

Full Length Research Paper

Effect of Persian galbanum (*Ferula gummosa* L.) extract on seed germination and growth of some weeds

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Persian galbanum seeds have hard dormancy, and washing has a good effect on seed dormancy breaking; it seems that Galbanum seeds are a source of inhibitor material. In this study, we evaluated the allelopathic effects of seed, stem and root aqueous extract of Galbanum in three concentrations (10, 5 and 2.5%) on seed germination and seedling growth of three weeds (common amaranth, purslane and wild barley) with pre and post-emergence method. In pre-emergence experiment, bioassays showed that all organ extracts in most of the concentrations significantly decreased germination percent, germination rate, radicle length and hypocotyl length. In post-emergence method, root (except wild barley) and stem length, dry and wet weight decreased in most of the treatment, viability reduced in some treatment of receiver plant. Also, total protein root content of wild barley plantlet enhanced by exposing them to stem extract (after 48 h), but polyphenol and peroxidase enzyme activity decreased. This study exhibited that galbanum has inhibitor material that decreased seed germination and seedling growth of receiver weeds. So, galbanum is an allelopathic plant that can be considered as a source of allelochemical. Also, galbanum organs can be used as an organic herbicide. Further, one of the main reasons for the hard dormancy of galbanum is the presence of the inhibitor compound.

Key words: Allelopathy, plant aqueous extract, organic herbicide, protein content, enzymes activity.

INTRODUCTION

Galbanum (*Ferula gummosa*, Apiaceae) is a perennial plant (Zargari, 1989) and native to some Middle East country including: Afghanistan, Iran, Pakistan, Turkmenistan and Turkey that grows in the region above 1800 to 6000 m high (Bernard et al., 2007). In the primary years, galbanum grows vegetatively and in the last year (5 to 7 year old) produce a stem (until 2 m height) that is the origin of flowers and after seed ripening the plant will die (Bernard et al., 2007). Galbanum is one of the most important rangeland products of Iran, with a high export demand due to a large number of applications within both traditional medicine and industry (Islami-Manuchehri, 1994). The gum obtained from the aerial organs of Galbanum in Iranian ancient medicine is used for

stomach pain, chorea, and wound-healing remedy (Zargari, 1989). Also, in recent years, it has been demonstrated that compounds obtained from Galbanum seeds have antinociaceptive and anticonvulsant properties (Sayyah et al., 2002).

Germination percent of *Ferula gummosa* is very low, after 45 days and the best treatment (washing and chilling at 5°C) cause 26.1% germination (Nadjafi et al., 2005). Hypothesis of this experiment is that the seed of galbanum has allelochemical materials that dissolve in water by washing, so by examination of aqueous extract on seed germination and growth of some weeds, we can demonstrate that galbanum is a source of inhibitory materials, also root and stem in the last year can be used as organic herbicide (the seed is required for regeneration), further one of the main reason that prevent galbanum seed germination is the inhibitor compound. Allelopathic compound may be used as new chemistry production, instead of a persistent compound derived of

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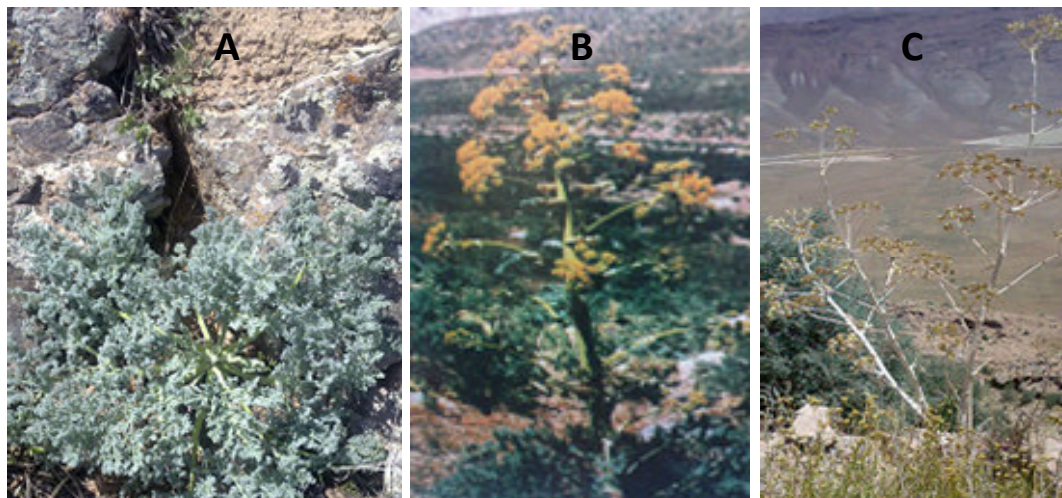


Figure 1. Galbanum in vegetative stage (A), flowering stage (B) and seeds physiological ripening stage (C).



Figure 2. Preparation of the aqueous extract of galbanum, intact root (1) chopped root (2) organs powdered (3) stirring of mixture (4) aqueous extracts filtering by cheesecloth (5) stock solution (6).

petroleum that is such a public health concern (Anaya, 2006). Recently, compounds with selective toxicity that can decompose easily by the plant or soil micro-organisms are considered (Anaya, 2006).

MATERIALS AND METHODS

Preparation of plant material and aqueous extracts

Persian galbanum organs include seed, root and stem after physiological ripening of seed (in this stage, galbanum does not have any leaves) were collected from the mountain of Tafresh in the center of Iran (Figure 1). Weed seeds were collected from the institute of KhoramAbad plant protection. Plant materials after cleaning were separated into root, stem and seed. Root were

chopped 2 cm in diameter, stem 2 cm in length. Chopped organs and intact seeds were dried in oven 60°C for 48 h, and then powdered. Each 100 g of organs powder was mixed with 1 L distilled water and stirred (200 rpm) for 12 h. For filtering aqueous extracts, four layers of cheese cloth was used, then the resulting solution was centrifuged at 3000 rpm for 4h (Chon et al., 2002). The supernatant was filtered again by using one layer filter paper (no. 1). Final solution is used as stock and was diluted to make 5 and 2.5% concentration solution (Figure 2).

Pre-emergence experiment

Weed seeds (common amaranth, purslane and wild barley) were surface sterilized with 1% sodium hypochlorite for 15 min. Then, 25 sterilized seeds were placed in sterile Petri dishes (9 cm) which contains two layers of filter paper and 5 ml of the aqueous extracts

Table 1. Germination percentage and rate (seed per day) of receptor weeds by different concentration of galbanum aqueous extract and distilled water (control).

Treatment	Germination percentage			Germination rate		
	Amaranth (%)	Purslane (%)	Wild barley (%)	Amaranth	Purslane	Wild barley
Control	51 ^a	49 ^a	79 ^a	4/07 ^a	3/95 ^a	6/32 ^a
Seed (10%)	0.00 ^c	70 ^d	0.00 ^e	0.00 ^c	0/27 ^{ef}	0.00 ^e
Seed (5%)	0.00 ^c	0.00 ^e	0.00 ^e	0.00 ^c	0.00 ^f	0.00 ^e
Seed (2.5%)	03 ^{bc}	25 ^{bc}	40 ^{de}	0/18 ^{bc}	1/45 ^c	0/19 ^e
Stem (10%)	0.00 ^c	21 ^c	50 ^{de}	0.00 ^c	1/15 ^{de}	0/21 ^e
Stem (5%)	0.00 ^c	0.00 ^e	0.00 ^e	0.00 ^c	0.00 ^f	0.00 ^e
Stem (2.5%)	0.00 ^c	45 ^a	13 ^d	0.00 ^c	3/18 ^{ab}	0/59 ^d
Root (10%)	10 ^b	0.29 ^{bc}	30 ^c	0/71 ^b	2/02 ^{cd}	1/29 ^c
Root (5%)	0.00 ^c	0.21 ^c	0.00 ^e	0.00 ^c	1/23 ^d	0.00 ^e
Root (2.5%)	50 ^{bc}	38 ^{ab}	42 ^b	0/38 ^{bc}	2/86 ^{bc}	2/34 ^b

Means within each column followed by the same letter are not significantly different at the 5% level as determined by Duncan's multiple range test.

(2.5, 5 and 10%) or distilled water (control). All Petri dishes were placed in a room at 25°C temperature. After 48 h germination, counting started and seed were considered as germinated when the radicle appeared at least 1 mm, also rate germination, radicle and hypocotyls lengths of seedling after 10 days was calculated (Golbashy and Zarabi, 2009). Experiment was carried out using a completely randomized design with four replications. Means of treatment compared with Duncan's multiple range test method at 5% probability level. Maguire formula (1962) used for calculation of germination rate:

$$GR = (E1/D1 + E2/D2 + \dots + Ef/Df)$$

That $E1, E2, \dots, Ef$ are the number of germinated seeds on the first, second, . . . , and final day of counting and $D1, D2, \dots, Df$ are the days after planting on the first, second, . . . , and final day of counting.

Post-emergence experiment

All weed were planted in sterilized medium (pit and perlite) until plantlet grow to 5 leaf stage, in this stage, root of similar seedling separated from the medium slowly and was washed by distilled water carefully, then the root of two seedlings were placed in an experiment pipe (15 ml) that contain different concentrations of extract or distilled water (control), cotton was used for holding plantlet. After 24 h, the seedlings again were planted in a medium, which seem as the first medium. After 8 day viability percent, root and shoot length, seedling wet and dry weights were measured.

Experiments were conducted in a completely randomized design with tree replications.

Total protein and enzyme activity

The root of wild barley seedling was exposed to different concentrations of stem extract and distilled water as control. After 48 h, 1 g of seedling root was homogenized in 3 ml of 0.05 M phosphate buffer, with pH 7 and centrifuged at 14,000 rotations min^{-1} for 20 min at 4°C; then supernatant was kept in the refrigerator

and used for measuring total protein (Bradford, 1976). Also, we used Janda's method (2003) for measurement of polyphenol oxidase and peroxidase activity.

RESULTS

Pre-emergence experiment

Germination percent and rate

In pre-emergence experiment, galbanum organs extract in all concentrations significantly decreased the germination percentage (except 2.5% stem and root for purslane) and germination rate (except 2.5% stem for purslane) per three weeds (Table 1).

Hypocotyl and radical length

Hypocotyl length decreased significantly in all treatments (except for 2.5% stem and root for purslane and 10% stem for amaranth). Radical length decreased significantly in all treatments for all weeds. In purslane and amaranth, radicle length compared to hypocotyls length (radicle/hypocotyl) was more decreased, especially in purslan seedling, so in some treatments only hypocotyls grew and radicle did not grow (Table 2).

Post-emergence experiment

Shoot and root length

In post emergence experiment, the effect of aqueous extract on reducing shoot and root length of amaranth

Table 2. Radicle length, hypocotyl length and radicle to hypocotyl ratio of receptor weeds in different concentrations of galbanum aqueous extract and distilled water (control) in pre-emergence experiment.

Treatment	Radicle length			Hypocotyl length			Radicle/Hypocotyl		
	Amaranth	Purslane	Wild barley	Amaranth	Purslane	Wild barley	Amaranth	Purslane	Wild barley
Control	2/14 ^a	1/64 ^a	7/11 ^a	4/03 ^a	1/56 ^a	5/02 ^a	1/88 ^a	0/95 ^a	0/70 ^{ab}
Seed (10%)	0.00 ^b	0/07 ^d	0.00 ^d	0.00 ^c	0.00 ^c	0.00 ^e	0.00 ^c	0.00 ^d	0.00 ^c
Seed (5%)	0.00 ^b	0.00 ^d	0.00 ^d	0.00 ^c	0.00 ^c	0.00 ^e	0.00 ^c	0.00 ^d	0.00 ^c
Seed (2.5%)	0/17 ^b	0/58 ^c	0/61 ^d	0/05 ^c	0/11 ^c	0/78 ^{de}	0/14 ^c	0/21 ^b	0/93 ^a
Stem (10%)	0.00 ^b	0/24 ^{dc}	0/33 ^d	0.00 ^c	0/01 ^c	0/33 ^e	0.00 ^c	0/03 ^{cd}	0/24 ^{bc}
Stem (5%)	0.00 ^b	0.00 ^d	0.00 ^d	0.00 ^c	0.00 ^c	0.00 ^e	0.00 ^c	0.00 ^d	0.00 ^c
Stem (2.5%)	0.00 ^b	1/31 ^{ab}	2/67 ^c	0.00 ^c	0.00 ^c	1/58 ^{cd}	0.00 ^c	0.00 ^d	0/78 ^{ab}
Root (10%)	2/20 ^a	1/14 ^b	3/08 ^c	1/01 ^b	0/13 ^c	2/56 ^b	0/34 ^b	0/11 ^c	0/88 ^a
Root (5%)	0.00 ^c	0/22 ^{cd}	0.00 ^d	0.00 ^c	0.00 ^c	0.00 ^e	0.00 ^c	0.00 ^d	0.00 ^c
Root (2.5%)	0/61 ^c	1/53 ^{ab}	4/45 ^b	0/19 ^c	0/43 ^b	1/96 ^{bc}	0/09 ^c	0/29 ^b	0/44 ^{abc}

Means within each column followed by the same letter are not significantly different at the 5% level as determined by Duncan's multiple range test.



Figure 3. Inhibitory effect of seed extract (10%) in post-emergence method on growing purslane (right) in comparison with control (left).

and wild barley was more than purslane. This study showed that inhibitory effect of stem extract was more than other organs (Figure 3). Also root to shoot ratio in weeds did not decline in spite of pre-emergence experiment (Table 3).

Wet weight, dry weight and viability

Wet and dry weights in amaranth and purslane significantly decreased in all treatments in comparison

with control, but in wild barley decrease in wet and dry weights in many treatments was not significant. According to the obtained results, viability decreased by increasing the extract concentration, but amaranth viability significantly declined in all treatments (except for 25% of stem extract), also the lowest decrease in viability was observed in purslane (Table 4).

Enzyme activity

Root total protein of wild barley after 48 h exposure to stem aqueous extract was enhanced. But peroxidase and polyphenol oxidase activity decreased by incorporation of aqueous extract, and amount of activity decreased with the increase of extract concentration (Figure 4).

DISCUSSION

All aqueous extracts from all organs in different concentrations showed inhibitory effect on amaranth, purslane and wild barley. In pre-emergence experiment, radicle length decreased more than hypocotyl length, which was supported by (Turk and Tawaha, 2002). Among receiver weeds, amaranth showed more sensitivity to all extracts. The most inhibitory effect was detected in 5%, then 10% and the lowest inhibitory effect was detected in 2.5% extract concentration. But it is attributed that inhibitory effect of the extract increase with increase in concentrations (Turk and Tawaha, 2002; Saxena et al., 1996) and our result is against that, so more research is required to define the mechanism and

Table 3. Radicle length, hypocotyl length and radicle to hypocotyl ratio of receptor weeds exposed to different concentration of galbanum organs aqueous extract (24 h) and distilled water (control) after 8 days of replanting in post-emergence experiment.

Treatment	Root length			Shoot length			Root/Shoot		
	Amaranth	Purslane	Wild barley	Amaranth	Purslane	Wild barley	Amaranth	Purslane	Wild barley
Control	8/50 ^a	7/24 ^a	4/88 ^a	5/05 ^a	6/00 ^a	6/82 ^a	1/67 ^a	1/22 ^a	0/71 ^a
Seed (10%)	0/50 ^c	5/94 ^a	2/73 ^d	0/50 ^d	5/06 ^{ab}	5/44 ^{bc}	0/33 ^c	1/16 ^a	0/50 ^b
Seed (5%)	3/64 ^b	7/13 ^a	4/33 ^{ab}	3/77 ^b	5/68 ^{ab}	6/16 ^{ab}	0/96 ^b	1/27 ^a	0/70 ^a
Seed (2.5%)	3/88 ^b	6/50 ^a	4/25 ^{ab}	3/48 ^{bc}	5/45 ^{ab}	6/82 ^a	1/12 ^{ab}	1/19 ^a	0/63 ^{ab}
Stem (10%)	0.00 ^c	1/47 ^b	3/16 ^{cd}	0.00 ^d	2/91 ^c	5/12 ^c	0.00 ^c	0/50 ^b	0/61 ^{ab}
Stem (5%)	3/35 ^b	5/70 ^a	3/26 ^{cd}	2/91 ^c	4/48 ^b	5/56 ^{bc}	1/20 ^{ab}	1/28 ^a	0/59 ^{ab}
Stem (2.5%)	4/62 ^b	6/44 ^a	3/79 ^{bc}	3/19 ^{bc}	5/42 ^{ab}	6/86 ^a	1/44 ^{ab}	1/19 ^a	0/55 ^{ab}
Root (10%)	0.00 ^c	7/21 ^a	3/92 ^{bc}	0.00 ^d	5/07 ^{ab}	5/72 ^{bc}	0.00 ^c	1/43 ^a	0/86 ^a
Root (5%)	3/91 ^b	6/15 ^a	3/26 ^{cd}	3/08 ^{bc}	5/42 ^{ab}	5/83 ^{bc}	1/27 ^{ab}	1/13 ^a	0/56 ^{ab}
Root (2.5%)	4/80 ^b	5/71 ^a	3/58 ^{bcd}	3/66 ^b	4/92 ^{ab}	5/93 ^b	1/29 ^{ab}	1/16 ^a	0/60 ^{ab}

Means within each column followed by the same letter are not significantly different at the 5% level as determined by Duncan's multiple range test.

Table 4. Wet weight, dry weight and viability percent of receptor weeds were exposed to different concentration of galbanum aqueous extract (24 h) and distilled water (control) after 8 days of replanting.

Treatment	Wet weight			Dry weight			Viability percent		
	Amaranth	Purslane	Wild barley	Amaranth	Purslane	Wild barley	Amaranth (%)	Purslane (%)	Wild barley (%)
Control	446 ^a	1318 ^a	92 ^a	39 ^a	87 ^a	14 ^a	100 ^a	100 ^a	100 ^a
Seed (10%)	33 ^e	800 ^{de}	76 ^{abc}	3.3 ^e	45 ^{dc}	12 ^{abcd}	08 ^d	91 ^{ab}	66 ^{cd}
Seed (5%)	193 ^c	925 ^{bcd}	81 ^{ab}	16 ^{cd}	52 ^{bcd}	13 ^{ab}	58 ^{bc}	100 ^a	83 ^{abc}
Seed (2.5%)	188 ^c	1071 ^b	83 ^{ab}	19 ^{bcd}	60 ^b	13 ^{ab}	75 ^b	100 ^a	91 ^a
Stem (10%)	0 ^e	204 ^f	69 ^{bc}	0 ^e	13 ^e	9 ^{de}	0 ^d	66 ^c	5 ^d
Stem (5%)	121 ^d	700 ^e	80 ^{ab}	13 ^d	41 ^d	10 ^{cde}	41 ^c	91 ^{ab}	91 ^{ab}
Stem (2.5%)	256 ^b	993 ^{bc}	91 ^a	22 ^{bc}	58 ^b	12 ^{abc}	83 ^{ab}	100 ^a	100 ^a
Root (10%)	0 ^e	853 ^{cde}	83 ^{ab}	0 ^e	48 ^{bcd}	10 ^{cde}	0 ^d	100 ^a	66 ^{cd}
Root (5%)	198 ^c	900 ^{bcd}	63 ^c	18 ^{bcd}	56 ^{bc}	8 ^e	33 ^c	100 ^a	5 ^d
Root (2.5%)	294 ^b	786 ^{de}	76 ^{abc}	24 ^b	45 ^{cd}	11 ^{bcd}	58 ^{bc}	91 ^{ab}	75 ^{bc}

Means within each column followed by the same letter are not significantly different at the 5% level as determined by Duncan's multiple range test.

more details of galbanum allelopathic compound. Also, seed extract had the most inhibitory effect in comparison to stem and root extract on seed germination.

In post-emergence experiment, the inhibitory effect of treatment did not show an apparent model like the pre-emergence experiment; but in most treatments growing characteristics (shoot and root length, wet and dry weights and viability) decreased with the increase of extract concentration. Total protein content was enhanced by exposure to stem extract, but polyphenol and peroxidase activity decreased. This result was

supported by Terzi et al. (2003) who found that the total protein of cucumber plantlet exposed to 1 mM juglone increased until tow time. Bohm et al. (2006) found that soybean seedling peroxidase decrease after 24 h of exposure to juglone in more than 10 μ M concentration. Many enzyme functions that are important in plants decrease by application of allelochemicals (Turk and Tawaha, 2002; Rice, 1984).

In conclusion, galbanum is a source of allelopathic substance and galbanum can be classified as an allelopathic plant. This study showed that at least one of the

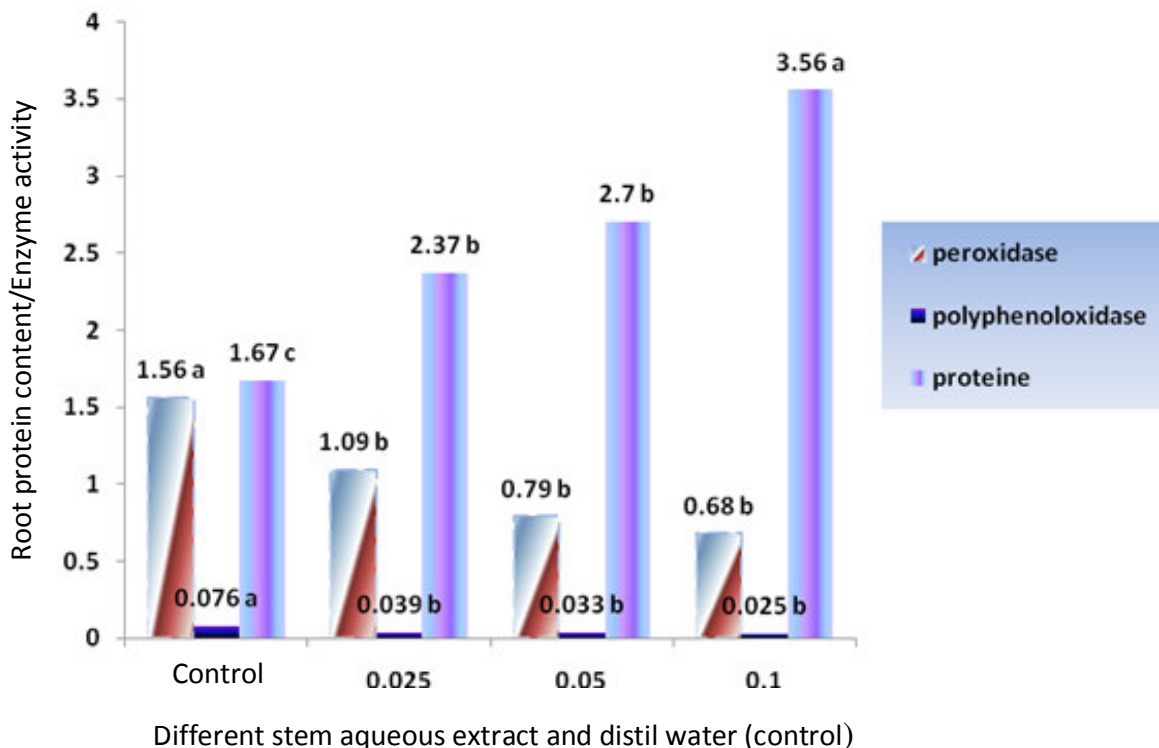


Figure 4. Effect of different stem aqueous extract and distilled water (control) after 48 h on wild barley root protein content (mg/g wet weight), soluble peroxidase activity ($\Delta OD 475/\text{min}/\text{mg}$) and soluble polyphenol oxidase activity ($\Delta OD 515/\text{min}/\text{mg}$). Means within each column followed by the same letter are not significantly different at the 5% level, as determined by Duncan's multiple range test. Means within each column followed by the same letter are not significantly different at the 5% level as determined by Duncan's multiple range test.

important reasons for the low percent of germination in galbanum, is the presence of inhibitor material in seeds of this plant.

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