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

**Abstract:**

Allelopathy is one of the main factors limiting the plants growth, aqueous extracts of aerial parts of *Artemisia herba-alba* were evaluated for their allelopathic effect on germination and seedling growth of *Raphanus sativus* and *Trigonella foenum-graecum* . Treatments were four

concentrations of 0, 20, 40, 80 % Artemisia extract. Treatments were evaluated using a completely randomized factorial experiment in three replications for each treatment in concentrations.

The results showed that aqueous extracts from aerial parts of wormwood plant were caused significant inhibition in germination of *Raphanus sativus* and *Trigonella foenum-graecum* seeds, and the degree of inhibition was concentration dependent. The germination and seedling growth of *Raphanus sativus* and *Trigonella foenum-graecum* decreased significantly as the concentration of the aqueous extracts of *Artemisia herba-alba* increased.

Results of the study indicated that Artemisia had strong allelopathic effects, , it prevented the germination and seedling growth of Radish seeds , while reduced germination of Fenugreek seeds to half, at highest concentrations (80%). Both plumule and radicle length were negatively affected by *A. herba alba* water extract.

plumule and radicle length of Radish was completely inhibited at the

highest concentration of aqueous extract of the donor species level (80%).

This inhibition was markedly in obvious Radish than in Fenugreek,

indicating that is *Raphanus sativus* more sensitive, to the allelophahtic effect of the different concentrations of the aqueous extract of the *A. herba alba* plant.

The present results also indicated the presence of water soluble allelochemicals in wormwood  that are able to inhibit growth of both Radish and Fenugreek.

**Keywords: Allelopathy; Germination; *Artemisia herba-alba*; Aqueous extract.**

**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 neighbouring plants . (Rice, 1984; Weir *et al*., 2004; Inderjit, *et al*., 2006).

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 .(Rice, 1984;  Lovett and Ryuntyu, 1992;  Rizvi and  Rizvi, 1992).

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, (Hussain and Reigosa,  2011).

Phenolic compounds are one in every of the biggest group of secondary metabolites, consisting of four main groups divided in keeping with the number of phenol rings and the structural parts that bind those rings, including flavonoids, phenolic acids, tannins, saponins,  cinnamic acid coumarins, terpenoids,  quinones, and lignans ( Balasundram *et al* 2006). some secondary metabolites are considered as natural pesticides against pathogens, bacteria, fungi, insects, and weeds. (Soltoft *et al* 2008).

Plants or organisms that harness these compounds are referred to as “donor species”, while those that are influenced in their growth and development are called “target or recipient species”.

Allelopathy includes plant-plant, plant-microorganisms, plant-virus, plant-insects, and plant-soil-plant chemical interactions.
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( Inderjit and Keating, 1999).
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 (Scrivanti *et al*, 2003).

*Artemesia herba-alba* Asso. (Asteraceae.), which recently classified as subgenus Seriphidium (Torrell *et al*, 2003), 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 (Alavi, 1983). 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 (Oran and Al-Eisawi, 1998).

Medicinal plants may contain bioactive compounds that possess inhibitory activity.

The seed of *A herba-alba* contain phytotoxic chemicals(Al-Charchfchi *et al*., 1987).These chemicals were toxic to the germination and seedling growth of other plants (Alam *et al*., 2001).

The allelopathic consequences of the genus Artemisia have been broadly investigated (Barney *et al*, 2005; Delabays, 2001; Simon *et al*, 1990) , but a little is known about *A. herba-alba* especially in Libya.
important studies that investigated the allelopathic properties of *A. herba alba* and concluded that it is a remarkably allelopathic species, especially with the increase of concentration.
According to Arroyo *et al*. (2016), the application of *A. herba-alba* aqueous extract reduced seedling emergence from the seed bank by 50%. Other authors reported similar results regarding the exhibition of allelopathy by many herbaceous, aromatic shrubs and trees species in

regions that have similar environmental conditions to the Mediterranean ecosystems (Thompson, 2005; and Scognamiglio *et al*., 2013). It seems that most species of the genus Artemisia have general positive tendency towards allelopathy yet with different extents between its species.
Examples of this tendency are documented by several recent studies.
A large number of secondary metabolites are produced in Artemisia of family Asteraceae that is widely recognized due to its medicinal potential.

Over 250 species of Artemisia are distributed throughout the world

(Guvenalp *et al*.,1998 ), and several studies have reported allelopathic

tendency of this genus most of these studies were carried out by making the plant extracts. (Friedman *et al*., 1977; Hoffman and Hazlett, 1977; Kelsey *et al* ., 1978 ).

*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 hypothesed that this species has allelopathy effects and can be used as determination factor for the growth of some plants.
The present research is to explore the allelopathic effects of aqueous extract from the aerial parts (leaves and stems) of *Artemisia herba-alba* (donor species) growing in Al-Gabal Al-akhdar on germination efficiency of two plants *Raphanus sativus* and *Trigonella foenum-graecum*, (recipient species) under laboratory conditions.

## 1.Material and Methods:

The seeds of Radish and Fenugreek were obtained from local market. Seeds were kept in the containers which they were supplied, and stored in the laboratory at room temperature until required for sowing.

### 2.1. Plant Material Collection

*A. herba-alba* samples were collected from the south of Al-Jabal Al-Akhdar - EL–Byda city – Libya ( Marawa region) , in October 2018, collected only areal parts.
The plant were classified and authenticated according to Jafri and EL-ghadi (1978). In Depatment of Botany, Faculty of Science , Omar Al-Mukhtar University, EL- Byda, Libya.

### Preparing the aqueous extract of *Artemisia herba-alba*

After the collection of the plants, they were dried in a shady place at room temperature for ten days.

At the Botany department laboratory of Omar Al-Mukhtar University, the dried areal parts (leaves and stems) were ground into powdered form, then 50 g of the powder were diluted into 500 ml of distilled water (Bajalan et al., 2013). Next to that, this mixture was left on a Shaker for 24 hrs in room temperature at speed of 120 rpm (Ghorbani *et al*., 2008). four-folded cotton fabric was used as a filter to separate rough solid particles from solution, and then it was centrifuged with the speed of 2000 rpm for 15 minutes (Bajalan *et al*., 2013).

Three concentrations of solutions were prepared based on volume/volume percent (v/v. %) water extract was diluted with distilled water to prepare solutions of different concentrations (20, 40, and 80%) in addition to the distilled water as a control (0%) Elshatshat (2010).

To achieve this experiment, 60 treated seeds were randomly collected and randomly distributed on 12 Petri dishes (90 mm diameter), five seeds per dish. Each treatment three Petri dishes (three replicates).

The parameters of interest in this part of the study was the germination percentage and seedling growth after the application of (distilled water, 20, 40, and 80% of *Artemisia herba-alba*).

All dishes were placed in a low temperature incubator at 21 $^{o}$C for seven days Two ml of each level of the donor species extract (20, 40 and 80%) were added daily to three replicates.

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 (PL) and radicle length (RL) were recorded after one week at the end of the experiment.

Relative reduction or stimulation of seed germination and radicle length as affected by the allelopathic substance were calculated . （ Salhi *et al*. 2011).

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 ± Standard Division (SD). P-value below 0.05 was considered to be statistically significant.

**RESULTS:**

**Germination percentage (GP):**

The germination percentage of both Radish and Fenugreek was significantly affected by the increase at different concentration levels of Artemisia aqueous extract after seven days of germination.

Results of data statistical analysis of both plant receptors had been shown in tables 1, 2 , these results showed that ***Artemisia herba-alba***. extracts have deterrence effect potential on germination and early growth of *Raphanus sativus* and *Trigonella foenum-graecum*. The germination

of both plant receptors was influenced in a different way by diverse concentrations of ***A. herba-alba*** aqueous extracts .

The concentrations (20, 40 and 80%) of aqueous extracts considerably suppressed the germination compare to control treatment.

The total percentage of Radish seed germination was decreased by increasing the concentration of Artemisia aqueous extract, at control GP value was about (93.33 %) . The percentage was reduced to (56.67%) at 20% and to ( 40%) at 40% respectively.

The maximum allelopathic effect was recorded in 80% Artemisia aqueous extract concentration, which completely inhibited Radish seed germination . (Table1).

The allelopathic effects of ***A***. ***herba-alba*** aqueous extracts on Fenugreek seeds were also was evaluated. The total percentage of Fenugreek seed germination was decreased by increasing the concentration of Artemisia aqueous extract, at control 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. (Table2).

It is clear from these findings that, inhibitory response varied with the concentration of aqueous extract. and at higher concentration (80%) , the extract prevented the germination and seedling growth of Radish seeds. while reduced germination of Fenugreek seeds to less than half.

**Plumule length (PL)**

Findings of PL of *Raphanus sativus* imply the downbeat effect of the allelopathic substances on seedling stage (Table 1).
Evidently, PL was significantly reduced (P< 0.05) either 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 at20 % , and to 2.2 cm at 40 %, the maximum allelopathic effect was recorded in 80% Artemisia aqueous extract concentration, which completely inhibited PL.
The allelopathic effect of Artemisia aqueous extract on PL of *Trigonella foenum-graecum* indica is illustrated in (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.
Statistically, the applied concentrations of Artemisia aqueous extract was highly significant (P< 0.05) Actually, 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 2.1cm respectively).

**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 extracts concentrations have possessed a significant inhibitory effect on radical growth (P< 0.05). 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. 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 (P< 0.05). At control, the values of RL were 4.4 cm. higher concentrations of *A. herba-alba* aqueous extracts were notably active disturbing radicle emergence. at 20, and 40 % concentrations, RL decreased to3.5 and 3.2 cm. Constantly, it continues reduction till it attained a value of about 1.8 cm at 80 % concentration level.

Table1. 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 Mean ± SD | GP % | RL(cm) Mean ± SD | PL(cm) Mean ± SD |
|---|---|---|---|---|
| 0 | 18.67$^{a}$ ±0.47 | 93.33 | 6.1$^{a}$ ± 0.09 | 4.2$^{a}$ ± 0.16 |
| 20 | 11.33$^{b}$ ±0.94 | 56.67 | 2.3$^{b}$ ± 0.08 | 2.7$^{b}$ ± 0.14 |
| 40 | 8$^{c}$ ± 0.82 | 40 | 1.5$^{c}$ ± 0.05 | 2.2$^{c}$ ± 0.12 |
| 80 | 0$^{d}$ | 0 | 0$^{d}$ | 0$^{d}$ |

Data are expressed as mean ± 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 Mean ± SD | GP % | RL(cm) Mean ± SD | PL(cm) Mean ± SD |
|---|---|---|---|---|
| 0 | 19.67$^{a}$ ± 0.47 | 98.33 | 4.4$^{a}$ ± 0.24 | 5.9$^{a}$ ± 0.09 |
| 20 | 16$^{b}$ ± 0.82 | 80 | 3.5$^{b}$ ± 0.05 | 4$^{b}$ ± 0 |
| 40 | 12.67$^{c}$ ± 1.2 | 63.33 | 3.2$^{b}$ ± 0.12 | 3.6$^{b}$ ± 0.21 |
| 80 | 9.33$^{d}$ ± 0.94 | 46.67 | 1.8$^{c}$ ± 0.16 | 2.1$^{c}$ ± 0 .14 |

Data are expressed as mean ± 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).

**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 (Saberi *et al* 2012).

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 (Kohli *et al*., 2008).

There by *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 (Daizy *et al*., 2007). (Fateh et al., 2012 ; Shang and Xu,2012).

Aqueous extract of plants may contain phenolics such as ferulic acid P-coumaric, vanillic, caffeic, chlorogenic and others. (Hussain and khan, 1988; Habib and Abdul Rehman, 1988). These phenolics inhibit the germination process (Williams and Hoagland, 1982; AL-Charchafchi *et al*., 1987), which was due to their interference with indol acetic acid metabolism, or synthesis of protein and ion uptake by the plants (Hussain and khan, 1988). Therefore, *A. herba alba* might release some soluble phenolic allelochemicals to the environment (Xu *et al*., 2003), 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 Escudero *et al*., (2000) and Periotto *et al*., (2004) 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. S. A. Elshatshat (2010) 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. (Akramghaderi *et al*., 2001), *Amaranthus retroflexus* L, and *Convolvulus arvensis* L. (Tabatabaee Zadeh *et al*., 2014), *Atriplex canescens*, *Agropyron elongatum* and *Agropyron desertorum* (Bagheri and Mohammadi, 2011),

According to above researches, it can be firmly concluded that grenus

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 (Lydon *et al*., 1997; Macro and Babera, 1990; Klyman, 1985). 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 (Maighany, 2003). 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.

**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.

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