Pak. J. Bot., 52(4): 1421-1425, 2020. DOI: http://dx.doi.org/10.30848/PJB2020-4(14)

ECOLOGICAL AND BIOCHEMICAL STUDIES ON *ARTEMISIA ABSINTHIUM* IN AL-BAHA CITY, SAUDI ARABIA

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Abstract

Artemisia absinthium is a known economic plant due to its high essential oil content. Besides playing a vital role in protecting plants, essential oils have several important uses in medicine, chemical and food-processing industries, and cosmetics. This study investigates the biochemical characteristics of *A. absinthium* and the soil where it thrived well. Soil and plant samples were collected from different sites at Al-Baha City, Saudi Arabia. Soil samples were analyzed for their physicochemical properties. Gas chromatography-mass spectrometry (GC-MS) was utilized to identify the most abundant secondary metabolites in the water extract of the *A. absinthium* shoot. Then the extract was tested for its bioactivity on seed germination and seedling growth of *Cucumis sativus*. The results showed that the pH of soils where *A. absinthium* thrived was 7.88 (moderate alkalinity). Additionally, the soils had high organic matter content (approximately 48%). The soil consisted mainly of nitrogen (45.7% of the total soil minerals); potassium and calcium levels were recorded at around 190 mg/kg and 125 mg/kg of soil, respectively. Approximately 6.9% of the total soil minerals recorded consisted of magnesium. The phosphorus level was the lowest (11.04 mg/kg of soil). GC-MS analysis of *A. absinthium* water extract revealed a total of 23 components, with the main ones including davanone (58.5%) and (-)-camphor (16.0%). Although *A. absinthium* is rich in secondary compounds, its water extracts did not significantly influence the germination or growth of *C. sativus* seedlings.

Key words: *Artemisia absinthium*, Soil properties, Gas chromatography-mass spectrometry analyses, Bioactivity, *Cucumis sativus*.

Introduction

The genus *Artemisia* belongs to the family *Asteraceae* (*Compositae*), and it is composed of 200 known species. These species typically thrive in arid regions, and they are invariably found as small fragrant shrubs or herbs (Qureshi and Arshad, 2002; Bertea CM, Freije *et al.,* 2005; Verdian-Rizi, 2008). According to Sharopov *et al.,* (2012), *Artemisia absinthium* L. (Asteraceae) has been extensively studied. It has been widely used for its medicinal properties in Europe, the Middle East, North Africa, and Asia (Ibrar *et al.,* 2007; Ebrahimzadeh *et al.,* 2010; Arnat *et al.,* 2010; Šaric-Kundalic *et al.,* 2010). It grows naturally on uncultivated, arid ground, rocky slopes and wastelands, and dry soil (Anon., 2009). They are also used to produce aperitifs, bitters, and spirits (Mishra *et al.,* 1999). Most of these plants yield essential oils, some of which are used in the cosmetic and pharmaceutical industries. The leaves of some species, on the other hand, are used as culinary herbs (Qureshi and Arshad, 2002; Bertea CM, Freije *et al.,* 2005; Verdian-Rizi, 2008).

Essential oils have several applications in the medical, pharmaceutical, cosmetic, and chemical and food-processing industries (Yentema *et al.,* 2007). They naturally have a vital role in protecting plants owing to their antibacterial, antiviral, antifungal, and insecticidal properties. They are also reported to attract certain insects, subsequently, facilitating the dissipation of pollens and seeds or repelling other unwanted insects (Abad *et al.,* 2012). In medicine, essential oils have been reported to be a potential source of anticancer compounds and to have antioxidant activity, which is helpful for scavenging free radicals (Hussain *et al.,* 2008; Shabbir *et al.,* 2009). Several studies have described the composition of essential oils and the biological activities of various species of *Artemisia* (Setzer *et al.,* 2004; Cha *et al.,* 2005). Furthermore, some authors suggested that *Artemisia* was potentially anticarcinogenic and had several health-promoting benefits owing to their antioxidant properties (Liu *et al.,* 2008).

Several substances, including allelochemicals, have been identified in essential oils (Dudai *et al.,* 1999). A plant can be affected either directly or indirectly and positively or negatively by other plants growing in the vicinity through the release of allelochemicals into the environment, also known as allelopathy (Rice, 1984). According to Inderjit and Duke (2003), most allelochemicals are secondary metabolites of plants. The amount of secondary metabolites in the plant is determined by the plant's genetic and environmental conditions during its growth. These substances have been found to alter the growth and development of plants (Chou, 2006). Inderjit & Duke (2003) stated that the most frequently reported allelopathic effects happen early in the life cycle of the plant, and it influences seed germination, coleoptile elongation, as well as shoot and root development.

The primary objective of this study is to investigate the properties of the soil in which *A. absinthium* thrives. Secondary objectives are to identify the allelochemicals in *A. absinthium* extract using gas chromatography-mass spectrometry (GC-MS) and to assess the effect of this extract on *Cucumis sativus* seed germination and seedling growth.

Materials and Methods

Sample collection: The leaves of *Artemisia absinthium* were collected from farm sites at Al-Baha, Saudi Arabia, in 2018. The recorded temperature was between 25.5–

30ºC, and the annual rainfall was about 10 mm in 2018. The average relative humidity was recorded at 63–99%. Meanwhile, the average elevation above sea level was estimated at 1014.9 m. Three measurements were taken from each of the sites.

Soil pH: Approximately 10 mL of distilled water was added to 10 g of soil, and the mixture was stirred and set to stand for 10 minutes. Next, it was filtered, and the pH of the solution was determined (Conklin, 2005).

Soil organic matter: Approximately 5 g of soil was oven-dried at 110°C for two hours and allowed to cool in a desiccator. The soil sample was weighed and placed in a furnace at 500°C for three hours. It was then left to cool overnight and then placed again in a desiccator prior to weighing (Wilde *et al.*, 1972).

Soil moisture: A one hundred gram sample of soil was collected and dried in an oven at 105°C for 24 hours. The sample was weighed and dried in the oven again, and the process was repeated until no further change in weight loss was documented (Yousef, 1999; Conklin, 2005).

Soil minerals: Macro- and microelements were analyzed using an atomic absorption flame emission spectrophotometer (Allen, 1974).

GC-MS: The oil samples were analyzed using a TRACE GC Ultra Gas Chromatograph (THERMO Scientific Corp., United States) and a THERMO mass spectrometer detector (ISQ™ Single Quadrupole Mass Spectrometer) at the Laboratory of Medicinal and Aromatic Plants, National Research Center, Egypt. The GC-MS system had a TR-5MS column (30 m x 0.32 mm, 0.25 µm film thickness). Helium was utilized as the carrier gas at a flow rate of 1.3 mL/minute and split ratio of 1:10 at different temperatures during the process: 80°C for one minute, then progressively increasing the temperature at a rate of 4°C/minute to 300°C, and holding it for one minute. The injector was maintained at a temperature of 220°C, whereas that of the detector was kept at 200°C. A mixture composed of 1 µL sample and hexane at volume ratio of 1:10 was also injected. Electron ionization was performed at 70 eV using a spectral range of m/z 40–450.

Qualitative and quantitative experiments: Two analytical methods were used to identify most of the compounds: (a) KI, Kovats indices for n-alkanes (C9–C22) and (b) mass spectra (authentic chemicals, Wiley spectral library collection, and National Institute of Standards and Technology Library). The compounds in the oil were isolated and then ascertained by matching the mass spectra of samples against the data in the National Institute of Standards and Technology Library.

Effects of *A. absinthium* extract on *C. sativus*

Aqueous extracts: Approximately 100 g of ground dried tissue from *A. absinthium* was soaked separately in 100 mL distilled water in sterile flasks at room temperature. Thereafter, it was centrifuged at 1500 rpm for 30 minutes; the supernatant was filtered through one layer of filter paper (Whatman no. 1). After filtration, these stock solutions were stored at 4°C. For bioassay, stock extracts (100%) were diluted with distilled water to give final concentrations of 10, 15, 25, and 50%.

Bioassay: Viable seeds of *C. sativus* were thoroughly washed with tap water and surface-sterilized with 95% ethyl alcohol for 10 minutes before being rinsed with sterilized distilled water five times. Ten seeds of *C. sativus* were placed in sterile 9 cm Petri dishes that contained two layers of Whatman No. 1 filter paper, and 5 mL of aqueous extracts were sprinkled on them. The Petri dishes were kept in the dark at 28°C for one week until germination. The emergence of 2 mm radical was used as the criterion for germination. The treatments were randomly replicated three times. After seed germination, seedlings were grown for 15 days under 16-hour-light photoperiod at 150 Imol s^{-1} m^{-2} using cool white fluorescent lights at 26/22°C day/night temperatures. At the end of the incubation period, the radical and plumule length of *C. sativus* was measured in three randomly picked seedlings. Seedlings were dried in an oven at 60°C for 24 hours and weighed.

Statistical analysis: The statistical package Statistix 7.0 was used to analyze the data. The Kruskal-Wallis test was used to compare continuous variables. An $\alpha = 0.05$ was used for all statistics.

Results and Discussion

Soil physicochemical characteristics: The pH value of soil where *A. absinthium* thrived was moderately alkaline. Most plants thrive well in soils with pH values between 6.0 or 6.5 to 7.4 (Anon., 2011) (Table 1). The pH value influences the electrical conductivity of soil. The more acidic or basic the soil is, the higher the electrical conductivity. This helps to determine the soil quality (Smith & Doran, 1996).

Table 1. Physiochemical properties (mean ± standard error) of soil where *Artemisia absinthium* thrived.

Physicochemical analysis		Mean (± Standard error)
Soil pH value		7.88 ± 0.04
Soil organic matter (%)		48.32 ± 0.36
Soil moisture (%)		48.23 ± 0.13
Electrical conductivity (uS/cm)		333.67 ± 2.18
Soil type	Coarse sand (%)	36.53 ± 0.87[a]
	Fine sand (%)	19.64 ± 0.51[a]
	Silt (%)	35.15 ± 0.55[a]
	Clay (%)	8.68 ± 0.90

[a] Significant using a paired t-test ($\alpha = 0.05$)

Table 2. Mean (± standard error) of elements analysis in the soil where *Artemisia absinthium* thrives.

Soil minerals	Content (mg/kg)
Nitrogen	313.23 ± 8.75[a]
Phosphorus	11.04 ± 0.52
Potassium	188.47 ± 0.33[a]
Calcium	125.77 ± 0.82[a]
Magnesium	47.3 ± 0.81[a]

[a] Significant using a paired t-test ($\alpha = 0.05$)

Table 3. Gas chromatography-mass spectrometry analysis of *Artemisia absinthium* plant extracts.

No.	Compounds	RT	% Area
1.	β-myrcene	7.81	0.01
2.	cis-β-terpineol	9.2	0.26
3.	Linalool oxide	9.79	0.05
4.	L-linalool	10.28	2.75
5.	Cis-sabinene hydrate	11.32	0.11
6.	(-)-Camphor	12.35	15.99
7.	(+)-Isomenthone	12.66	0.21
8.	p-menthone	13.03	0.08
9.	Isoborneol	13.32	0.53
10.	Terpinene-4-ol	13.63	5.39
11.	α-terpineol	14.33	0.47
12.	Bornyl acetate	17.92	2.4
13.	Myrtenyl acetate	19.06	0.17
14.	trans-Caryophyllene	23.38	0.18
15.	Isopinocarveol	24.45	0.29
16.	(E)-Ethyl cinnamate	26.68	0.11
17.	Butylated hydroxytoluene	26.91	0.82
18.	Davana ether	27.79	0.18
19.	(±)-trans-Nerolidol	29.26	4.01
20.	Davanone	30	58.48
21.	6-epi-shyobunol	32.42	0.09
22.	β-Eudesmol	32.9	0.13
23.	Chamazulene	35.69	5.04
	Total		**97.8**

Table 4. Effect of *Artemisia absinthium* plant extracts on the germination and growth of *Cucumis sativus* plumule and radical.

Treatment	Number of seeds germinated	Plumule length (mm)	Radical length (mm)
Control	8.67 ± 0.33^{a}	14.53 ± 0.09^{a}	5.00 ± 0.29^{a}
10 %	2.67 ± 0.67^{a}	9.83 ± 0.73^{ab}	4.17 ± 0.44^{a}
15 %	3.33 ± 0.33^{a}	3.23 ± 0.50^{b}	2.80 ± 0.17
25 %	3.00 ± 0.58^{a}	3.77 ± 0.43^{ab}	5.27 ± 0.43^{a}
50 %	5.33 ± 0.33^{a}	5.23 ± 0.49^{ab}	6.50 ± 0.32^{a}

[a] Significant using a paired t-test ($\alpha = 0.05$)

In this study, soil organic matter was recorded at around 48% of total soil analyzed. Soil organic matter is considered essential for soil function and quality (Beare *et al.,* 1994). According to Fenton *et al.,* (2008), high organic matter content in soil contributes to soil productivity in several ways. Soil organic matter influences nutrient turnover and cation exchange, suppresses plant disease, and affects soil water retention and soil system fertility (Willemse, 2016).

Soil element analysis: The tested soil sample consisted mainly of nitrogen (45.7%) (Table 2). Potassium and calcium levels were recorded at around 190 mg/kg and 125 mg/kg of soil, respectively, and approximately 6.9% of the total soil minerals recorded consisted of magnesium. Phosphorus levels were the lowest recorded (11.04 mg/kg of soil).

Biomass of *A. absinthium*: The dry shoot weight was 0.96 ± 0.01 g, with 0.60 ± 0.01% consisting of organic matter, while the dry root weight was 1.65 ± 0.02 g, with 0.75 ± 0.02% of the total weight consisting of organic matter.

GC-MS analysis of *A. absinthium* plant extract: A total of 23 components were found, with the main components being davanone (58.5%) and (-)-camphor (16.0%) (Table 3). Other main components that were identified include terpinene-4-ol (5.4%), chamazulene (5.0%), (±)-trans-nerolidol (4.0%), l-linalool (2.8%), and bornyl acetate (2.4%). According to Abad *et al.,* (2012), the harvesting season affects the quality and yield of essential oils obtained from *Artemisia* plants. Other factors that affect the quality and yield of the plant include the fertilizer used, soil pH, process and conditions for drying, geographical area, chemotype or subspecies, plant part or genotype selected, and technique used to extract the oils.

In a study investigating the composition of the essential oil of wildly growing *A. absinthium* thriving in the north of Iran, Rezaeinodehl and Khangholi (2008) identified 28 compounds, representing 93.3% of the oil. Additionally, they reported that most of the compounds identified were monoterpenes. The major compounds included β-pinene (23.8%), *trans*-anethole (21.1%), and *trans*-α-ocimene (20.6%). Other major compounds identified in the oil included limonene (12.4%), α-pinene (5.1%), *cis*-β-ocimene (4.8%), allylveratrol (2.2%), β-pinene (0.8%), and α-terpinolene, bornyl acetate and bicyclogermacrene (0.5% each). Our results are consistent with those of other investigators who reported that > 10% of the components of *A. absinthium* (grown in different areas, including Turkey, Canada, and Iran) consisted of essential oils (Kordali *et al.,* 2005; López-Lutz *et al.,* 2008; Nibret & Wink, 2010).

Some species of *Artemisia* have been reported to have a strong aromatic smell, which is primarily due to high concentrations of volatile terpenes, particularly in the leaves and flowers of the plants (Abad *et al.,* 2012). In several studies conducted worldwide, investigators demonstrated considerable variation in the amounts of terpene compounds in the essential oils extracted from different strains of *Artemisia*. Further investigations suggested that the variations observed in the volatile components of *Artemisia* probably occur during plant development (Abad *et al.,* 2012).

In general, essential oils have potent bioactivity due to the presence of various secondary metabolites, which have different mechanisms of action (Abad *et al.,* 2012). These oils contain several volatile molecules, including terpenes, phenolic-derived aromatics, and aliphatic compounds, which vary depending on the extraction technique used, especially when distillation is performed on aromatic plants (Abad *et al.,* 2012). At least nine different chemotypes have been identified based on the composition of essential oils extracted from *A. absinthium* (Orav *et al.,* 2006). These chemotypes are characterized by two or three principal components that are present in higher amounts (20–70%) and other components present in very small quantities (Bakkali *et al.,* 2008). The principal components endow essential oils with their biological properties and comprise two groups with

distinct biosynthetic origins: the principal group comprises terpenes while the other comprises aromatic and aliphatic compounds, which all have a low molecular weight (Abad *et al.*, 2012).

Effect of *A. absinthium* extracts on the germination and growth of *C. sativus* plumule and radical: The treatment of *C. sativus* seeds with *A. absinthium* extracts showed that the germination rate was not significantly different from untreated seeds (Table 4**)**. Plumule lengths were significantly different between untreated seeds and seeds treated with 15% concentration of *A. absinthium* extract.

Biomass of *C. sativus* treated with *A. absinthium* extracts: The extracts of *A. absinthium* did not inhibit the growth of *C. sativus* seedlings. No differences in dry shoot or root weight was recorded (Table 5). Moreover, the organic matter content was not significantly different between treated and untreated seedlings.

Conclusion

Overall, *A. absinthium* thrived in moderately alkaline soil (pH = 7.88) that contained a high organic matter content. Additionally, the soil consisted mainly of nitrogen, potassium, and calcium levels. Approximately 6.9% of the total soil minerals consisted of magnesium. The phosphorus level was the lowest (11.04 mg/kg of soil). Twenty-three different chemical compounds were identified in *A. absinthium* water extract using GC-MS, with davanone (58.5%) and (-)-camphor (16.0%) being the main components identified. Seed germination or seedling growth of *C. sativus* was not significantly influenced by *A. absinthium* water extracts.

Acknowledgment

We thank King Abdul Aziz University (Jeddah, Saudi Arabia) for supplying the equipment and support on other aspects of the work.

Table 5. Biomass of *Cucumis sativus* (mean ± standard error) plants untreated and treated with different concentrations of *Artemisia absinthium* extracts.

Conditions	Parameters			
	Dry shoot weight (g)	Dry shoot organic matter (%)	Dry root weight (g)	Dry root organic matter (%)
Control	0.097 ± 0.00[a]	0.067 ± 0.009[a]	0.02 ± 0.006[a]	0.017 ± 0.003[a]
10 %	0.097 ± 0.02[a]	0.043 ± 0.003[a]	0.001 ± 0.0003[a]	0.030 ± 0.012[a]
15 %	0.04 ± 0.008[a]	0.027 ± 0.003[a]	0.02 ± 0.0058[a]	0.037 ± 0.012[a]
25 %	0.09 ± 0.006[a]	0.077 ± 0.009[a]	0.02 ± 0.0068[a]	0.033 ± 0.003[a]
50 %	0.08 ± 0.006[a]	0.053 ± 0.003[a]	0.01 ±0.0037[a]	0.017 ± 0.003[a]

[a] Significant using a paired t-test ($\alpha = 0.05$)

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(Received for publication 15 December 2018)