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Volatile oils content of some species of *Artemisia* growing under different environmental conditions and its effect on germination of seeds of some plants

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***Abstract*:** *The essential oils from the aerial parts of Artemisia herba alba and A. Judica growing wild in Tabuk region at North of Saudi and Saint Catherine analyzed by GC-MS analysis. The goal was to study the effect of different environmental conditions on the content of total phenolics and on the chemical composition of essential oils of two species of Artemisia, as adaptability indicator to prove that the plants may react with environmental stresses by the production of these compounds, which is reflected on the potency of its allelopathic effect as a competitive strategy of these species. The present research indicated that the total phenolic contents and chemical composition of essential oils of both species of Artemisia are influenced by the difference in the environmental conditions and the geographic location, which resulted in a difference in the potency of allelopathic activity of Artemisia extracts. The allelopathic effects of aqueous and oil extracts of both species of Artemisia on the germination of wheat (Triticum aestivum L.), maize (Zea mays L.) and Leucaena leucocephala seeds were studied. Higher extracts of both species showed maximum inhibition on the germination of the tested seeds in comparison with a control. In the present research, we have found that all oil extracts at the concentrations of 3 and 5µl/ml inhibited seed germination of wheat and Leucaena , as well as seedling growth (significant inhibition of radicle and plumule elongation of both species). Maize was found comparatively tolerant to aqueous extracts of Artemisia than wheat and leucaena. A.judica exhibited greater phytotoxicity than A.herba alba. It can be suggested that the essential oils and aqueous extracts of both species of Artemisia have the potential to be used as a natural herbicide and their allelopathic activity was significantly affected by environmental condition*s.

Keywords: *Allelophathic effect, Artemisia herba alba, Artemisia judica, phenolic content, volatile oils,*

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I. Introduction

Medicinal and aromatic plants are important sources of secondary metabolites, which have important applications in control of plant and human diseases, cosmetics and in the pharmaceutical industry [1]. All *Artemisia* species are rich in essential oils and used as traditional herbal medicines and pharmaceuticals. Furthermore, *Artemisia* has anti-tumor [2], anti-hepatotoxic [3], anti-inflammatory [4], antioxidant [5] and antidiabetic effects [6].

The genus *Artemisia* belongs to family Asteraceae. Asteraceae comprises about 1,000 genera and over 20,000 species. Within this family, *Artemisia* is included in the tribe Anthemideae and comprises over 500 species of aromatic shrubs and herbs distributed over the Northern Hemisphere and a few species are distributed in the Southern Hemisphere and are mainly found in Asia, Europe and North America [7]. For the present study two species of *Artemisia*; *Artemisia herba alba* Asso. and *Artemisia judica* L. were selected to determine the effect of different environmental conditions on the content of total phenolic and the composition of essential oils. Also to examine the reflection of this effect on the potency of the allelopathic effect as a competitive strategy of these plants.

Artemisia judaica L.and *Artemisia herba alba* Asso. grow wildly in Tabuk region at the northwestern part of Saudi Arabia and Sinai at Egypt. For many years, *A. judica* and *A. herba alba* are used in Egypt and Saudi Arabia as traditional medicinal herbs and showed allelopathic activities [8] [9] and the aerial parts of these species are used for the treatment of coughs, the common cold, rheumatic pain, and have an antidiabetic effect [10].

The adaptability of the plants to environmental stress can be tested through essential oil contents, which helps the plant to tolerate different environmental stress conditions; drought, intense radiation, high temperature and heavy metal contents. The population with the composition of essential oil has a higher adaptive value and a

high chance of survival. No information is currently available concerning the effect of different environmental conditions on physiological responses and essential oil production of *Artemisia* species. The volatile oil of *Artemisia* species has the allelopathic effect and the ability to inhibit the growth of seedling and reduce its survival [11].Others [12][13][14] have found that inhibitory substances are terpenoid and phenolic compounds. The goal was to study the effect of different environmental conditions on the content of total phenolics and the chemical composition of essential oil of both species of *Artemisia* growing wild in Saudi and Egypt, as adaptability indicators and to prove that the plants may react with environmental stress by the production of phenolic content and essential oils, which is reflected on the potency of their allelopathic effect and the competitive strategy of these species.

II. Materials and Methods

2.1 Plant materials

The aerial parts of *Artemisia herba alba* Asso. and *Artemisia judica* L. were collected from Tabuk region at the north part of Saudi Arabia and from Wadi El Arbaeen and Wadi El Sheikh at Saint Catherine, Egypt in June 2016. The plant was identified and authenticated in the Desert Research Center.

2.2 Ecological studies

Description of the study areas and their meteorological data

Saudi Arabia has an area of approximately 2,026,213 km2 and comprises almost two-thirds of the Arabian Peninsula area. The dominant terrestrial ecosystem in Saudi Arabia is desert, which is characterized with low annual rainfall and elevated temperature, especially during summer time (can reach up to 50°C) [15][16]. Although most of Saudi Arabia is an arid region, it has a considerably high diversity of plant species. A total of about 2,253 species in 132 families have been reported in this area, of which 20% are rare plants. Some of these plants are endemic to this region and show medical importance [17]. Based on the biogeography theory, the flora of Saudi Arabia has strong connections with that of North Africa, East Africa, the Mediterranean countries and the Irano Turanian countries [18]. The plants were collected from Tabuk, Northern Province of Saudi Arabia (latitude 28˚ 22' 59" N; 26˚ 34' 59" E, altitude 773m). Tabuk region is characterized by highly variable environmental conditions from extremely low to high temperatures .These episodic variations in temperature significantly affect the medicinal properties of the plants. The average temperature in Tabuk is 21.6 °C. Precipitation here averages 46 mm. Precipitation is the lowest in June, with an average of 0 mm. Most precipitation falls in November, with an average of 13 mm. At an average temperature of 30.6 °C, July is the hottest month of the year. In January, the average temperature is 10.8 °C , it is the lowest average temperature of the whole year. Dry period extended from June to September in 2016. The difference in precipitation between the driest and wettest months is 13 mm. The average temperatures vary during the year by 19.8 °C.

The second habitats where the plants were collected are Wadi El Arbaeen and Wadi El Sheikh in Saint Catherine at South Sinai, Egypt. South Sinai is characterized by an arid to extremely arid climate and irregularity in rainfall. The climate is influenced by the orographic impact of the high mountains [19][20]. Saint Catherine region is situated in the southern part of Sinai and is a part of the upper Sinai massif. It has been recognized by the IUCN as one of the most important regions for floral diversity in the Middle East, containing 30% of the entire flora of Egypt and a great proportion of its endemic species. Within the Protectorate, more than 400 species of higher plants have been recorded, of which 19 species are endemic, 10 are extremely endangered and 53 are endangered. It is located between 33˚ 55' to 34˚ 30' East and 28° 30' to 28° 35' North. The soil is formed mainly from mountains weathering, thus it is mainly granitic in origin. The soil layer is generally shallow where the bedrock is close to the surface. Catherine is considered the coolest area in Sinai and Egypt as a whole due to its high elevation [21]. During 2016, the average annual temperature in St Catherine was 14.1 °C , July is the warmest month of the year, the average temperature is 21.4°C. While in January the average temperature is 5.5°C, it is the lowest average temperature all over the year. The average temperatures vary during the year by 15.9 °C. The rainfall here averages 35 mm. The driest month is May, with 0 mm of rain. Most precipitation falls in January, with an average of 6 mm. Dry period extended from May to September in 2016. There is a difference of 6 mm of precipitation between the driest and wettest months.The mean values of climatic factors for Saint Catherine were obtained from the Applied Agricultural Meteorological Laboratory in 2016.

2.3 Soil Analysis

For soil analysis, soil samples were collected at 3 random points from each Location at a depth of 0–25 cm. The electrical conductivity (EC) and pH for each sample were determined as a 1:2.5 dilution in deionized water according to Page (1987) [22].

2.3.1 Soil physical properties

Soil texture (Granulometric analysis) was determined through mechanical analysis by the sieve method [23].

2.3.2 Soil chemical analysis

Major cations such as sodium (Na), potassium (K) were determined in the 1:2.5 soil extract by a flame photometer (Jenway, PFP-7). While the concentration of calcium (Ca), magnesium (Mg) were determined by titration with ethylene diamine tetra-acetic acid (EDTA) according to the method of Rowell (1994). Chloride (Cl) was determined by titrating the soil extract against silver nitrate (0.5N) and 1% potassium chromate as an indicator [23].However, the content of carbonate (CO3) and bicarbonate ions (HCO3) was determined by titration, using 0.1N HCl and methyl orange as an indicator [24].

2.4 Isolation and analysis of essential oils

The plant materials were air dried and ground to fine powder. The essential oils were extracted using Soxhlet apparatus with petroleum ether: diethylether (1:1) for 4h. The solvent was evaporated under reduced pressure. The obtained oils were dried over anhydrous sodium sulfate and stored at 4°C until used for Gas Chromatographic/Mass Spectral (GC-MS) analysis. The *Artemisia* essential oils were analyzed by gas chromatography (Hewlett Packard 6890) mass spectrometry (Hewlett Packard 5972) (GC/MS) apparatus. The GC column was a 30 m (0.25 mm i.d., film thickness 0.25 µm) HP-5MS (5% diphenyl) dimethylpolysiloxane capillary column. Helium was used as the carrier gas at the rate of 20ml/min. The GC conditions were as follows: The column temperature was increased from 40°C to 220°C at a rate of 4°C/min; injector temperature, 250°C; injection volume, 1 µl; transfer temperature, 250°C. The effluent of the GC column was introduced directly into the ion source of the MS. Spectra were obtained in the EI mode with 70 eV ionization energy. The oil components were identified by matching of their retention indices and mass spectra with the Wiley 275.L mass spectral library [25] and the relative percentage was calculated by the software of the apparatus from the total area under the peaks.

2.5 Preparation of plant aqueous extracts

The aerial parts of *Artemisia herba alba* and *Artemisia Judica* were collected from three natural field population, dried in air and ground to fine powder. The aqueous extracts of both *Artemisia* sp were obtained by refluxing 10 g of powdered with 100 ml of distilled water for 24 hours and then filtered. The desired concentrations, 25%, 50%, 75%, and 100% were prepared through the addition of distilled water.

2.6 Determination of total phenolic content

The amount of total phenolic contents in the different concentration of prepared aqueous extracts were determined using Folin- Folin-Ciocalteu (FC) reagent according to the method of [26].Briefly, 2 ml of each aqueous extract were introduced into a test tube and mixed up with 1.0 ml of Folin-Ciocalteu's reagent and 0.8 ml of sodium carbonate (7.5%) were added and mixed thoroughly in the solution. The test tube was allowed to stand for 30 min and the absorbance of each solution was determined at 765 nm against the blank (Unicam UV 300). The amount of total phenolics was determined as gallic acid equivalent (GAE) and expressed as mg GAE/g dry weight.

2.7 The allelopathic effect of *Artemisia* sp. aqueous extracts and essential oils

The effect of different concentration of *A.herba alba* and *A.judica* aqueous extracts and essential oils on the germination of wheat (*Triticum aestivum* L.), maize (Zea mays L.) and *Leucaena leucocephala* seeds. These seeds were sterilized with 15% sodium hypochlorite for 20 min, then, were rinsed with distilled water. Ten seeds were placed in each sterile Petri dish (9 cm) lined with Whatman's filter papers. Oil was dissolved in tween-water solution (1%: v/v), the oil treatments concentrations were 0,1, 3 and 5 µl/ml. The emulsions of 8 ml were added to each Petri dish as per treatments [27].The same method was followed when treating seeds with different concentrations of water extract. The distilled water was used as a control. For each treatment, three replicates were used and the experiment was done using a complete randomized design. The experiment was conducted at room temperature (25°C) and daylight. Germination rate was examined on the second day, while Radicle and Plumule lengths were determined on the 6th day for wheat and *Leucaena* seeds and after 9 days for Zea Mays seeds.

2.8 Statistical analysis

Data were statistically analyzed by using Model GLM of SAS program software version 9.1 (SAS 1998)[28]. The effects of different plant extract concentrations in two localities on germination, radical and plumule growth stages were tested comparing with control plant. Duncan Multiple Range test was used to test the level of significance among the means between different treatments. Where, $\alpha = 0.05$ (Statistical analysis was carried out according to Snedecor and Cochran (1989) [29].

III. Results and Discussions

3.1 Soil analysis

Physicochemical characters of Tabuk soil at Saudi, Wadi El Sheikh and Wadi Al Arbaeen soils at Saint Catherine, Egypt were represented in Tables (1) and (2). Soil samples collected from the different locations showed great variation in texture, their texture was Silt loam at Tabuk and sand at Wadi El Sheikh and Wadi Al Arbaeen soils. The soil of Tabuk is characterized by high percentages of silt (57.39%), while at Saint Catharine soil, its percentage ranged between 2.52 (Wadi Al Arbaeen) to 9.21 % (Wadi El Sheikh). Others [30][31]reported that South Sinai soils are gravelly in wadis and plains, sand to loamy sand in texture, alkaline, nonsaline to slightly saline and this agrees with our results. The pH values fluctuated in the basic range. Generally, no significant differences in soil pH due to location changes were noticed. Results of this study showed that the soil of Wadi El Sheikh is characterized by high content of Cl^- (6 meq/L) and high content of essential nutrients; Ca^{++} (19 meq/L), Mg^{++} (16 meq/L), Na^+ (6 meq/L) and K^+ (5 meq/L). Also, the highest values of $HCO3^-$ (17.5 meq/L) and CaCO3 (13.78%) were recorded in the soil of Wadi El Sheikh, while their lowest values (3.23meq/L) and (5.32%) were detected in the soil of Wadi Al Arbaein, respectively. Results showed that there was no considerable variation in the content of Ca^{++}, K^+ and Cl^- between Tabuk and Wadi Al Arbaeen soils ,where the values of Ca^{++} were 3.50 and 3.65 meq/L and K^+ were 0.57 and 0.72 meq/L while the values of Cl^- were 1.50 and 1.90 meq/L in Tabuk and Wadi Al Arbaeen soils, respectively.

Table 1. Physical proprieties of the soil supporting *Artemisia* sp. at Saudi and Egypt habitats

Locations		Soil Depth (cm)	Soil particles distribution (%)		Soil Texture
Saudi	Tabuk	0-30	Sand Silt Clay	40.60% 57.39% 2.01%	Silt Loam
Egypt	Wadi ElSheikh (1)	0-25	Fine Gravel Coarse Sand Sand Silt Clay	27.62% 18.35% 40.77% 9.21% 4.05%	Sand
	Wadi El Arbaeen (2)	0-25	Fine Gravel Coarse Sand Sand Silt Clay	50.25% 22.34% 22.95% 2.52% 1.94%	Sand

Table 2. Chemical properties of the soil supporting *Artemisia* Sp at Saudi and Egypt habitats

Locations	pH (1:2.5)	EC dS/m	Cation (milliequivalent/Liter)				Anion (milliequivalent/Liter)			$CaCO_3$ %
			Ca^{++}	Mg^{++}	Na^+	K^+	Cl^-	CO_3^{--}	HCO_3^-	
Tabuk	8.02	0.76	3.50	3.05	1.09	0.57	1.50	-	4.85	12
Wadi ElSheikh	7.79	4.5	19.0	16.0	6.0	5.0	6.1	-	17.5	13.78
Wadi El Arbaeen	8.16	0.93	3.65	1.02	3.5	0.72	1.90	-	3.23	5.32

3.2 Essential oils

The chemical composition of the volatile oil is shown in Table (3), the obtained results showed that external environmental conditions can affect the chemical composition, where the major component of the essential oil of *A. herba alba*, collected from Tabuk was Isoaromadendrene epoxide (9.8%). While at Wadi Al Arbaeen, the major one of *A. herba alba* was camphor. The highest concentrations of camphor (4.65 and 4.6%) were detected in the samples of oil of *A. herba alba* and *A. judica* collected from Catherine, while the lowest concentrations (0.26 and 0.23%) were detected from the same plants collected from Tabuk, respectively. Whereas *A. judica* essential oils from Tabuk and Wadi El Sheikh were found to be largely composed of lilac alcohol, its concentrations were 11.58 and 9.02 %, respectively. The lowest values of lilac alcohol (4.1 and 6.5%) were detected in the essential oil of *A. herba alba* from Wadi Al Arbaeen and Tabuk, respectively. Others [32][33] reported that *A. judica* essential oil collected from Sinai Peninsula is rich in piperitone (27-46%), cis – ethyl cinnamate (5-6%), trance ethyl cinnamate (8-13%), ethyl-3propionate (0.2-0.5) and chrysanthenone (5-6%), which not detected and measured in this study due to the difference in the extraction methods or the

difference in the analysis technique [34]. These variations in the composition and in the content of volatile oils may also related to different factors, such as plant age, season and the effect of different environmental conditions [33][35]. Also, the quality of essential oil are affected by pH of the soil, the geographic location, growth at different altitudes or the plant genotype.

Table 3. Chemical constitution of the volatile oil of *Artemisia herba alba* and *A.judica*

No	Compounds	RT	*Artemisia herba alba* (Percent Composition)		*Artemisia judaica* (Percent Composition)	
			Saudi (Tabuk)	Egypt (Wadi Al Arbaeen)	Saudi (Tabuk)	Egypt [49] (Wadi El Sheikh)
1	p-Cymene	5.16	0.50	0.11	0.19	0.59
2	8 hydroxymenthol	5.4	-	-	0.09	0.02
3	Geranyl- vinyl ether	5.6	-	0.03	0.02	0.09
4	trans-Isoeugenol	6.16	0.04	0.04	-	0.65
5	Camphor	8.29	0.26	4.65	0.23	4.6
6	Verbenol	8.4	6.95	-	-	-
7	Carvone oxide,	8.78	-	0.03	-	0.09
8	(+)Borneol	8.99	0.32	0.32	0.08	-
9	Terpineol	9.28	0.37	0.38	-	-
10	R Limonene	10	0.15	0.03	0.12	0.08
11	D Verbenone	10.39	0.25	0.12	-	0.34
12	Hotrienol	10.6	0	-	0.52	-
13	Carvenone	12.17	2.81	2.01	0.19	0.72
14	4–Terpinenyl- acetate	13.1	0.04	-		-
15	Cisp mentha (7,8 dien 2-ol)	13.27	0.14	0.05	-	-
16	5-Caranol,	13.6	0.38	0.30	0.09	0.86
17	7-trans-sesquisabinene hydrate	18.08	0.16	0.06	0.02	0.06
18	Davana ether	19.6	-	-	0.37	-
19	Spathulenol	20.67	0.68	1.11	0.64	0. 72
20	Caryophyllene oxide	20.76	0.08	0.14	0.12	0.72
21	Lilac alcohol C	21.17	6.5	4.1	11.58	9.02
22	Methyl jasmonate	22.57	0.64	0.15	0.71	1.21
23	α-Santoline -alcohol	22.80	0.39	-	0.61	0.62
24	Trans- Z Bisabolene epoxide	27.30	0.21	0.09	0.78	1.6
25	Epicederene -oxide	32	-	0.11	0.04	0.06
26	à-Santonin	32.22	0.39	0.39	-	0.52
27	Corymbolon	34.43	0.29	0.08	-	0.16
28	Aromadendrene oxide	34.73	0.44	0.35	0.29	2.4
29	Isoaromadendrene epoxide	35.31	9.8	0.28	0.78	0.25
30	6-epishyobunol	35.79	0.17	0.5	0.09	0.14
31	Cedran-diol	38.44	0.42	0.14	0.08	0.12

3.3 Total phenolic content

The total phenolic content at different concentrations of aqueous extracts of *A. herba alba* and *A. judica* collected from Saint Catherina and Tabuk is Shown in Table (4). The results revealed that the value of total phenolic content was increased gradually with the increase in the concentration of plant aqueous extract. The lowest value of total phenolic content was 2.03 mg GAE g^{-1}at a concentration of 25% of *A. herb alba* aqueous extract, collected from Wadi Al Arbaeen and gradually increased to 4.13 mg GAE g^{-1} at a concentration of 50% ,to 6.53 mg GAE g^{-1} at 75% and to 8.27 mg GAE g^{-1} at a concentration of 100%. Whereas, in aqueous extracts of *A. herba alba* collected from Tabuk ,the values of total phenolic content reached to 8.10, 8.50, 10.87 and15.87 mg GAE g^{-1} at the same concentrations, respectively. The highest values of total phenolic content were detected in the aqueous extracts of *A. Judica* collected from Tabuk, its values were 12.50, 13.47 and 23.37 mg GAE g^{-1} at the concentrations of 50, 75 and 100% of its aqueous extracts, respectively.

The obtained results indicated that the content of plant from total phenolic was affected by environmental conditions since its values were higher at the plant collected from Saudi. Severe environmental conditions tend to increase the content of phenolic compounds in the plant. The phenolic metabolism in higher plants is induced in response to environmental stress conditions such as infection by microbial pathogens, mechanical wounding, and excessive UV or high visible light levels [36][37].Where phenolics have several functions in plants as antibiotics, natural pesticides, protective agents against ultraviolet (UV) light [38] and protection against vascular disorders, which may be caused by oxidative damage of cell membranes. The phenolic compounds such as; ferulic acid, P-coumaric, vanillic, caffeic, chlorogenic and others inhibit the germination process due to the interference with the metabolism of indol acetic acid or the uptake of ion or the synthesis of protein [39].However, Modallal and Al-Charchafchi, (2006)[40] reported that the phenolic

compounds of *A.herba alba* were the responsible agent for its phytotoxic effect on germination and seedling growth of *Anabasis setifera*.

Table 4. Total phenolic contents of *Artemisia herba alba* and *A.judica* aqueous extracts

Country	Plant species	Conc. of Aqueous Ext.	Total Phenolics (mg GAE g-1)
Saudi (Tabuk)	*Artemisia herba alba*	25%	8.10±0.12g
		50%	8.50±0.17g
		75%	10.87±0.12f
		100%	15.87±0.12b
	Artemisia judica	25%	8.37±0.20 g
		50%	12.50±0.17d
		75%	13.47±0.19c
		100%	23.37±0.15a
Egypt (Wadi Al Arbaeen)	*Artemisia herba alba*	25%	2.03±0.09 m
		50%	4.13±0.12 l
		75%	6.53±0.12 i
		100%	8.27±0.15 g
Egypt (Wadi El Sheikh)	*Artemisia judica*	25%	4.87±0.18 k
		50%	5.57±0.12 j
		75%	7.47±0.15 h
		100%	11.60±0.12e
S.O.V.			**

Results are means ±S.E(n=3) -±S.O.V.: Source of Variance - **: P≤ 0.01 is highly significant. - Means with the same letter in the same column are not significantly different.

3.4 Allelopathic effect of aqueous and oil extract of *Artemisia* sp

The effect of *Artemisia* sp water and oil extracts on the germination percentage of wheat (*Triticum aestivum* L) and their effects on the elongation of the root (Radical) and shoot (plumule) are shown in Table (5), Plate (1),(2).The obtained results revealed that all extracts significantly reduced radical and plumule lengths and the increase in the concentration of aqueous and oil extracts leads to reduce the length of radical and plumule. The highest inhibitory effect was found at a concentration of 100% of aqueous extracts and the concentrations of 3 and 5 µl /ml of oil extracts. The germination percentage reached 76.67 and 16.67 % by treatment of wheat grains with aqueous extracts of *A. herba alba* collected from Tabuk at the concentrations of 25 and 50 % and reached 75.37 and 21.67% in case of treatment with the same concentrations (25 and 50%) of that collected from Wadi Al Arbaeen. The oil extracts of *A. herba alba* at a concentration of 1µl/ml from Tabuk and Wadi Al Arbaeen significantly reduced the percentage of germination of wheat grains to 31.67 and 26.67%, respectively. Also, the treatments of wheat grains with the same concentrations (25 and 50%) of *A. Judica* aqueous extracts showed the same behavior and caused a significant decrease in the percentage of germination to 73.33 and 13.33% at Tabuk and to 73.51 and 16.67% at Wadi El Sheikh, respectively. The treatment of wheat grains with the lowest concentration of oil extracts (1µl/ml) of *A. judica* collected from Tabuk and Wadi Al Arbaeen resulted in significantly reduction in the percentage of germination to 30 and 23.33%,respectively. Meanwhile, the germination of wheat were inhibited by the treatment with the concentrations of 75 and 100% of aqueous extracts of both species of *Artemisia* collected from Saint Catherine. The lengths of radical and plumule of wheat were significantly reduced to (0.63 and 2.92 cm) and (0.14 and 0.28 cm) by treatment with aqueous extracts of *A. herba alba* collected from Tabuk at the concentrations of 50 and 75 %, respectively. Also, their lengths were reduced to (2.37 and 5.97cm) at a concentration of 50% and completely reduced by treatment with higher concentrations (75 and 100%) of *A. herba alba* aqueous extract from the other habitat. A complete inhibition in the elongation of radical and plumule was obtained by the treatment the seeds of wheat with aqueous extracts of *A. judica* at a concentration of 100% at Tabuk and 100 and 75% at Wadi El Sheikh. The results revealed that *A.herba alba* and *A.Judica* allelopathy have an inhibitory effect and resulted in a reduction in the length of radical and plumule of the germinated seeds of wheat.

Table 5. Effects of aqueous and essential oil extracts of both species of *Artemisia* on the germination of wheat

Country	Plant Species	Treatments	Conc. of Treatments	Wheat Germination %	Radical Length (cm)	Plumule Length (cm)
		Water	Control	83.33±1.67 a	4.57±0.12 a	9.83±0.17 a
		Aqueous	25%	76.67±1.67 b	3.07±0.07 cd	5.08±0.15 d

Tabuk	*A. herba alba*	Extract	50%	16.67±1.67 g	0.63±0.02 h	2.92±0.26 f
			75%	3.17±0.17 h	0.14±0.02h	0.28±0.01i
			100%	0±0	0±0	0±0
		Volatile Oils	1µl/ml	31.67±1.67 c	1.83±0.07 fg	2.13±0.07 g
			3µl/ml	0±0	0±0	0±0
			5µl/ml	0±0	0±0	0±0
	A. judica	Aqueous Extract	25%	73.33±1.67 b	2.16±0.05 ef	5.15±0.18 d
			50%	13.33±1.67 g	0.57±0.03 h	1.19±0.12 h
			75%	4.17±1.67 h	0.27±0.02 h	0.53±0.01 i
			100%	0±0	0±0	0±0
		Volatile Oils	1µl/ml	30.00±0 cd	1.37±0.09 g	1.93±0.09 g
			3µl/ml	0±0	0±0	0±0
			5µl/ml	0±0	0±0	0±0
Wadi Al Arbaeen	*A. herba alba*	Aqueous Extract	25%	75.37±1.67 b	3.57±0.35 bc	9.40±0.10 a
			50%	21.67±1.67 f	2.370±0.37ef	5.97±0.09 c
			75%	0±0	0±0	0±0
			100%	0±0	0±0	0±0
		Volatile Oils	1µl/ml	26.67±1.67de	2.57±0.23 de	2.63±0.13 f
			3µl/ml	0±0	0±0	0±0
			5µl/ml	0±0	0±0	0±0
Wadi ElSheikh	*A. judica*	Aqueous Extract	25%	73.51±1.67 b	3.80±0.12 b	7.73±0.32 b
			50%	16.67±1.67 g	3.03±0.29 cd	4.23±0.15 e
			75%	0±0	0±0	0±0
			100%	0±0	0±0	0±0
		Volatile Oils	1µl/ml	23.33±1.67 ef	1.97±0.15 f	2.03±0.03g
			3µl/ml	0±0	0±0	0±0
			5µl/ml	0±0	0±0	0±0
S.O.V.				**	**	**

Results are means ±S.E(n=3) -±SE: Standard Error -S.O.V.: Source of Variance - **: P≤ 0.01 is highly significant. - Means with the same letter in the same column are not significantly different.

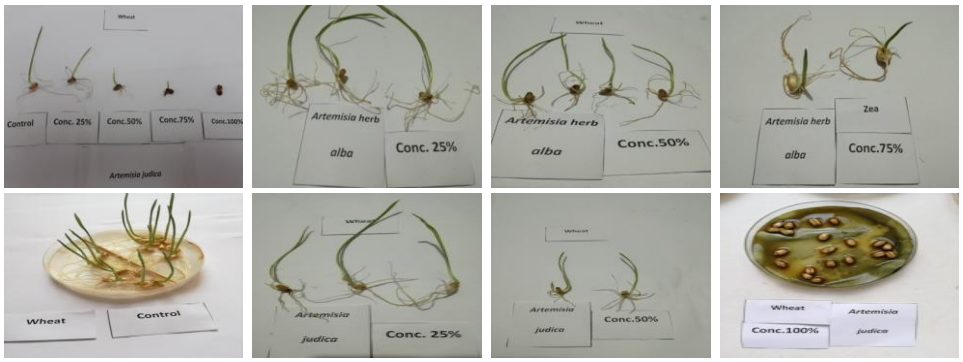

Plate 1.

Effects of aqueous extracts of both species of *Artemisia* on the germination of wheat

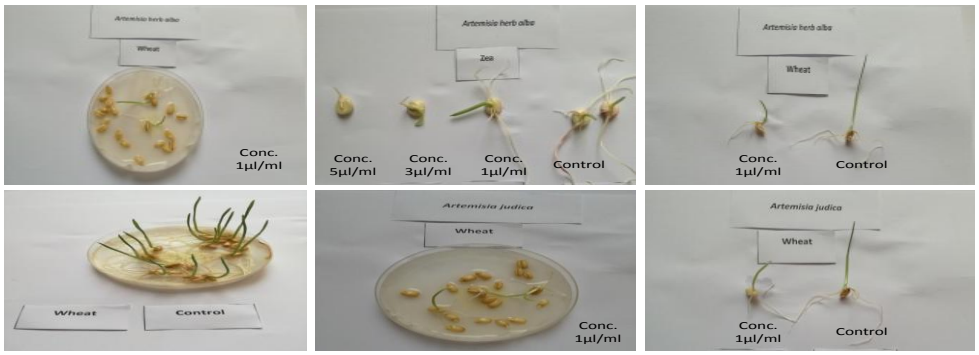

Plate 2.
Effects of essential oil extracts of both species of *Artemisia* on the germination of *Zea mays* and wheat

As Shown in Tables (6), (7) and Plates (2),(3),(4) the treatments of Zea mays and *Leucaena leucocephala* seeds with the aqueous extracts of *A.herba alba* collected from different habitats at the concentrations of 75 and 100% lead to reduce the percentage of germination of *Zea mays* to 53 and 31.67% at the collected plant from Tabuk and reduced to 71.67 and 41.67% from that collected from Wadi Al Arbaeen. While the treatments with a concentration of 5µl/ml of all oil extracts of *Artemisia*, collected from both species at different habitats inhibited the germination of all the investigated seeds. The high concentration of aqueous extracts treatment (100%) showed a significant effect on the germination percentage of wheat, *Zea mays*, and *Leucaena* , but Maize was found comparatively tolerant to aqueous extracts of *Artemisia* than the other seeds and this may be due to more resistance mechanisms that have been evolved in this plant. Chon et al. (2002)[41] mentioned that the growth of root tip of some plants nearly inhibited to escape from allelochemicals absorption. The aqueous extract of both species at the concentration of 50 and 75% caused a significant decrease in the lengths of radical and plumule of the seedling in comparison with that of 25% and control. The lengths of radical and plumule of *Zea mays* were significantly reduced to (5.51 and 6.08 cm) and (4.09 and 3.94 cm) by the treatment with the aqueous extracts of *A. herba alba* collected from Tabuk at the concentrations of 50 and 75% respectively. Also, the treatment with the extracts of that collected from Wadi Al Arbaeen significantly reduced the lengths of radical and plumule to (8.60 and 5.33 cm) and (7.23 and 3.33cm) at the same concentrations, respectively. The obtained results indicated that there was a significant reduction in the elongation of the germinated seed of *Zea mays* treatment with *A. judica* aqueous extracts collected from two habitats. Generally, the results in this research revealed that the seeds of wheat and *Leucaena* were more sensitive and more affected by the treatment with the aqueous and oil extracts of the *Artemisia* plants. In this present research, we have found that the strength of the phytotoxic effect of oil extracts was greater than that of aqueous extracts and all oil extracts at the concentrations of 3 and 5µl/ml inhibited seed germination of wheat and *Leucaena* , as well as seedling growth (significant inhibition of radicle and plumule elongation of both species).

Table 6. Effects of aqueous and essential oil extracts of both species of *Artemisia* on the germination of *Zea mays*

Location	Plant Species	Treatments	Conc. of Treatments	Zea mays Germination (%)	Radical Length(cm)	Plumule Length (cm)
Tabuk	*A. herba alba*	Water	Control	95.00±2.89 a	13.83±0.38a	8.63±0.73 a
		Aqueous Extract	25%	80.00±2.89 cd	6.23±0.01 f	7.40±0.14 b
			50%	76.67±1.67 de	5.51±0.16 g	6.08±0.17cd
			75%	53.33±1.67 ghi	4.09±0.18 i	3.94±0.31f
			100%	31.67±1.67 l	1.29±0.05 m	1.92±0.12 m
		Volatile Oils	1µl/ml	48.33±1.67 ij	6.60±0.10 ef	3.07± 0.07 ghij
			3µl/ml	31.67±1.67 l	3.17±0.09 jk	1.17± 0.09 on
			5µl/ml	0±0	0±0	0±0
	A. judica	Aqueous Extract	25%	73.33±1.67 e	4.82± 0.18 h	7.33± 0.11 b
			50%	61.67±1.67 f	4.46±0.06 hi	5.78±0.22 cd
			75%	43.33±1.67 jk	4.12±0.10 i	5.06±0.15 e
			100%	33.33±1.67 l	1.78± 0.12 l	2.45±0.21 jkl
		Volatile Oils	1µl/ml	41.67±1.67 k	4.50± 0.17 hi	2.73±0.13 hijk
			3µl/ml	21.67±1.67 m	2.73±0.17 k	1.10±0.06 on

			5µl/ml	0±0	0±0	0±0
Wadi Al Arbaeen	*A. herba alba*	Aqueous Extract	25%	86.67±1.67 b	9.90±0.21 b	6.30± 0.12 c
			50%	83.33±1.67 bc	8.60±0.06 c	5.33±0.24 de
			75%	71.67±1.67 e	7.23±0.12 d	3.33±0.17 fgh
			100%	48.33±1.67 ij	4.27±0.15 i	2.33±0.24 kl
		Volatile Oils	1µl/ml	56.67±1.67 fgh	6.47±0.03 ef	3.43±0.07 fg
			3µl/ml	41.67±1.67 k	3.43±0.07 j	1.57±0.09 mn
			5µl/ml	0±0	0±0	0±0
Wadi El Sheikh	*A. judica*	Aqueous Extract	25%	81.67±1.67 bcd	7.60±0.21 d	3.33±0.09 fgh
			50%	76.67±1.67 de	6.77±0.15 e	3.17±0.17 ghi
			75%	51.67±1.67 hi	3.50±0.29 j	2.27±0.15 kl
			100%	41.67±1.67 k	2.83±0.17 k	2.03±0.03 ml
		Volatile Oils	1µl/ml	58.33±1.67 fg	4.90±0.21 h	2.57±0.07 ijkl
			3µl/ml	43.33±1.67 jk	3.13±0.07 jk	1.60±0.10 mn
			5µl/ml	4.83±0.17 n	0.37±0.03 n	0.77±0.06 o
S.O.V.				**	**	**

Results are means ±S.E(n=3)-_+SE: Standard Error -S.O.V.: Source of Variance - **: P≤ 0.01 is highly significant. - Means with the same letter in the same column are not significantly different.

Table 7. Effects of aqueous and essential oil extracts of both species of *Artemisia* on the germination of *Leucaena*

Locations	Plant Species	Treatments	Conc. of Treatments	*Leucaena* Germination%	Radical Length (cm)	Plumule Length (cm)
Tabuk	*A. herba alba*	Water	Control	81.66±1.67 a	3.96±0.09a	3.30±0.17 a
		Aqueous Extract	25%	46.67±1.67 b	2.47±0.15 d	2.53±0.15 b
			50%	31.67±1.67 e	2.17±0.03 e	0.93±0.03 ef
			75%	8.00±1.15 hi	1.13±0.09 f	0.87±0.09 ef
			100%	0±0	0±0	0±0
		Volatile Oils	1µl/ml	11.67±1.67 h	0.57±0.07 hi	0.27±0.03 h
			3µl/ml	0±0	0±0	0±0
			5µl/ml	0±0	0±0	0±0
	A. judica	Aqueous Extract	25%	36.67±1.66 d	3.17±0.08 c	2.47±0.15 cb
			50%	23.33±1.67 f	1.93±0.12 e	0.53±0.03 gh
			75%	4.17±0.16 ij	0.53±0.03 i	0.47±0.03 gh
			100%	0±0	0±0	0±0
		Volatile Oils	1µl/ml	9.33±0.67 h	4.20±0.15 a	3.30±0.17a
			3µl/ml	0±0	0±0	0±0
			5µl/ml	0±0	0±0	0±0
Wadi Al Arbaeen	*A. herba alba*	Aqueous Extract	25%	45.00±2.89cb	3.17±0.17 c	2.53±0.03 b
			50%	41.67±1.67cd	2.93±0.06 c	2.03±0.03 d
			75%	36.67±1.67d	2.07±0.03 e	1.10±0.06 e
			100%	0±0	0±0	0±0
		Volatile Oils	1µl/ml	18.33± 1.67g	0.73±0.15ghi	0.47±0.03 gh
			3µl/ml	0±0	0±0	0±0
			5µl/ml	0±0	0±0	0±0
	A. judica	Aqueous Extract	25%	48.33±1.67 b	3.53±0.03 b	2.23±0.15cb
			50%	38.33±1.67 d	2.03±0.09 c	0.67±0.09 fg
			75%	28.33±1.67 e	0.87±0.03fgh	0.50±0 gh
			100%	0±0	0±0	0±0
		Volatile Oils	1µl/ml	16.67±1.67 g	0.90±0.06 c	0.37±0.07gh
			3µl/ml	0±0	0±0	0±0
			5µl/ml	0±0	0±0	0±0
S.O.V.				**	**	**

Results are means ±S.E(n=3)±SE: Standard Error -S.O.V.: Source of Variance - **: P≤ 0.01 is highly significant. - Means with the same letter in the same column are not significantly different.

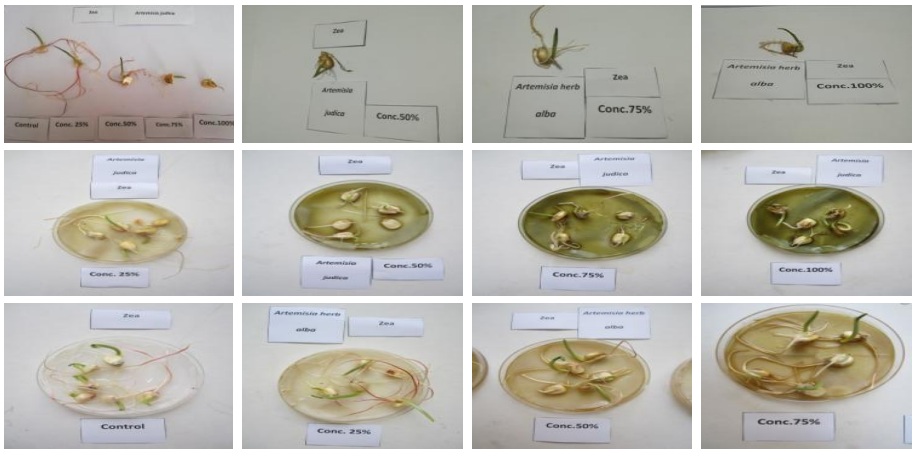

Plate 3.

Effects of aqueous extracts of both species of *Artemisia* on the germination of *Zea mays*

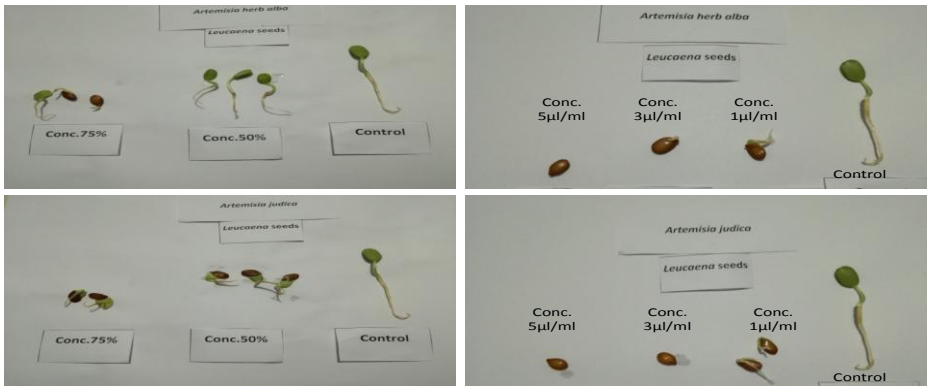

Plate 4.

Effects of aqueous and essential oil extracts of both species of *Artemisia* on the germination of *Leucaena*

The allelopathic activity of *Artemisia* essential oil is likely due to the high concentration of camphor, terpineol and caryophyllene oxide. Others [12][13][14] have found that inhibitory substances which have allelopathic effects are terpenoids and phenolic substances. Monoterpene vapors lead to accumulation of lipid globules in the cytoplasm which may cause the reduction in the mitochondria and disruption in the surrounding membrane.Others [42][43][44] reported that α-terpineol (5.6%) has allelopathic activity and may contribute to the allelopathic effect of *Artemisia indica* oil from Nepal. Moreover, wheat was found more sensitive to aqueous extracts than maize and *Leucaena* which suggests the ability of *Zea mays* to tolerate the negative effects of allelochemicals of *Artemisia* extracts. Reduction in germination caused by allelochemicals of extracts can be attributed to their effects on cell membrane permeability, enzymatic abnormalities, pH changes in medium, alteration growth hormones and metabolic reaction, the absorption capacity of the tested seeds to water and nutrient [45][46]. Results of this study are in close agreement with those of Siyar *et al.* (2018)[47], who reported that the aqueous extracts of two plants (*Artemisia annua* and *Taraxicum officinalis*) had inhibitory effects on the germination of wheat and maize and wheat was more affected by leaf extracts of *A. annua* than maize.

IV. Conclusion

The present research concludes that the content of total phenolic and chemical composition of essential of both species of *Artemisia*; *A. herba alba* and *A .judica* collected from Tabuk and Saint Catherine habitats are influenced by the difference in the environmental conditions and the geographic location, which is reflected on the potency of their allelopathic effect and the competitive strategy of these species. The obtained results indicate that both species of *Artemisia* had inhibitory effects on the germination of maize, wheat, and *Leucaena* seeds. Wheat and *Leucaena* were severely affected by extracts of *Artemisia*. Higher concentration of aqueous and oil extracts of both species showed maximum inhibition in germination. The allelopathic effects of *Artemisia* aqueous and essential oil extracts not only lead to inhibited seed germination but also they cause a reduction in the elongation of radical and plumule. The strength of the inhibitory effect of oil extracts was significantly greater than that of aqueous extracts. The inhibitory substances in *Artemisia* species which have allelopathic effects are terpenoids and phenolic substances. The *Artemisia* species could be used as a natural herbicide and their allelopathic activity was significantly affected by environmental conditions.

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