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Full Length Research Paper

Physiological effects of allelopathic Activity of *Artemisia monosperma* on common bean (*Phaseolus vulgaris* L.)

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Abstract

The objective of this study was to determine the impact of allelopathic potentials of aqueous extract of *Artemisia monosperma* aerial parts on germination and metabolite accumulation in common bean seedlings. *Artemisia monosperma* collected from Al-Thomamah region, Saudi Arabia and the experimental design was a complete randomized with four replicates. The results showed that the quantitative analysis of aqueous extract of *Artemisia* contained the phenolic compounds and flavonoids that might be implicated as allelochemicals agents. The germinated seedlings after 8 days were inhibited with the increase in concentration of aqueous Artemisia aerial parts, but the phenolic compounds of the common bean seedlings had stimulatory effects with high level of concentration of Artemisia aerial parts extract. Degradation of storage carbohydrates of common bean seedlings was significantly retarded with increasing the concentration of Artemisia aerial parts extract. It seems that the retardation was attributed to the activity inhibition of amylase enzyme that reduced the contents of reducing and non-reducing sugars. However, the polysaccharides remained at high level when compared with those of the control. While, inhibition of protease activity led to accumulation of free amino acids and protein contents in germinated seedlings of common bean. Although aqueous extract of Artemisia shoots contain allelopathic chemicals and phytotoxic, this effect led to increase in unsaturated fatty acids than saturated fatty acids in germinated common bean seeds.

Keywords: Allelopathy, *Artemisia Monosperma*, Seedling Growth, Phenolics.

INTRODUCTION

Plants may affect other plants growing in their vicinity in a stimulatory or inhibitory manner through released biologically active compounds often termed as allelopathics, allelocompounds or allelochemicals. This phenomenon is termed as allelopathy, receiving an increased attention recently and is considered to be applied in practice for weeds and pest managements (Prasanta et al., 2003). Allelopathic effects are common for many plant species and can be observed at any level of biological organization (Gawronska et al., 2000; Gawronska, 2003; Chon et al., 2003; Gholami et al., 2011; Al-Rabiah, 2012). Several Asteraceae species have been reported for having allelopathic effects on other plant species, reducing seed germination and emergence of subsequent small grain crops when grown in rotation (Miky, 2008; Abu-Romman, 2011). Plant extract that is not decomposed was thought to contain secondary compounds with allelochemical activity or phytotoxic which cause growth inhibition (An et al., 1993). However allelopathy may alter the available resources in the environment (Wardle et al., 1998). Allelochemicals are believed to be a joint action of several secondary metabolites including phenolic compounds (Dalton, 1999), flavonoids (Berhow and Voughn, 1999), juglone (quinone) (Jose and Gillespie,

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1998), terpenoids (Langenheim, 1994) and non protein amino acids like alkaloids (Harborne, 1988). 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; Khanh et al., 2007)

Phenolics are broad variety of compounds with a wide array of allelechemical activities and most important in allelopathy. Phenolic compounds are water soluble and leach from leaves, stem and roots into the soil solution (Katase, 1993; Zhu and mallik, 1994).

A wide array of biologically active constituents is produced by plants in the genus *Artemisia* (Moussavi-Nik et al., 2011; Lixf et al., 2010, Modallal and Al-Charchafchi, 2006). The volatile oil of *Artemisia ordosica* resulted in inhibition the growth and photosynthetic activity of *Palmellococcus miniatus* (Yang et al., 2012).

Artemisia monosperma is a common perennial shrub in deserts of Saudi Arabia (Chaudhary, 1999) and one of the most common medicinal species of *Artemisia*. It is widely used in folk medicines (Stavri et al., 2005). *Artemisia monosperma* contains bioactive compounds e.g. sterols, terpenes, flavonoids, saponins and tannins (Kanitah, 2011).

The purpose of this study was to assess the allelopathic effects of *Artemisia monosperma* on seed germination characteristics, primary growth and biochemical changes associated with germinated common bean seeds.

MATERIALS AND METHODS

Samples of *Artemisia monosperma* were collected from Al-Thomamah region, Saudi Arabia during April 2011 and identified by plant taxonomist. A voucher specimen has been deposited at the Herbarium of Botany Department, Faculty of Science, King Saud University.

Four different weights (1, 2, 3 and 4 grams) of dry *Artemisia monosperma* aerial parts that each weight were separately soaked in 100ml of double distilled water for 48 hours at room temperature. The mixture was filtered through Whatmann paper No. 1 filter paper to obtained 1%, 2%, 3% and 4% w/v. Four levels beside the control were chosen for the germination experiments of this study.

Seeds of common beans were obtained from the Agriculture Research Centre Giza, Cairo. Then, their surfaces sterilized with 5% aqueous solution of sodium hypochlorite for 2 minutes, rinsed five times with distilled water and dried between two paper towels. Twenty seeds were soaked in 50 ml of each level of *Artemisia* extract for 24 hours then washed with distilled water. These seeds were then placed in petri dishes on Whattman No.1 filter paper under laboratory conditions at 25^{o} C. Ten ml of solutions were added to petri dishes and germination was counted after 2, 4, 6 and 8 days from starting the experiment. Seeds were considered as germinated when the radical extended through the seed coat.

Phenolic content was estimated in the ethanolic Potassium hydroxide extract. Paper chromatography was carried out on Whattman No.1 filter paper using solvent systems H_2O: HOAc (47:3) and BAW (4:1:5). Samples analyzed by using HPLC (Harborne, 1984).

Total amino acids were hydrolyzed with 6N HCl in sealed tubes for 24 hours at 120^{o} C (Black burn, 1986). The samples were estimated using amino acid analyzer (Eppendorf). Fatty acids were extracted by petroleum ether (40-60^{o}C), then saponification was done by alcoholic 40% NaOH, methylation with diazomethane and methyl esters then analyzed by using GLC (PYEUNICAM) (IUPAC, 1999).

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 activities were assayed in the crude extract of fresh tissues using the method modified from that described by Afifi et al., (1986), whereas the estimation of protease enzyme was made using the method of Ong and Gaucher (1973).

All samples estimated variability were treated with one way ANOVA test using Co stat 2.0 Statistical analysis software and the means were compared using Least significant difference (LSD).

RESULTS AND DISCUSSION

The results of mean comparisions for effect of aqueous extract of *Artemisia monosperma* aerial parts on germination percentage of common bean seeds

Shown in Table 1 clearly revealed that the two aqueous extracts of *Artemisia monosperma* aerial parts (1 and 2 %) stimulated the germination percentage of common bean seeds. On the other hand, the other two concentrations (3 and 4%) of aerial parts extract of *Artemisia* had great inhibitory effects on the germination percentage and early seedling growth of common bean seeds.

This indicates that the aqueous extract contained growth inhibiting allelochemicals and their effects were dependent on the extract of *Artemisia* concentration. These results were in agreement with Abdel-Fattah et al., (2011) who observed that allelopathic effects can cause both stimulatory and suppressive effects at lower and higher concentrations respectively.

The same results were obtained by Seyed et al., (2011) who showed that in different extract concentration of *Artemisia annua*, the most germination percentage is related to control and the least was related to 100 % of the extract.

Also, other scientists such as Adrian et al., (2000); Lixf et al., (2010) and Yang et al., (2012) revealed that the inhibitory effect of *Artemisia* water extract was directly related to the allele chemical concentration.

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

Extract Concentration (w/v)	Germination time (days) %			
	2 days	4 days	6 days	8 days
Control	46.5	57.0	80	86.6
1%	50.1	79.0	85.7	93.0
2%	54.0	85.6	93.5	100
3%	45.5	69.5	68.5	80
4%	20	39	51	70
LSD 1%	2.110	2.520	2.543	2.962
LSD 5%	1.43	1.82	1.75	1.98

Table 2. Chemical content of 1% aqueous *Artemisia monosperma* aerial parts extract

	Costituents	*u*g/ml extract
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	200.9
10	Free amino-N-	0.85
11	Free ammonia	20.95

Table 3. Effect of aqueous extract of *Artemisia monosperma* aerial parts on the phenolic contents of germinated common bean seeds

	Phenolic contents	Control	2%	4%	
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	1.6	2.1	
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

Analysis of 1% aqueous extract of *Artemisia* showed that the presence of five known flavanol glycosides and three galloylglucose in different concentrations, in addition to reducing sugars, free ammonia and free amino N (Table 2). In this respect, flavanoids may leach from shoots, leaf litter or roots into the soil solution and inhibit seed germination and radical elongation (Berhow and Voughn, 1999). The flavanoids also show antagonistic properties with plant hormones Indol Acetic Acid (IAA) metabolism, or protein synthesis and ion uptake by the plants (Hussain and Khan, 1988).

Interestingly the present results revealed that shoot aqueous extract was more capable for inhibiting root length of common bean than shoot length. 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. The phenolics were the responsible agents for allelopathic effects of *Artemisia monosperma* extracts on common bean. Phenolic compounds exist as free forms esters or as glycosides when combined with sugars and is water soluble that may be indirectly related to chemicals that are finally responsible for the observed allelepathic effects (Seal et al., 2004). Table 2.

Table 3 showed that the decreasing of total phenolic compounds for common bean at 2 % concentration of *Artemisia* shoots rather than increasing at the 4 % concentration of *Artemisia*. These phenolic compounds are water soluble and leach from shoots and roots into

Table 4. Effect of aqueous extract of *Artemisia monosperma* aerial parts on the carbohydrate and protein contents (mg/g dry wt.) of growing common bean.

Content/ Concentration	Soluble sugars		Total soluble sugar	Polysaccharides	Total carbohydrates	Protein
	Reducing	Non-Reducing				
Control	20.31 ± 0.32	35.28 ± 0.27	55.59 ± 0.04	125 ± 0.80	180 ± 0.79	160.2 ± 0.91
2%	15.41 ± 0.12	30.35 ± 0.31	45.76 ± 0.24	143.1 ± 0.88	188.8 ± 0.98	190.4 ± 2.3
4 %	10.43 ± 0.23	26.2 ± 0.25	36.63 ± 0.28	156.1 ± 0.47	192.7 ± 0.51	195.4 ± 0.35
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

Note: All units are mg/g

Table 5. Effect of aqueous extract of *Artemisia monosperma* aerial parts on the activities of amylase and protease enzymes (mg/g dry wt.) of growing common bean.

Content/ Concentration	Amylase	Protease
Control	2.46 ± 0.03	0.81 ± 0.02
2%	2.31 ± 0.02	0.61 ± 0.02
4 %	0.76 ± 0.02	0.42 ± 0.02
LSD at 1%	1.03	0.21
LSD at 5 %	0.11	0.14

Note: All units are mg/g

the soil solution and are taken by the roots and translocated through the plants (Seal et al., 2004). Fordonski and Rutkowski (1988) declared that common bean contain the antioxidant activity such as Flavanoids, Phenolic acids and other phenols.

The early seedling of the plant contains complex compounds of carbohydrates, proteins and lipids. When seed germination starts, the stored compounds in the cotyledons are broken down by hydrolytic enzymes activities. As shown in Table 4, the aqueous extract of *Artemisia* aerial parts inhibited the amylase activity, an effect which increased with the increase in the extract concentration. This reduction caused decrease in the level of the total soluble sugars (reducing and non-reducing) and the increase in the level of polysaccharides as compared with the corresponding control values (table 5). The same findings were obtained by El- Darier (2002), Pandey and Mishra (2005), El- Khawas and Shehata (2005).

In this investigation, proline and phenyl-alanine contents were increased by raising the extract concentration as compared with control treatment (Table 6). This concept was confirmed in the present study where the biosynthesis of phenolics released from the aqueous extract of *Artemisia monosperma* is derived from the shikimic acid pathway. Also, flavanoids contains structural groups originated from both the Shikimic Phenylalanine (El-Khatib et al., 2004). Thus a higher level of amino acids was assumed for the increasing in protein contents (Table 5). 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 were observed by a number of investigators (El- Khatib and Hegazy, 1999; El- Khawas and Shehata, 2005).

Results in Table 7 showed that at every reduction in saturated fatty acids, there was a regular increasing in unsaturated one, especially those treated with aqueous extract of *Artemisia* aerial parts with concentration (4%). The previous increasing may be related to the accumulation of Myristic acid (14:0), Palmitic acid (16:0), Oleic acid (18:1), Linoleic acid and linolenic acid (18:2, 18:3) in 2% concentration of *Artemisia* aerial parts. The absence of Erucic acid (22:0) is a typical characteristic of control samples and 2% concentration shoots extract of *Artemesia*.

Also Barat et al., (2011) showed that the highest germination percentage of seed weeds occurred in control which was decreased with increasing extract concentration.

These results are in agreement with An et al., (1993).They 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.

It is familiar that both biotic and abiotic stresses

Table 6. Effect of aqueous extract of *Artemisia monosperma* aerial parts on the total amino acids (mg/g dry wt.)of germinated common bean.

Contents/ Concentration	Amino Acids Glu.	Arg.	Prol.	Orn.	His.	N-Leu.	Asp.	Threo.	Lys.	Iso Leu.	Meth.	Gly.	Ser.	Cyst.	Ala.	Val.	Shikmic acid family Ph.ala	Tyr.	Total amino acids
Control	4.35	0.73	0.85	-	0.12	-	2.11	0.15	0.25	0.75	0.13	0.11	0.45	0.62	0.81	0.73	0.53	0.12	12.81
2%	14.12	1.89	0.63	-	0.16		1.61	0.95	0.99	2.60	0.16	0.41	1.53	0.51	2.42	2.12	1.65	0.56	32.31
4%	17.0	5.31	3.0	-	0.31	-	5.91	1.35	1.38	2.10	0.39	1.63	2.43	2.40	1.34	1.25	1.93	1.19	48.9

Note: All units are mg/g

Table 7. Effect of aqueous extract of *Artemisia monosperma* on the total fatty acids (%) of germinated common bean.

Contents/ Concentration	Saturated (%) Caprylic (8:0)	Capric (10:0)	Louric (12:0)	Myristic (14:0)	Palmatic (16:0)	Streric (18:0)	Arachidic (20:0)	Erucic (22:0)	Total	Unsaturated (%) Oleic (18:1)	Linoleic (18:2)	Linolenic (18:3)	Total
Control	0.33	0.01	0.08	-	14.0	7.5	2.13	-	24.05	41.5	32.0	-	73.5
2%	0.66	-	1.02	19.10	18.95	-	1.28	-	41.01	90.80	70.9	23.2	184.9
4%	0.25	0.78	1.45	0.91	15.4	0.04	2.25	0.82	21.65	37.11	37.6	-	74.71

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). The common bean in this study tended to accumulate amino acids, proline and unsaturated fatty acids which may be considered an adaptive mechanism to increase stress tolerance.

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