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An investigation of allelopathic effects of *Artemisia herba-alba* Asso, aqueous extract on common bean (*Phaseolus vulgaris* L.)

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Abstract

The objective of this study was to investigate the impact of allelopathic potentials of aqueous extract of *Artemisia herba-alba* aerial parts on germination and secondary metabolites accumulation in common bean seedlings. Quantitative analysis of aqueous extract of Artemisia showed the phenolic compounds and flavonoids that might be concerned as allelo-chemicals agents. After 8 days, the germinated common bean seedlings were significantly inhibited with the increase in concentration of Artemisia extract. However, the phenolic compounds in common bean seedlings increases when the concentration of Artemisia extract increased. Degradation of storage carbohydrates of common bean seedlings was significantly increased with increasing the concentration of Artemisia extract. It seems that the enhancement of total carbohydrates was attributed to the inhibition of amylase activity that reduced the contents of reducing and non-reducing sugars. However, the polysaccharides remained at high level when compared with those of the control. Inhibition of protease activity led to accumulation of free amino acids and protein contents in germinated seedlings of common bean. Although, 4% aqueous extract of Artemisia shoots contains allelopathic chemicals and phytotoxic, this effect led to an increase in unsaturated and saturated fatty acids in germinated common bean seedlings.

الملخص العربي

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تهدف هذه الدراسة إلي تحديد تأثير قوة الاليلوباثي للمستخلص المائي للأجزاء الهوائية (المجموع الخضري) لنبات الشيح على إنبات وتراكم النواتج الثانوية لشتلات نبات الفاصوليا، أظهر التحليل الكمي للمستخلص المائي لنبات الشيح احتوائه على مركبات فينولية وفلافونيدات وهي تعتبر عوامل أليلوكيميائية، بعد ثمانية أيام وجد أن زيادة تركيز المستخلص المائي لنبات الشيح قد أدى إلى تثبيط نمو الشتلات النامية وأدى إلى زيادة نسبية للمركبات الفينولية الموجودة في شتلات الفاصوليا، كما أدى إلى زيادة معنوية للكربوهيدرات المخزنة في شتلات الفاصوليا بزيادة تركيز المستخلص المائي للمجموع الخضري لنبات الشيح، ويبدو إن هذه الزيادة في الكربوهيدرات يعزى إلى تثبيط إنزيم الأميليز الذي أدى إلى خفض في السكريات المختزلة والغير مختزلة ، ومع ذلك ظلت السكريات في مستواها العالي مقارنة بالشتلات الغير معاملة بالمستخلص (Control)، في حين أدى التثبيط في الأنزيم البروتيني إلى تراكم في الأحماض الأمينية الحرة والبروتينات الموجودة في شتلات الفاصوليا، وعلى الرغم من تركيز 4 % من المستخلص المائي للمجموع الخضري لنبات الشيح له تأثيرات كيموآليلوباثيه وسمية نباتية فإن هذا التأثير أدى إلى زيادة في الأحماض الدهنية المشبعة والغير مشبعة في شتلات الفاصوليا النامية.

Keywords *Allelopathy, Artemisia herba-alba, Seedling Growth, Secondary metabolites*.

1. Introduction

Artemisia, one of the larger genera in the family Asteraceae and the largest genus in the tribe Anthemideae, comprises from 200 to more than 500 taxa at the specific or subspecific level. Many Artemisia species have a high economic value in several fields, *Artemisia herba-alba is* a common perennial shrub in semi- deserts of Middle East (Zohary,1973) and it is one of the most common medical species of *Artemisia herba alba*. It is widely used in folk medicine by Bedouins (Al-Shamaony *et al.*, 1994) as hot and cold drink. Medicinal plants may contain bioactive compounds that possess inhibitory activity. *Artemisia herba alba* has mucilaginous cells which contain phytotoxicity chemicals (Al-Charchafchi *et al.*, 1987) such as sterols, terpenes, flavonoids, saponins and tannins (Kanitah, 2011). These chemicals were toxic to the seed germination , seedling growth and other physiological factors of the plants (Alam *et al.*, 2001) or even auto-toxic to same plant species (Al- Charchafchi *et al.*, 1987).

The effects of one plant on other plant through the release of chemical compounds in the environment can be defined as allelopathy (Hussain and Khan 1988). Allelopathy is an important mechanism of plant interference mediated by the addition of plant produced phytotoxins to the plant environment and competitive strategy of plants. Allelopathy involving secondary metabolites is produced by plants, microorganisms, viruses, and fungi that influences growth and development of biological systems (Oussama, 2003). Over whelming evidence suggests that plant phenolic play a major role in allelopathy (An *et al.*, 2000). Many researchers have found that the inhibitory substances involved in allelopathy that are terpenoids and phenolic substances (Alexa *et al.*, 2004; Chaves and Escudero, 2006; Khan *et al.*, 2007). A wide array of biologically active constituents is produced by plants in the genus Artemisia (Lixf Wang et al., 2010 ; Modallal and Al- Charchafchi, 2006; Moussavi-Nik *et al.,* 2011).

This study was undertaken to assess the allelopathic effects of aqueous extract of *Artemisia herba-alba* aerial parts on seed germination, growth and biochemical changes associated with germinated common bean (*Phaseolus vulgaris* L.**)** seeds.

2. Materials and Methods

2.1. Laboratory experiment:

Aerial parts of *Artemisia herba-alba* were collected from Wadi Derna in Libya during June 2012, dried and four different weights of 1, 2, 3 and 4 g were separately soaked in 100 ml of double distilled water for 48 hours at room temperature. The mixture was filtered through Whatmann paper No. 1 to obtain 1%, 2%, 3% and 4% (w/v). Four concentrations beside the control were chosen for the experimental study.

Seeds of common beans (*Phaseolus vulgaris* L.**)** were surfaces sterilized with 5% aqueous solution of sodium hypochlorite for 2 minutes, rinsed five times with distilled water and dried between two paper towels. The experimental design was complete randomized with four replicates. Twenty seeds were soaked in 50 ml of each concentration of Artemisia extract for 24 hours then washed with distilled water. Another twenty seeds were soaked in 50 ml distilled water served as control. The treated and untreated seeds were then placed in petri dishes on Whattman No.1 filter paper under laboratory conditions. Ten ml of extract solutions were added to petri dishes and germination was counted after 2, 4, 6 and 8 days from starting the experiment. Seeds were considered germinated when the radical extended through the seed coat.

2.2. Biochemical measurements:

Phenolic contents were extracted in ethanolic potassium hydroxide with refluxing, separated on paper chromatography and determined by HPLC (Harborne, 1984). Total amino acids were hydrolyzed with HCl (6N) in sealed tubes for 24 hours at 120 °C and analyzed using amino acid analyzer (Black burn, 1986). Fatty acids were extracted by petroleum ether (40-60 °C), saponification was done by alcoholic 40% NaOH, methylation with diazomethane and methyl esters then analyzed by using GLC (IUPAC, 1979). The total carbohydrate was determined according to the method of Nelson (1944) and Somogyi (1952). Protein content was estimated by Lowry *et al.* (1951). Amylase and protease activities were assayed in the crude extract of fresh tissues using the method of Afifi *et al.* (1986) and Ong and Gaucher (1973).

2.3. Statistical analysis:

Differences between treatments and control were determined by one- way analysis of variance (ANOVA) followed by Least Significant Differences (LSD) for comparing means. All statistical analysis were performed using Costat version 2 software programme.

3. Results and Discussion

The results of mean effect of aqueous extract of *Artemisia herba-alba* aerial parts on germination percentage of common bean seeds are shown in Table (1). The results clearly that the two concentrations of aqueous extracts of *Artemisia herba-alba* aerial parts (1 and 2 %) significantly enhanced the germination percentage of common bean seeds through the entire time of experiment. The other two concentrations (3 and 4%) of Artemisia extract had highly inhibitory effects on the germination percentages of common bean seeds.

Table 1. Effect of aqueous extract of *Artemisia herba-alba* aerial parts on germination percentage of common bean seeds.

Treatments	Germination (%) at different times in days*			
	2	4	6	8
Control	46.5	57.0	80.0	86.6
1%	50.1	79.0	85.7	93.0
2%	54.0	85.6	93.5	100.0
3%	45.5	69.5	68.5	80.0
4%	20.0	39.0	51.0	70.0
LSD 1%	2.1	2.5	2.5	2.9
LSD 5%	1.43	1.82	1.75	1.98

*Average based of four replicates

The inhibitory effect was pronounced in 4% extract than in 2% extract and this indicates that the aqueous extract contained growth inhibiting allelochemicals and their effects were dependent on the extract of Artemisia concentration. These results are in agreement with the results of Abdel-Fattah *et al.* (2011); Adrian *et al.* (2000) and Lixf Wang *et al.* (2010). They showed that Artemisia extracts decreased the percentages of seed germination of several crops.

Table 2. Chemical constituents of 1% aqueous *Artemisia herba-alba* aerial parts extract.

No.	Constituents	Concentration (µg/ml)
1	Quercetin 3-O-glucoside	8.39
2	Quercetin 3-O-galactoside	8.35
3	Quercetin 3-O-glucosylgalactoside	6.21
4	Quercetin 3-O-rutinoside	9.31
5	Isorhamnetin 3-O-rutinoside	4.51
6	1,3,6 tri-O-galloyl-β-glucopyranose	5.93
7	1,6 di-O-galloyl-β-glucopyranose	4.98
8	1-O-galloyl-β-glucopyranose	3.39
9	Reducing sugars	200.90
10	Free amino-N-	0.85
11	Free ammonia	20.95

Table 3. Effect of aqueous extract of *Artemisia herba-alba* aerial parts on the concentrations of phenolic contents (µg/ml) of germinated common bean seeds

No.	Phenolic contents	Control	2% extract	4% extract
1	Quercetin 3-O-glucoside	0.17	2.45	3.75
2	Quercetin 3-O-galactoside	0.19	2.12	3.43
3	Quercetin 3-O-glucosylgalactoside	0.20	1.60	2.10
4	Quercetin 3-O-rutinoside	0.97	1.97	2.25
5	Isorhamnetin 3-O-rutinoside	0.47	1.03	1.98
6	1,3,6 tri-O-galloyl-β-glucopyranose	0.85	1.03	2.95
7	1,6 di-O-galloyl-β-glucopyranose	0.18	0.81	1.96
8	1-O-galloyl-β-glucopyranose	0.17	0.45	1.65

Table (2) shows the constituents of 1% aqueous *Artemisia herba-alba* aerial parts extract. The data analysis showed that the presence of five known flavanol glycosides (4.51- 9.31 µg/ml) and three galloyl glucose (3.93- 5.93 µg/ml), reducing sugars (200.90 µg/ml), free amino-N- (0.85 µg/ml) and free ammonia (20.95 µg/ml). The flavanoids show antagonistic properties with plant hormones Indol Acetic Acid (IAA) metabolism, protein synthesis and ion uptake by the plants (Hussain and Khan, 1988). In the present study, the results revealed that the aqueous aerial parts of Artemisia extract was more capable for inhibiting roots of common bean than shoots. This may be due to the direct contact between the roots and phenolic compounds of the aqueous extract which may in turn inhibit cell division (Rietjens and Alink, 2003) which is highly active in meristematic tissues for the growing roots. In this respect, flavanoids may leak from shoots, leaf litter or roots into the soil solution and inhibit seed germination and radical elongation (Berhow and Voughn, 1999). The phenolics are responsible agents for allelopathic effects of *Artemisia herba-alba* extracts on common bean. Phenolic compounds present as free forms esters or as glycosides when combined with sugars that may be indirectly related to chemicals which responsible for allelepathic effects (Seal *et al.,* 2004).

Table (3) indicated that the total phenolic compounds for germinated common bean seeds were increased with increasing the concentration of Artemisia extract. These phenolic compounds are water soluble and leach from shoots and roots.

Results in Table (4), indicated that the total soluble sugars in germinated common bean seeds were significantly decreased with increasing the concentration of Artemisia extract. Whereas the opposite effect was noticed with polysaccharides, total carbohydrates and protein contents when compared to the control.

Table 4. Carbohydrates and protein contents (mg/g dry weight) in common bean seedlings treated with aqueous extract of *Artemisia herba-alba* aerial parts.

Treatments	**Soluble sugars**		**Total soluble sugar**	**Poly-saccharides**	**Total carbohydrate**	**Protein**
	Reducing	**Non-Reducing**				
Control	20.31	35.28	55.59	125.00	180.00	160.20
2% extract	15.41	30.35	45.76	143.10	188.80	190.40
4 % extract	0.43	26.20	36.63	156.10	192.70	195.40
LSD at 1%	2.32	2.69	3.12	2.14	2.62	3.52
LSD at 5 %	1.51	1.58	1.87	1.39	1.65	1.98

Each value represents mean of four replicates

The aqueous extracts of Artemisia was inhibited the activities of amylase and protease, this effect increased with increasing the extract concentration. The reduction of amylase caused decreases in the level of the total soluble sugars (reducing and non-reducing) and the increase in the level of polysaccharides as compared with the control values (Table 5). The same findings were obtained by El-Darier (2002), Pandey and Mishra (2005) and El- Khawas and Shehata (2005).

Table 5. Amylase and protease enzyme activities (Unit/mg protein) in common bean seedlings treated with different concentrations of aqueous extract of *Artemisia herba-alba* aerial parts.

Treatments	Amylase	Protease
Control	2.46	0.81
2% extract	2.31	0.61
4 % extract	0.76	0.42
LSD at 1%	1.03	0.21
LSD at 5 %	0.11	0.14

In the present investigation, proline and phenyl-alanine contents were increased by increasing the extract concentration as compared with control treatment (Table 6).

Table 6. Total amino acids (mg/g dry weight) of germinated common bean affected by aqueous extract of *Artemisia herba-alba* aerial parts

Treatments		Control	2% extract	4% extract
Amino Acids	Glu.	4.35	14.12	17.00
	Arg.	0.73	1.89	5.31
Prol.		0.85	0.63	3.00
Orn.		-	-	-
His.		0.12	0.16	0.31
NLeu.		-	-	-
Asp.		2.11	1.61	5.91
Threo.		0.15	0.95	1.35
Lys.		0.25	0.99	1.38
IsoLeu.		0.75	2.60	2.10
Meth.		0.13	0.16	0.39
Gly.		0.11	0.41	1.63
Ser.		0.45	1.53	2.43
Cyst.		0.62	0.51	2.40
Ala.		0.81	2.42	1.34
Val.		0.73	2.12	1.25
Shikmic acid family	Ph.ala	0.53	1.65	1.93
	Tyr.	0.12	0.56	1.19

Total Amin acids	12.81	32.31	48.90

This concept was confirmed where the biosynthesis of phenolics released from the aqueous extract of *Artemisia herba-alba* is derived from the shikimic acid pathway. Also, flavanoids contains structural groups originated from the Shikimic phenyl-alanine (El-Khatib *et al.*, 2004). Thus, a higher level of amino acids was assumed for the increasing in protein contents as shown in Table (4). The high level of protein content was accompanied by a great decline in the protease activity that plays an important role in the hydrolysis of reserve proteins during germination of common bean seeds. Similar results obtained by other investigators (El-Khatib and Hegazy 1999; El- Khawas and Shehata 2005).

Table 7. Total fatty acids (saturated and unsaturated) as percentages of germinated common bean affected by aqueous extract of *Artemisia herba-alba* aerial parts

Treatments		**Control**	**2% extract**	**4% extract**
Saturated (%)	Caprylic (8:0)	0.33	0.66	0.25
	Capric (10:0)	0.01	-	0.78
	Louric (12:0)	0.08	1.02	1.45
	Myristic (14:0)	-	19.10	0.91
	Palmatic (16:0)	14.00	18.95	15.40
	Streric (18:0)	7.50	-	0.04
	Arachidic (20:0)	2.13	1.28	2.25
	Erucic (22:0)	-	-	0.82
	Total	24.05	41.01	21.65
Unsaturated (%)	Oleic (18:1)	41.50	90.80	37.11
	Linoleic (18:2)	32.00	70.90	37.60
	Linolenic (18:3)	-	23.20	-
	Total	73.50	184.90	74.71

Results in Table (7) showed that at any reduction in saturated fatty acids, there was a regular increasing in unsaturated one, especially those treated with 4% aqueous extract of Artemisia aerial parts. This increasing effect may be related to the accumulation of Myristic acid (14:0), Palmitic acid (16:0), Oleic acid (18:1), Linoleic acid (18:2) and linolenic acid (18.3) in 2% concentration of Artemisia extract. The absence of Erucic acid (22:0) is a typical characteristic of control samples and 2% Artemisia extract. These results are in agreement with An *et al.* (1993). Who showed that any secondary compound with allelochemical activity can cause both stimulatory and inhibitory effects. This pattern is common and it related to concentration:

stimulation at low concentrations and suppression for higher concentrations. During growth period often cause to increase production of secondary metabolites (Einhelling, 1996; Weir *et al.* 2004) and other metabolic compounds as free amino acids, proline, sugars, organic solutes (Ercisli *et al.* 2005). This study tended to accumulate amino acids, proline and unsaturated fatty acids which may be considered an adaptive mechanism to increase stress tolerance and seed germination, growth and biochemical changes associated with germinated common bean seeds.

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