation, PH, Organic matter, K₂O, P₂O₅, total salt and CaCO₃.

Collection of Soil Samples: Soil samples were collected and prepared for analysis as per (25). Soil samples were sieved through 2 mm sieve before laboratory analysis.

(i). **Soil Texture:** Particle size distribution of soil samples was determined according to the hydrometer method as per (10).

(ii). **Water saturation percentage:** Soil samples were saturated with pure water based on the principles as per (13).

(iii). **Soil pH:** Soil pH was determined in the saturation paste using a pH meter as reported by (47).

(iv). **Electrical Conductivity:** The electrical conductivity of soil samples was measured in the saturation extract by an electrical conductivity meter as per (48).

(v). **Total lime:** Total calcium carbonate content of soil samples was calculated by the volume of CO₂ in the Scheibler calcimeter (20).

(vi). **Organic matter:** The modified form of Walkley Black method was used as per (33).

(vii). **Available phosphorus (P):** Available P concentration of soil samples was determined by the method as per (31).

(viii). **Exchangeable potassium (K):** The potassium, which is one of the exchangeable cations, concentration of soil samples was extracted by 1N (pH 7.0) ammonium acetate as described by (22) and the extraction was analyzed by a flame spectrophotometer.

VII. Statistical Analysis of Data: Significance of the differences between the treatments was assessed by analysis of variance (ANOVA) test and the mean values were compared using the DUNCAN test. The statistical analyses were performed using SPSS (Ver.15.0, SPSS) software.

RESULTS AND DISCUSSION

Soil properties and Chemical composition

S. absconditiflora samples were collected from 10 different locations of Kırşehir, from different altitudes (Table 1). The soil of the research area was alkaline clay loam, calcareous CaCO₃ content: 3.78 to 67.45 %, soil pH : 7.58 to 8.30, Organic matter: 1.39 % to 3.71%, Potassium : 88.47 kg da⁻¹ K₂O, poor in phosphorus 4.06 kg da⁻¹ P₂O₅ (Table 2).

Table 2. Physico-chemical properties of soil samples collected from different locations of *Salvia absconditiflora* populations

| Sample Number | Locations | Saturation water (%) | pH | Total salt (%) | CaCO ₃ (%) | Organic matter (%) | K ₂ O (kgda ⁻¹) | P ₂ O ₅ (kgda ⁻¹) |
|---------------|-----------------------------|----------------------|------|----------------|-----------------------|--------------------|--|---|
| 1 | Çiçekdağ entry | 72.6 | 8.25 | 0.015 | 67.454 | 1.871 | 113.589 | 3.256 |
| 2 | Centry/Campus | 54.50 | 7.58 | 0.018 | 26.85 | 1.79 | 65.52 | 2.01 |
| 3 | Akçakent/ Hamzabey Vill. | 58.3 | 8.09 | 0.008 | 14.074 | 3.501 | 98.766 | 3.914 |
| 4 | Kaman exit | 52.8 | 7.69 | 0.009 | 3.776 | 3.712 | 55.206 | 11.553 |
| 5 | Mucur exit | 62.7 | 8.22 | 0.015 | 22.313 | 1.6 | 160.02 | 4.573 |
| 6 | Akçakent entry | 61.6 | 8.3 | 0.010 | 27.806 | 1.509 | 73.205 | 1.807 |
| 7 | Akpınar | 59.95 | 7.91 | 0.011 | 3.776 | 2.777 | 91.053 | 1.543 |
| 8 | Çiçekdağ exit | 67.1 | 8.28 | 0.014 | 31.238 | 2.656 | 125.84 | 4.046 |
| 9 | Kaman-Sarıyahşi | 42.9 | 8.16 | 0.007 | 7.037 | 1.388 | 88.028 | 2.07 |
| 10 | Mucur entry | 50.6 | 8.04 | 0.012 | 49.089 | 1.569 | 72.146 | 6.68 |

The distribution of plants even of same specie depends on the soil characteristics and ecological factors. Different soil characteristics, altitudes, and ecological factors may cause differences in morphological and chemical structures of plants. Soil pH (5.3-8.2), organic matter (2.3-7.2%), and sand (38.7-60.7%) contents of the areas where *Salvia desoleana* populations were highly variable. *Salvia* species grow on rocky slopes, alkaline soils and lime rich soils with low groundwater levels (38).

Chemical composition: In the essential oils of *S. absconditiflora* plants collected from different locations, 36-components were identified. Essential oil composition varied among populations (Table 3). Camphor (0.52-58.64%), Bicyclo [2.2.1] Heptan-2-One, 1.7.7 (21.94-30.16%) and viridiflorol (3.42-25.2%) were the main components according to GC/MS analysis. The Bicyclo (2.2.1) Heptane-2-One in *S. absconditiflora* essential oil collected from the regions 1,4,5 and 10; Viridiflorol in the region 2 and Camphor in the sampling locations of 3,6,7,8 and 9 were the main components (Table 3). Eucalyptol (27.64%), camphor (29.87%), α -pinene (11.91%) and borneol (6.57%), were identified as the primary components out of total 32 components of *S. cryptantha* essential oil collected from Tokat province (53). Identified camphor (18.1%), 1,8-cineole (eucalyptol) (17.8%) and bornyl acetate (11.4%) as the primary components of total 54 components of *S. cryptantha* essential oil (1).

Similarly, camphor (19.1%), 1,8-cineole (16.4%), borneol (11.9%), viridiflorol (11.5%) in naturally distributed *S. cryptantha* essential oil in Kayseri (2) and Valence (31,80%), 1,8-cineole (17.43%), camphor (13.73%) and Isoberneol (10.79%) in *S. cryptantha* essential oil collected from Konya were identified as the primary components (24). The predominant compounds of *Salvia cryptantha* essential oil were determined as 1,8-cineole (21%), camphor (19.1%), α -pinene (12.5%), camphene (8.7%) (15). The identified 13 components in the essential oil of above-ground parts of *S. cryptantha* collected from the Ereğli-Ivriz region and 10 components in the flower (6). In the same study, Valencene (24.34-26.53%), 1,8-Cineole (30.38-36.28%) and camphor (12.29-14.72%) were reported as the main components. Viridiflorol (21.4%) was also reported in the essential oil of *Salvia crypthanta* (28). The vegetative conditions (flowering time, harvesting time, post-harvest processes and drying conditions), analysis methods and geographic, climatic and environmental factors of plants grown resulted in the differences for the essential oil compositions obtained in the present study and as well as in other studies (3,32,41,42). The essential oil compositions of plants are also closely related to soil properties (pH, organic matter, salinity, etc.) where plants are grown (38). In this study, the soil properties of the sampling locations where *S. absconditiflora* populations were distributed significantly varied (Table 2) and therefore differences in essential oil compositions were observed.

Allelopathic potential

The seed germination, root and shoot growth of *Amaranthus retroflexus* L. and *Lepidium sativum* L. plants were adversely affected by *S. absconditiflora* essential oil. The effects differed depending on the dose of essential oil and the test plant, and the negative effect increased with the increased dose. The increase in *S. absconditiflora* essential oil dose decreased the seeds germination and seedlings root and shoot growth of *Lepidium sativum* (cress) than control.

Table 3. Chemicals Constituents (%) identified by GCMS in essential oil of *Salvia absconditiflora*

| No. | RT* | RRI** | Compounds | Site/Sample Number | | | | | | | | | |
|--------------|--------|-------|--|--------------------|--------------|--------------|--------------|--------------|--------------|---------------|--------------|--------------|--------------|
| | | | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| 1 | 12.510 | 1387 | β -caryophyllene | 1.6 | - | - | - | - | - | 6.92 | 0.17 | - | - |
| 2 | 12.546 | 1394 | Valencene | 0.27 | - | - | - | - | - | - | 2.72 | 1.86 | - |
| 3 | 13.642 | 1405 | Naphthalene | - | - | 9.5 | 0.24 | 1.31 | - | 6.82 | 0.23 | 3.96 | - |
| 4 | 12.956 | 1417 | Muurolene < Gamma-> | - | 6.31 | - | 12.93 | 3.37 | 7.22 | - | 4.03 | - | 2.57 |
| 5 | 13.529 | 1427 | Bisabolene | 8.25 | - | - | - | 16 | - | - | - | - | 10.49 |
| 6 | 13.540 | 1428 | Gurjunene<Beta-> | - | 16.94 | - | - | - | - | - | - | - | - |
| 7 | 14.765 | 1429 | Camphor | 20.71 | 10.52 | 58.64 | 16.9 | 16.34 | 53.18 | 37.91 | 55.64 | 47.33 | 12.44 |
| 8 | 14.971 | 1433 | Bicyclo[2.2.1]Heptan-2-One, 1,7,7 | 29.06 | 21.94 | - | 28.12 | 30.16 | - | - | - | - | 24.45 |
| 9 | 15.966 | 1453 | Myrtenol | - | - | - | - | - | 5.44 | - | 2.66 | 4.06 | 0.53 |
| 10 | 17.168 | 1475 | n-icosane | - | - | - | - | - | - | - | - | - | 4.13 |
| 11 | 17.265 | 1478 | Sesquisabinene hydrate | - | - | 0.33 | 0.5 | - | 0.28 | - | - | - | - |
| 12 | 17.603 | 1482 | Ledol | - | 0.54 | 1.5 | 3.16 | 3.56 | - | - | - | - | 1.24 |
| 13 | 17.609 | 1484 | Cubebol | 0.52 | - | - | - | 0.3 | - | - | - | - | - |
| 14 | 18.799 | 1508 | Germacrene D | 1.64 | - | 0.65 | 0.41 | - | - | - | 1.07 | - | - |
| 15 | 19.417 | 1520 | Viridiflorol | 13.85 | 25.2 | 10.54 | 21.02 | 10.67 | 15.71 | 23.24 | 3.42 | 19.83 | 16.73 |
| 16 | 20.052 | 1532 | Bicyclo[4.4.0]dec-1-ene | - | 0.36 | - | - | - | - | - | - | - | 0.78 |
| 17 | 20.092 | 1534 | Longifolene | 0.92 | - | - | - | - | - | - | - | 1.6 | 1.01 |
| 18 | 20.315 | 1538 | Alloaromadendrene oxide | - | - | 1.57 | 0.32 | - | - | - | - | - | - |
| 19 | 20.555 | 1542 | Spathulenol | 5.02 | 1.92 | - | 0.48 | - | 0.75 | 0.97 | - | - | 3.48 |
| 20 | 20.590 | 1544 | Caryophyllene oxide | - | - | 2.39 | 2.1 | 2.62 | 3.47 | 7.74 | 5.59 | 3.29 | - |
| 21 | 21.253 | 1559 | Cadinol<Alpha-> | - | 1.11 | - | 1.45 | 0.72 | 0.72 | 0.31 | 1.36 | 0.47 | - |
| 22 | 21.425 | 1560 | Humulene epoxide II | - | - | 3.43 | - | - | - | - | - | - | 1.16 |
| 23 | 21.849 | 1568 | Azulene | - | - | 1.38 | - | - | - | - | - | - | 0.39 |
| 24 | 21.894 | 1572 | Eudesmol<Alpha-> | 1.96 | 1.82 | - | 1.43 | 1.64 | 1.43 | 1.27 | 2.36 | 1.11 | 1.35 |
| 25 | 22.117 | 1576 | 7-Methoxy-2-Methyl quinoline - 5,8-Dione | 0.3 | 0.34 | - | - | 0.51 | - | - | - | - | - |
| 26 | 22.264 | 1580 | Eudesmol<Beta-> | 6.66 | 4.83 | 3.71 | 3.65 | 3.92 | 3.18 | 4 | 6.97 | 2.83 | 3.58 |
| 27 | 22.758 | 1588 | Valeranone | 4.79 | 3.58 | 2.52 | 2.57 | 4.17 | 2.83 | 3.43 | 3.13 | 5.2 | 6.58 |
| 28 | 23.245 | 1598 | 1H-Cycloprop[A] Naphthalen -6-Ol | 0.48 | 0.34 | 0.66 | 0.35 | - | 0.45 | 1.3 | 1.67 | 0.86 | - |
| 29 | 23.479 | 1602 | Valerenol | 0.51 | 0.48 | 1.51 | 1.12 | 0.53 | 2.2 | - | 2.79 | - | - |
| 30 | 24.486 | 1621 | Ledene oxide-(II) | - | - | 0.89 | 0.95 | 0.32 | 1.31 | - | 1.56 | 1.06 | - |
| 31 | 24.488 | 1642 | Vulgarol A | - | - | 0.2 | 0.2 | 0.18 | 0.24 | 0.21 | 0.28 | 0.2 | 0.16 |
| 32 | 26.569 | 1663 | Manool | 1.6 | 1.97 | - | - | 0.22 | - | 0.12 | - | 1.55 | 2.72 |
| 33 | 27.159 | 1687 | 1-Naphthalenol | - | 0.22 | - | - | - | - | - | - | 0.29 | 0.26 |
| 34 | 27.937 | 1689 | 1,2-Benzenedicarboxylic acid | - | - | 0.1 | 0.11 | 0.15 | 0 | 0.2 | 0.11 | - | - |
| 35 | 30.780 | 1746 | 9H-Benzimidazol [1,2-d][1,2,3]triazol | - | 0.19 | - | - | 0.15 | - | - | - | - | 0.15 |
| 36 | 35.003 | 1826 | Abietal | - | - | - | 0.27 | - | 0.18 | - | 0.21 | 0.23 | - |
| Total | | | | 98.14 | 98.61 | 99.52 | 98.28 | 96.84 | 98.59 | 94.436 | 97.57 | 95.94 | 93.19 |

*RT: Retention times **RRI: Relative Retention Indices

The essential oil at 10 μ l/petri dish decreased the germination of cress seeds by 80% than control, while germination was completely inhibited at 20 μ l/petri dish dose (Figure 3, Figure 5). The growth of seedlings of cress sprout was decreased by 84.44 % at 10 μ l/petri dish essential oil dose, while 20 μ l/petri dose completely inhibited the growth. At 10 μ l/petri dose, the root growth was inhibited drastically by 97.88 %, whereas, the root growth was completely inhibited at 20 μ l/petri dose (Figure 4, Figure 5). *S. absconditiflora* essential oil was phytotoxic to seeds germination of cress but the inhibitory effect was lower than *A. retroflexus*.

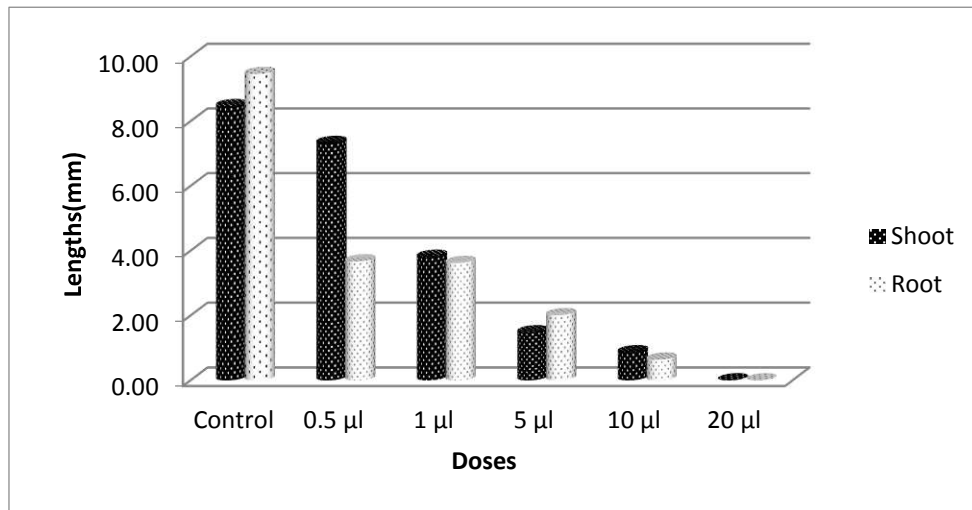


Figure 2. Effects of *Salvia absconditiflora* essential oil on root and shoot lengths of *Amaranthus retroflexus*

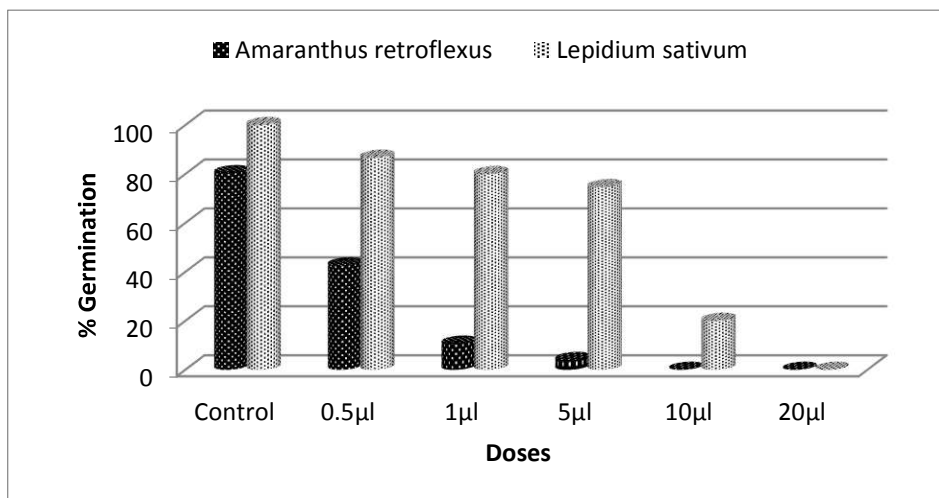


Figure 3. Effects of *Salvia absconditiflora* essential oil on germination % of the test plants

The *S. absconditiflora* essential oil decreased the root and shoot growth of *A. retroflexus* at higher doses than control. Its essential oil was very phytotoxic to seed germination of *A. retroflexus*. The essential oil at 5 μl /petri dose inhibited the seed germination of *A. retroflexus* by 53.10 % than control treatment and the seed germination completely inhibited at 20 μl /petri dose (Figure 3, Figure 5). The 10 μl /petri dose of essential oil drastically inhibited (89.73 %) the shoot growth of *A. retroflexus* than control. While 20 μl /petri dose completely inhibited the shoot growth. Similarly, the 10 μl /petri dose drastically inhibited (92.33 %) the root growth, while root growth was completely inhibited at 20 μl /petri dose (Figure 2, Figure 5).

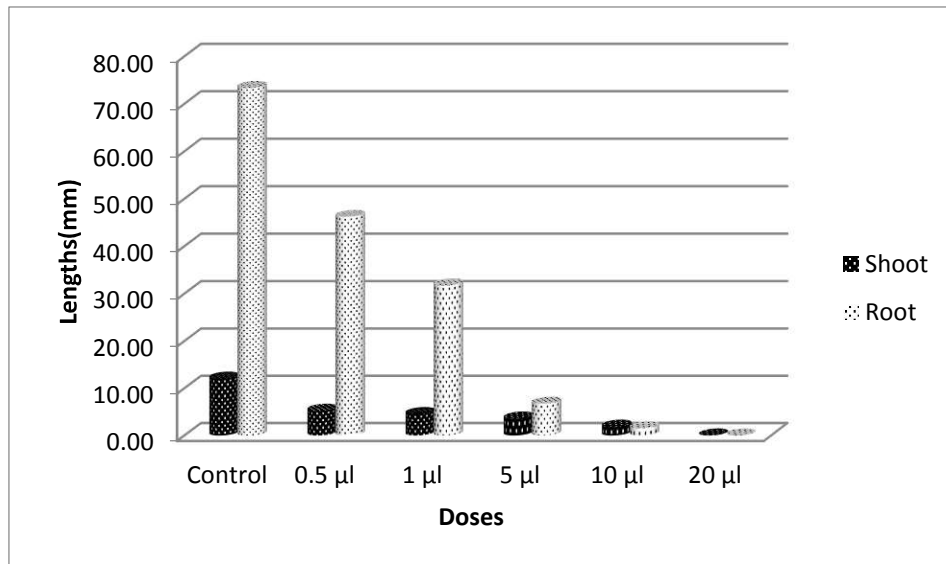


Figure 4. Effects of *Salvia absconditiflora* essential oil on root and shoot lengths of *Lepidium sativum*

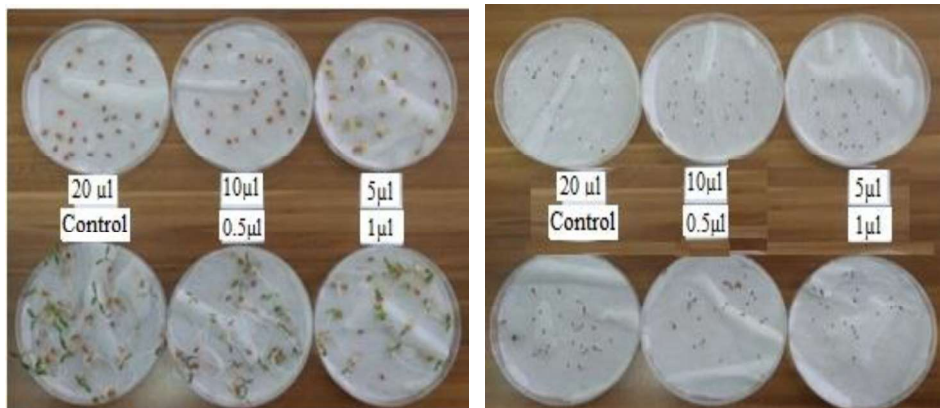


Figure 5. Phytotoxic effect of *S. absconditiflora* essential oil on germination of *Amaranthus retroflexus* and *Lepidium sativum* plants (7th day).

The phytotoxic effects of above-ground exudates of *Salvia namaensis* Schinz, *S. fallax* Fernald, *S. disermas* L., *S. chamaedryoides* Cav., *S. confertiflora* Pohl., *S. x jamensis* J. Compton, *S. burchanani* Hedge, *S. wagneriana* Polak, *S. scabra* Linn.fil., *S. miniata* Fernald, *S. cacaliaefolia* Benth., *S. adenophora* Fernald, *S. rutilans* Carriere species on *Papaver rhoeas* L. and *Avena sativa* L. have been reported (8). Similarly, it indicated the inhibitory effects of *Salvia officinalis* essential oil on seed germination and root growth of *Lepidium sativum* L.(cress) (9). The methanol extracts obtained from *Salvia macrochlamys* Boiss. et Kotschy aboveground inhibited the germination of *Portulaca oleracea* seeds (18). In this study, antifungal and allelopathic effects of *S. absconditiflora* essential oil have been demonstrated. Allelopathic potential of dried leaves of *Salvia officinalis* L. reported on *Lycopersicon esculentum* Mill. (tomato), *Panicum maximum* Jacq. (guinea grass) and *Salvia hispanica* L. (chia) in greenhouse condition (12). In another study, Allelopathic effect of *Salvia plebia* R. Brown are reported on growth and germination of *Zea mays* var. 30-25 Hybrid, *Triticum aestivum* var. Pirsabak-04 and *Sorghum bicolor* L. plant (23).

Antifungal potential

The efficacy of *S. absconditiflora* essential oil on plant pathogens *Sclerotinia sclerotiorum* and *Alternaria solani* fungi was investigated *in-vitro*. The *S. cryptantha* essential oil has slight phytotoxic effects on the mycelium growth of *S. sclerotiorum*. The mycelium growth was inhibited by 9.3 % with 10 μ l/petri dish dose, whereas the mycelium growth was completely inhibited with 20 μ l/petri dish dose. The essential oil had significantly influenced the sclerot formation of the *S. sclerotiorum* pathogen. Accordingly, sclerot formation of the pathogen was completely inhibited at 7 μ l/petri dose (Table 4, Figure 6, Figure 7).

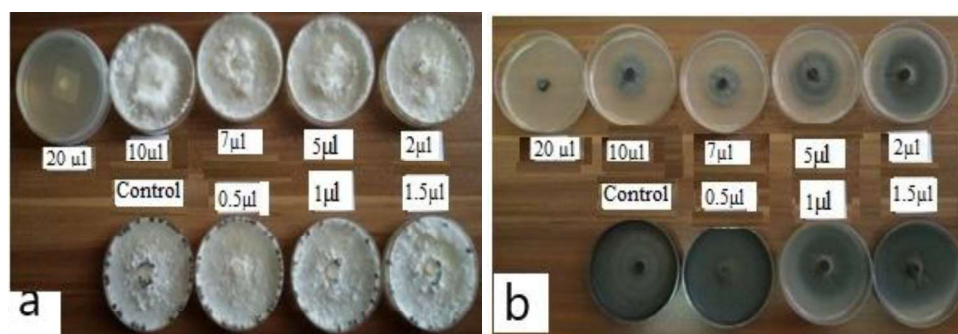


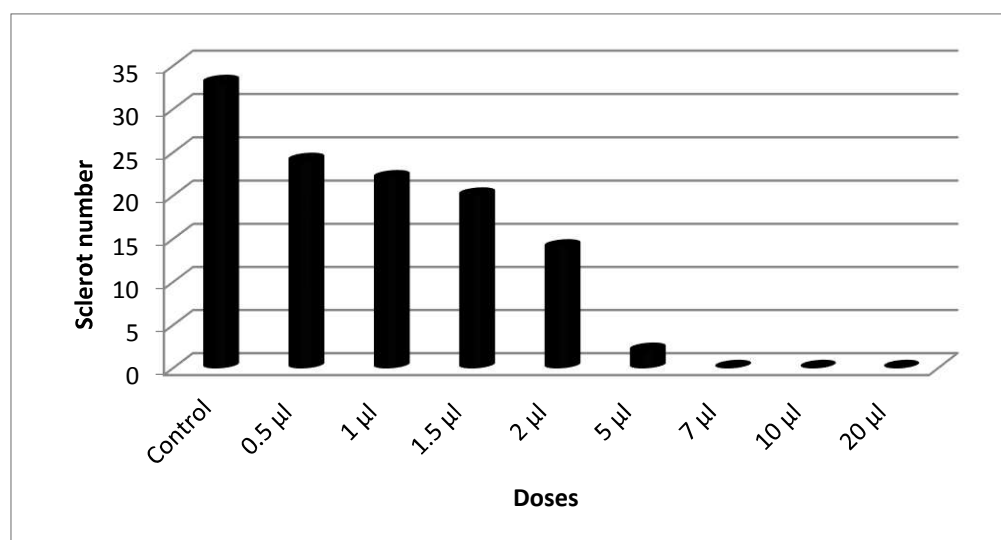
Figure 6. Effects of *Salvia absconditiflora* essential oil on mycelium growth of *S. sclerotiorum*(a) and *A. solani*(b) pathogens (7th day)

In contrast to the *S. sclerotiorum* pathogen, *Salvia absconditiflora* essential oil showed higher antifungal effects against the *Alternaria solani* pathogen. The effects of essential oil increased in relation to the increase in application dose of essential oil. *Salvia absconditiflora* essential oil inhibited the *A. solani* mycelium growth by 54.40v% with at 10 μ l/petri dose compared to control and *A. solani* mycelium growth was completely inhibited at 20 μ l/petri dose (Table 4, Figure 6).

Table 4. Effects of *Salvia absconditiflora* essential oil on mycelium growth of *S. sclerotiorum* and *A. solani* pathogens

| Doses(μ l) | Control | 0.5 | 1 | 1.5 | 2 | 5 | 7 | 10 | 20 |
|------------------------|-----------------------------------|----------------------------------|----------------------------------|-----------------------------------|-----------------------------------|----------------------------------|----------------------------------|----------------------------------|---------------------------------|
| <i>S. sclerotiorum</i> | 60.00 \pm 0.00 ^{a*} | 60.00 \pm 0.00 ^a | 60.00 \pm 0.00 ^a | 60.00 \pm 0.00 ^a | 60.00 \pm 0.00 ^a | 60.00 \pm 0.00 ^a | 60.00 \pm 0.00 ^a | 54.42 \pm 0.08 ^a | 0.00 \pm 0.00 ^b |
| <i>A. solani</i> | 60.00 \pm 0.00 ^a | 60.00 \pm 0.00 ^a | 56.90 \pm 3.09 ^a | 54.74 \pm 2.46 ^{ab} | 47.98 \pm 0.75 ^{bc} | 41.89 \pm 2.75 ^c | 34.02 \pm 4.36 ^d | 27.36 \pm 0.27 ^d | 0.00 \pm 0.00 ^e |

* Means in the same column with the same letter were not significantly different by ANOVA (a = 0.05)

Figure 7. Effects of *Salvia absconditiflora* essential oil on sclerot formation (number) of *S. sclerotiorum*

The results demonstrated that *Salvia absconditiflora* has antifungal and allelopathic activity. Biological activity of *Salvia absconditiflora* essential oil and extracts on different insects, pathogens and test plants have also been reported in other studies. Plant extracts of *S. absconditiflora* were effective on gram-positive bacteria while ineffective on gram-negative bacteria and *Candida albicans* (50). The *S. absconditiflora* essential oil and methanol extract have antimicrobial and antioxidant activities (46). The *S. absconditiflora* essential oil showed insecticidal activity on two important pests, *Sitophilus granarius* L. and *S. oryzae* L. (27). The biological activity of *Salvia* species has been revealed in many studies. Similar results were found in antifungal studies with other species of *Salvia*. *Salvia officinalis* essential oil has antifungal effects on *Botrytis cinerea* and *Fusarium* sp. (51,52). Previous investigations on other *Salvia* spp. reported the antifungal activity of *S. somalensis* and *S. dolomitica* essential oils against *Aspergillus niger*, *Aspergillus flavus*, *Candida albicans* and others(17). In another study, extracts of *Salvia rhytidea* has been demonstrated the antifungal activity against different *Candida* isolates (40). Essential oils of *Salvia macrochlamys* and *Salvia recognita* were non-selective in inhibiting the growth and development of reproductive stroma of the plant pathogens *Colletotrichum fragariae*, *Colletotrichum acutatum* and *Colletotrichum gloeosporioides* (44).

CONCLUSIONS

The study showed that the essential oil compositions in *Salvia absconditiflora* populations vary depending on the location and soil characteristics. *S. absconditiflora* plant showed antifungal effects on *S. sclerotiorum* and *A. solani* pathogens and allelopathic effects on *Amaranthus retroflexus* and *Lepidum sativum* plants. This and similar studies will help to develop natural pesticides with the potential to replace synthetic pesticides.

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