



## **Evaluation of Allelopathic Potential of *Artemisia herba-alba* on Germination and Seedling Growth of *Raphanus sativus* and *Trigonella foenum-graecum***

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### **Author's contribution**

*The sole author designed, analysed, interpreted and prepared the manuscript.*

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### **ABSTRACT**

The allelopathic effects of the aqueous extracts of aerial parts of *Artemisia herba-alba* at different concentrations (20, 40, 80%) were evaluated on germination and seedling growth of *Raphanus sativus* (Radish) and *Trigonella foenum-graecum* (Fenugreek) after 7 days "in vitro". *Artemisia* had strong allelopathic effects, it prevented the germination and seedling growth of Radish seeds, at highest concentrations (80%). Results obtained showed significant inhibition in germination percentage (GP), plumule length (PL) and radicle length (RL) of both Radish and Fenugreek seeds, and the degree of inhibition was concentration dependent. At 20 and 40% the germination percentage of Radish seed was reduced to (56.67%) and to (40%), while the germination of Fenugreek seeds was reduced to (80%) and to (63.33%) respectively. This inhibition was markedly in obvious Radish than in Fenugreek, indicating that is *Raphanus sativus* more sensitive, to the allelopathic effect of the different concentrations of the aqueous extract of the *A. herba alba* plant.

**Keywords:** *Allelopathy; germination; Artemisia herba-alba; aqueous extract.*

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## 1. INTRODUCTION

Allelopathy as an ecological phenomenon, has been defined as any direct or indirect effects of one plant, including micro-organisms, on another through the release of phytochemical compounds into the environment, these biochemical materials are called allelochemicals that may affect the physiological processes of the plants such as respiration, cell division, water and nutrient uptake, oxidative stress and others. Most plant species, including wild plants, crops and trees are capable of producing such molecules into the environment to inhibit the development of neighboring plants [1,2,3]. The term allelopathy refers to any method involving secondary metabolites (allelochemicals) created by plants, or microorganisms, bacteria, viruses and fungi that influence the growth and development of agricultural and biological systems as well as positive and negative effects. Allelochemicals from plants are produced by any organ of the plant and discharged into the environment by volatilization, exudation from roots, leaching from stems and leaves or decomposition of plant material [4,5]. Allelochemicals are mainly secondary metabolites which usually associated with plant defense against herbivores and pathogens, these distinctive compounds may be linked to wide range of ecological functions [6]. Allelopathic effects can be stimulatory or inhibitory, depending on the identity of the active compound on the static and dynamic availability, persistence and fate of organics in the environment and on the particular target species [7]. Also, allelopathy is generally accepted as a significant ecological factor in determining the plant growth, succession, dominance, distribution, species diversity, structure and composition of plant communities [8]. *Artemisia herba-alba* Asso. (Asteraceae), which recently classified as subgenus *Seriphidium* [9], commonly known as the desert wormwood, is a dwarf, semi shrub growing widely in Al-Gabal Al-Akhdar in Libya and in, Northern Africa (Morocco, Tunisia Algeria), the Middle East Western Asia (Arabian Peninsula) and Southwestern Europe. The plant is a perennial, strongly aromatic herb, with many basal, erect and leafy stems covered with woolly hairs [10]. It is widely used as folk medicine and in particular for common uses such as relief of coughing, intestinal disturbances, colds and muscle tensions by the local population in different countries [11]. The allelopathic consequences of the genus *Artemisia* have been broadly investigated [12,13,14]. *Artemisia herba-alba*

grown in Libya was observed as dominant species in its natural micro habitat in Al-Gabal Al-Akhdar area and prevents growth of another plant species those grow closely to it. Therefore, we hypothesized that this species has allelopathy effects and can be used as determination factor for the growth of some plants. Therefore, the study was aimed to evaluate the allelopathic effect of *Artemisia herba-alba* (*Artemisia*) on germination and seedling growth of *Raphanus sativus* (Radish) and *Trigonella foenum-graecum* (Fenugreek).

## 2. MATERIALS AND METHODS

### 2.1 Sources of the Seeds

The seeds of Radish and Fenugreek used in this study were obtained from the local market. Al Bayda- Libya. The definition of the type of seeds through the Herbarium in the Department of Botany. And were kept in the containers which they were supplied, then stored in the laboratory at room temperature until required for sowing.

### 2.2 Plant Material Collection

*Artemisia herba-alba* samples were collected from the south of Al-Jabal Al-Akhdar EL-Bayda city – Libya ( Marawa region), in October 2018, collected only areal parts. The plant were classified and authenticated according to [15] through the Herbarium in the Department of Botany, Faculty of Science, Omar Al-Mukhtar University, EL- Bayda, Libya.

### 2.3 Preparing the Aqueous Extract of *Artemisia Herba-alba*

A number of fresh samples from the aerial shoots of the donor species were collected from the natural habitats in the study area during the vegetative stage. The samples were air-dried then after ground in a Wiley Mill to fine uniform texture and stored in glass jars until use. Stock aqueous extract was obtained by soaking 50 g air-dried plant material in 500 ml of cold distilled water (10% w/v) at room temperature ( $20\pm 2^{\circ}\text{C}$ ) for 24 hours with occasional shaking. The mixture was left on shaker (heidolph titramax 101) for 24 h in room temperature at speed of 120 rpm four-folded cotton fabric was used as filter to separate rough solid particles from solution. The contents were then filtered with Whatman filter paper and then it was centrifuged in laboratory centrifuges (Thermo Electron Corporation, Sorvall RC 6 Plus) with the speed of 2000 rpm for 15 minutes [16].

## 2.4 Preparing the Aqueous Extract Concentrations

Three concentrations of solutions (20, 40, and 80%) were prepared from the stock solution based on volume/volume percent (v/v)% [17]. In addition to the distilled water as control. To prepare solutions of different concentrations, doses of *A. herba-alba* aqueous extract (20, 40 and 80 ml) were taken. Then, volume was completed to 100 ml by adding distilled water to obtain (20, 40, and 80%).

## 2.5 Treatment of Seeds with Plant Extracts

Sixty seeds of each species (Radish and Fenugreek) were distributed in 12 petri dishes (5 seed in each dish) on two layers of Whatman filter paper No.1. Five ml of each prepared aqueous extract (20, 40, and 80%) or distilled water as control (0%) were added in petri dishes (added daily). Three replicates were incubated in randomized complete block design at 20° C in an incubator. Before sowing, the seeds were surface sterilized with 2% sodium hypochlorite for 2 minutes then rinsed four times with distilled water. The sterilized seeds were soaked in aerated distilled water for 24 hours. The germination percentage (GP), plumule length (PL) and radicle length (RL) were recorded after one week at the end of the experiment [18]. A variety of parameters were used in this work to assess the effects of extracts on seed germination and seedlings development of test species. These parameters include:

1. **Germination Percentage (GP)** was calculated according to the following equation [19].

$$\% \text{Germination} = \left( \frac{\text{Number of germinated seeds}}{\text{Total number of Seeds}} \right) \times 100$$

2. **Plumule Length (PL) and Radicle Length (RL)**

Length of plumule and radicle system were measured in cm using a ruler.

## 2.6 Statistical Analysis

Statistical analysis was performed using a computer run program (Minitab software). One way ANOVA followed by Tukey, s HSD test was performed to show the statistical

significance among the means of the groups. Results were expressed as mean  $\pm$  Standard Division (SD). P-value below 0.05 was considered to be statistically significant [20].

## 3. RESULTS

### 3.1 The Allelopathic Effect of Artemisia on Radish Seeds

#### 3.1.1 Germination Percentage (GP)

The germination percentage of Radish seeds was significantly affected by the increase at different concentration levels of Artemisia aqueous extract after seven days of germination. Artemisia aqueous extracts have inhibitory effect on germination and early growth of *Raphanus sativus* and considerably suppressed the germination compare to control treatment. The total percentage of Radish seeds germination was decreased by increasing the concentration of Artemisia aqueous extract, at control (0%) GP value was about (93.33 %). While the percentage was reduced to (56.67%) and to (40%), at concentrations 20 and 40% *Artemisia herba-alba* aqueous extract, respectively. The maximum allelopathic effect was recorded in 80% Artemisia aqueous extract concentration, which completely inhibited Radish seed germination (Table 1).

#### 3.1.2 Plumule Length (PL)

Findings of PL of *Raphanus sativus* imply the inhibitory effect of the allelopathic substances on seedling stage. Evidently, PL was significantly reduced due to each main effect as treatment. Additionally, the value of PL was 4.2 cm at control level. Afterward, it reduced to 2.7cm at 20%, and to 2.2 cm at 40%, the maximum allelopathic effect was recorded in 80% Artemisia aqueous extract concentration, which completely inhibited PL (Table 1).

#### 3.1.3 Radicle Length (RL)

Decreased was observed among Radish RL assessment in seeds culture (Table 1). The control value was 6.1cm. Elevated *A. herba-alba* aqueous extract concentrations have possessed a significant inhibitory effect on radical growth. At 20% *A. herba-alba* aqueous extracts concentration, it was 2.3cm. Upon applying the highest *A. herba-alba* aqueous extracts

concentration (80%), it was completely inhibited RL (Table 1).

### 3.2 The Allelopathic Effect of Artemisia on Fenugreek Seeds

#### 3.2.1 Germination Percentage (GP)

The allelopathic effects of *A. herba-alba* aqueous extracts on Fenugreek seeds were also evaluated, the total percentage of Fenugreek seed germination was decreased by increasing the concentration of Artemisia aqueous extract, at control (0%) GP value was about (98.33%). The percentage was reduced to (80%) at 20% and to (63.33%) at 40%, while recorded (46.67%) at (80% v/v) *A. herba-alba* aqueous extracts concentration (Table 2).

#### 3.2.2 Plumule Length (PL)

Evaluation of PL correlated with higher Artemisia aqueous extract concentrations has demonstrated their inhibitory influence on

*Trigonella foenum-graecum* growth process (Table 2). The plumule elongation was not completely inhibited by the extract but it was less at higher concentration levels. Obviously, all allelopathic concentrations have reduced PL. at control level PL of Fenugreek was 5.9 cm. On the other hand, 20, 40 and 80% concentrations were considered as inhibitory concentrations (the values about 4, 3.6 and 2.1 cm) respectively.

#### 3.2.3 Radicle Length (RL)

Compared to control, a gradual decrease in RL of Fenugreek seed was observed along gradual increase in *A. herba-alba* aqueous extracts concentrations. RL implication was significantly affected by the treatment. At control, the values of RL were 4.4 cm. higher concentrations of *A. herba-alba* aqueous extracts were notably active inhibiting radicle emergence. at 20, and 40 % concentrations, RL decreased to 3.5 and 3.2 cm respectively. Constantly, it continues reduction till it attained a value of about 1.8 cm at 80 % concentration level (Table 2).

**Table 1. Allelopathic effect of different concentrations of aqueous extract of *Artemisia herba-alba* on germination percentage (GP) and radicle (RL) and plumule (PL) length (cm) of *Raphanus sativus* L. (after 7 days)**

Extract concentration%	Seed germination	GP%	RL(cm)	PL(cm)
	Mean $\pm$ SD		Mean $\pm$ SD	Mean $\pm$ SD
0	18.67 <sup>a</sup> $\pm$ 0.47	93.33	6.1 <sup>a</sup> $\pm$ 0.09	4.2 <sup>a</sup> $\pm$ 0.16
20	11.33 <sup>b</sup> $\pm$ 0.94	56.67	2.3 <sup>b</sup> $\pm$ 0.08	2.7 <sup>b</sup> $\pm$ 0.14
40	8 <sup>c</sup> $\pm$ 0.82	40	1.5 <sup>c</sup> $\pm$ 0.05	2.2 <sup>c</sup> $\pm$ 0.12
80	0 <sup>d</sup>	0	0 <sup>d</sup>	0 <sup>d</sup>

Data are expressed as mean  $\pm$  SD of three replicate. Within each row, means with different superscript (a, b, c or d) were significantly different at  $p < 0.05$ . Where means superscripts with the same letters mean that there is no significant difference ( $p > 0.05$ )

**Table 2. Allelopathic effect of different concentrations of aqueous extract of *Artemisia herba-alba* on germination percentage (GP) and radicle (RL) and plumule (PL) length (cm) of *Trigonella foenum-graecum*. (after 7 days)**

Extract concentration%	Seed germination	GP%	RL(cm)	PL(cm)
	Mean $\pm$ SD		Mean $\pm$ SD	Mean $\pm$ SD
0	19.67 <sup>a</sup> $\pm$ 0.47	98.33	4.4 <sup>a</sup> $\pm$ 0.24	5.9 <sup>a</sup> $\pm$ 0.09
20	16 <sup>b</sup> $\pm$ 0.82	80	3.5 <sup>b</sup> $\pm$ 0.05	4 <sup>b</sup> $\pm$ 0
40	12.67 <sup>c</sup> $\pm$ 1.2	63.33	3.2 <sup>b</sup> $\pm$ 0.12	3.6 <sup>b</sup> $\pm$ 0.21
80	9.33 <sup>d</sup> $\pm$ 0.94	46.67	1.8 <sup>c</sup> $\pm$ 0.16	2.1 <sup>c</sup> $\pm$ 0.14

Data are expressed as mean  $\pm$  SD of three replicate. Within each row, means with different superscript (a, b, c or d) were significantly different at  $p < 0.05$ . Where means superscripts with the same letters mean that there is no significant difference ( $p > 0.05$ )

#### 4. DISCUSSION

Environmental and non-environmental stresses lead to the interactions in plants. Some of environmental stresses are allelopathic compounds which secrete by some plants and cause disturbance in life cycle and activate a series of biochemical reactions [21]. The present work was carried out as a study to investigate any possible allelopathic activity *A. herba alba* aqueous extract on germination and seedling growth of *Raphanus sativus* and *Trigonella foenum-graecum*, the results showed severe toxicity at high extract concentration and moderate toxicity at low concentration. The highest germination rate of Radish and Fenugreek seeds was obtained from distilled water treatment and the lowest rate was obtained from treatments lead to lack of germination, respectively. In general the results showed that when concentration of extract increases, traits significantly decrease, this can result from the increase in amount of allelochemicals and the toxicity characteristics [22]. *A. herba alba* aqueous extract may contain some phytotoxic substances that inhibits germination and growth of Radish and Fenugreek. These results correlated with the findings that Allelochemicals presented in the aqueous extracts of different plant species commonly identified as allelopathic agents, which have inhibitory and/or lethal effects on seed germination growth and development, reduction in seedling growth and have been reported to effect on different physiological processes through their effects on enzymes responsible for phytohormone synthesis and were found to associate with inhibition of nutrients and ion absorption by affecting plasma membrane permeability [23,24]. Aqueous extract of plants may contain phenolics such as ferulic acid P-coumaric, vanillic, caffeic, chlorogenic and others [25,26]. These phenolics inhibit the germination process [27,28], which was due to their interference with indol acetic acid metabolism, or synthesis of protein and ion uptake by the plants [25]. Therefore, *A. herba alba* might release some soluble phenolic allelochemicals to the environment [29], which has a growth inhibitory effect on new seedling of both *Raphanus sativus* and *Trigonella foenum-graecum* or other plant species. Our results are agreed with [30,31] who reported that seeds of some species can be suppressed using water extracts from Artemisia plants or another species and theses extracts can effect on germination behavior too. The results of this study showed that Artemisia extracts had

deterrent effects on the germination and growth indices of *Raphanus sativus* and *Trigonella foenum-graecum*. The seed germination traits and seedling growth were decreased by increasing the extract concentration. [17] concluded that *A. herba alba* aqueous extract at different concentrations suppressed the germination of monocot (wheat) and dicot (tomato) seeds. And this suppression was possibly due to the presence of allelochemicals in this plant. There are some reports about the inhibitory effects of different species of Artemisia on seed germination traits of *Triticum aestivum* L., *Brassica napus*, *Sinapis arvensis* L. [32] *Amaranthus retroflexus* L, and *Convolvulus arvensis* L. [33] *Atriplex canescens*, *Agropyron elongatum* and *Agropyron desertorum* [34]. According to above researches, it can be firmly concluded that genus Artemisia forms the plants whose allelopathic ability is proved between different species. In this genus, a wide range of active biological compounds are produced which included artemisinin, tannin, flavonoids, sesquiterpene lactone and other secondary metabolites such as coumarin, camphor and bornyl acetate which their toxicity for some other plants is proved [35,36,37] Coumarin prevents the cell from entering the as the first group of mitochondrial mitosis. Flavonoids have been introduced absorption inhibitor that may stop ATP production in mitochondria and affect the breathing [38]. Through preventing from the cell division and cell elongation in the germination stage, flavonoids and coumarin deter germination and reduce the length of root and shoot of the seeds.

#### 5. CONCLUSION

It can be concluded that *Artemisia herba-alba* water extract at different concentrations suppressed the germination of *Raphanus sativus* and *Trigonella foenum-graecum* seeds. And this suppression was possibly due to the presence of allelochemicals in this plant. Based on the results of this study: *Artemisia herba-alba* species have strongest allelopathic potential must be examined for their selective action on other specific plants including weeds and crops under field conditions, their allelopathic activity will be much more detailed. Analysis of possible allelochemicals in this plant is also required. The isolation and characterization of growth inhibitors, which might be responsible for the strong allelopathic potential of this species is needed. There is possibility of using these allelochemicals directly or as structural leads for

the discovery and development of environment friendly herbicides to control weeds.

## COMPETING INTERESTS

Author has declared that no competing interests exist.

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