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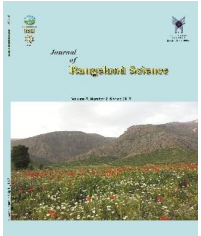

Research and Full Length Article:

Investigating Allelopathic Effects of *Artemisia sieberi* on Seed Germination and Seedling Growth Indices of Three Alfalfa Species

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Abstract. Allelopathy is one of the main factors limiting the plants growth. The present study investigated the effect of aqueous extract of leaves and fruit of *Artemisia sieberi* that is an important species for rangeland rehabilitation on seed germination and seedling growth of three alfalfa species (*Medicago sativa* cv. Nikshahri, *Medicago polymorpha* and *Medicago scutellata* cv. Robinson) in laboratory and glasshouse. Treatments were five concentrations of 0, 25, 50, 75 100% *Artemisia* extract. Treatments were evaluated using a completely randomized factorial experiment in four replications in germinator and pots separately (2014). Data were collected and analyzed for the germination percent, germination rate, seedling length, seedling weight and seed vigor index. Means comparisons were made using Duncan test. The results showed significant differences between extract concentrations of *Artemisia* on all of traits in both growth conditions ($P<0.05$). There was a decrease trend for all of the traits. Germination percent of *M. polymorpha* was stopped in low concentration of *Artemisia* extracts (25%) whereas there were linear decreasing trends for the other specie until irrigation by 100% extraction. *M. sativa* had a higher germination rate and *M. scutellata* had higher vigor index values in all the treatments. Results of the study indicated that *Artemisia* had strong allelopathic effects and prevents from the germination and seedling growth of alfalfa.

Key words: Allelopathy, *Artemisia sieberi*, *Medicago Spp.*, Seed germination Malayer

Introduction

The term of allelopathy was used for the first time by an Australian physiologist called Hans Molish in 1937. The word was derived from two Greek words of alleon meaning mutual and pathos meaning damage (Dehdari *et al.*, 2008) which represents the interaction of plants by their returned chemicals on each other. It shows the potential of allelopathic materials production at some plants and weeds such as *Triticum durum*, *Brassica nigra* and *Brassica napus* is proven (Oueslati, 2003; Turk & Tawaha, 2003; Asaduzzaman *et al.*, 2014). Oliveira *et al.* (2014) examined the phytochemical and potential allelopathic effect of 0, 1, 2.5, 5, 10 and 20% of aqueous and alcoholic extracts of *Palicourea rigida* leaves on seed germination and seedling growth of *Lactuca sativa* L. They found that at all of concentrations, germination rate was influenced by both aqueous and alcoholic extracts and root and shoot developments were stopped in 10 and 20% concentrations.

Meiners (2014) examined allelopathic effects of 65 plant species in northwestern areas of USA in laboratory conditions. They found that allelopathic potential of species was related to their growth form, and characteristics. Most of examined species showed remarkable allelopathic effects and in general, allelopathic potential of species decreased with life span, roughly following the successional transitions from short-lived to long-lived herbs and to woody species. Investigating the effects of soil under and around the invasive species of thyme (*Thymus vulgaris*) on germination and growth responses of non-native grass (*Bromus diandrus* Roth, *Dactylis glomerata* L., and *Vulpia myuros*) and native (*Anthosachne aprica* and *Poa colensoi*) species, Nielsen *et al.* (2015) found a small amount of thymol and carvacrol allelochemicals in the soil under thyme.

Artemisia genus belongs to the Asteraceae family which is one of the plant species and its allelopathic potential in different species is proven. One of the methods of rangelands improvement is seed sowing. *Artemisia sieberi* covers more than 50 million hectares of semi-desert and steppe areas of Iran. It is a good choice for the improvement actions. *Artemisia* scrublands as long period winter rangelands are grazed by sheep, goats and camels (Jouri and Mahdavi, 2010). This species grows well in an area with 200 mm rainfall and absorbs its necessary moisture from the soil horizons with its powerful rooting system (Azarnivand and Zare Chahooki, 2010). This specie is also grazed by livestock in rainy season because of its high content of essence in growing season. *Artemisinin*, *Sesquiterpene* lactone and other secondary metabolites such as Coumarin, Camphor and Bornyl acetate are active biological compounds of the genus which its toxicity is proved. Based on the studies of Yun and Han (1993), the active extract of *Artemisia princeps* plant prevents from the root growth of *Diarheno japonica* and *Chrysathemum beca*. Examining the allelopathic activity of annual *Artemisia* (*Artemisia annua*), Lydon *et al.* (1997) stated that the plant leaf tissue had inhibitory effects on seedling growth of mustard (*Sinapis arvensis*) and germination of Amaranth (*Amaranthus retroflexus* and *Chenopodium album*). This effect was associated with methyl chloride as well as artemisin. In studying the allelopathic effect of *Artemisia vulgaris*, Inderjit and Foy (1999) attributed the presence of more nitrogen and phosphorus in soils under *Artemisia* to high microbial activity for the phenolic compounds. Preston *et al.* (2002) investigated the inhibitory effect of *Artemisia tridenta* var Tridenta and identified the methyl jasmonate compounds as the most important inhibitor material in the essence of this specie for the germination of the plant tested. Kaur *et al.* (2010) conducted an experiment on five weed species of *Achyranthes aspera*, *Cassia occidentalis*, *Parthenium hysterophorus*, *Echinochloa crus-galli* and *Ageratum conyzoides* to assess the biological herbicide ability of *Artemisia scoparia*. The results showed a significant reduction in emergence and seedling growth of weeds when *Artemisia scoparia* essence was present. Treatment of volatile *Artemisia* essence led to lose chlorophyll and cell respiration of weeds and as a result, it made disorders in plant metabolism and photosynthesis. Yang *et al.* (2012) studied the chemical composition of *Artemisia ordosica* essence and their allelopathic effects on photosynthetic and antioxidant systems of *Palmellococcus miniatus*. The results showed the emission of volatile *Artemisia ordosica* essence through oxidation damage and *Palmellococcus miniatus* growth inhibition, which has a negative impact on its photosynthesis and growth.

Using the proposed species for rangeland improvement should not be in conflict with high quality and native species of rangelands. Alfalfa is one of high quality and native species of the country's arid and semi-arid regions i.e. region improvement by *Artemisia sieberi*; due to having abundant nutrients including protein, minerals, vitamins, particularly vitamins A and C and being rich in calcium, low percentage of cellulose, high yield and high palatability, it has a special advantage over other forage plants, and that is why it is called green gold and queen of forage plants or Lucerne (Sedghi *et al.*, 2014; Jouri and Mahdavi, 2010). Alfalfa (*Medicago sativa*) usually grows everywhere. This plant grows earlier than the other forage plants in spring. Considering the importance of *Artemisia* modifying species, its area under cultivation, alfalfa varieties in terms of nutritional value as well as few studies that have been conducted on the reaction of alfalfa species to the allelopathic effect of Ccultivated species in the rangeland improvement, this study was conducted to examine the allelopathic effects of leaves and fruit of *Artemisia sieberi* on seed characteristics germination and seedling growth of *Medicago sativa*, *Medicago scutellata* and *Medicago polymorpha.*

Materials and Methods

In this study, three species of alfalfa were used (*Medicago sativa* cv. Nikshahri, *Medicago polymorpha* and *Medicago scutellata* cv. Robinson). This study was conducted in natural resources department in Khatam Anbia Technology University, Behbahan, Iran. Seeds were sown in a 9-cm Petri-dish after being rubbed for 2 minutes with medium sandpaper and germination percent was determined as 100% after 6 days.

For preparing the extraction of *Artemisia*, the aerial organs of plant were dried in the open air for two weeks. Then, it was milled and mixed with the distilled water at a ratio of 1 to 3 (weight-volume) and stirred for an hour by a shaker (it was kept in fridge 4°C for 24 hours). It was again stirred for an hour and kept in the refrigerator for 24 hours and finally laid in a shaker for at least 2 hours. In order to remove the excess material, the centrifuge device was first used for 5 minutes at a rate of 2500 rpm and then, the additional materials were passed through a filter paper (Whatman No. 1) (Rezai *et al.*, 2007). The extract was prepared as 100% and 3 treatments of 25%, 50% and 75% were prepared by adding the distilled water. Distilled water was used as control treatment. In both conditions, two factorial experiments were conducted based on CRD with four replications. Seeds were disinfected by the benomyl fungicides. Then, 4 × 25 seeds of each species were placed in Petri dishes. Pots were filled by soil and then, 25 seeds were sown in each pot. Seeds in each Petri dish and pot were irrigated by different concentrations of *Artemisia* extracts and were placed in a germinator at 21°C for 16/8 hours of day light and darkness by cold fluorescent lamps (Razmjui *et al.*, 2008). The number of germinated seeds was counted on the basis of at least 2 mm radicle length daily for 14 days (Gholami *et al.*, 2011a, b). The germination percent, germination rate, seedling length, seedling fresh weight and seed vigor index of species in Petri dish and pot were separately recorded.

Germination Percent (Germ %): The average percentage of maximum seeds germinated during the test:

$$\text{Germ\%} = \frac{\text{Ng}}{25} \times 100 \qquad \text{(Equation 1)}$$

Where:
Germ = Germination Percent,
Ng = Number of Germinated Seeds

Germination Rate

$$R_s = \sum \frac{S_i}{D_i} \qquad \text{(Equation 2)}$$

Where:
R_s = germination rate (number of germinated seeds per day),
S_i = number of sprouts per day,
D_i = number of days (Maguire, 1962).

Seed Vigor Index

$$S_p = G_p(\text{MRL} + \text{MHL}) \qquad \text{(Equation 3)}$$

Where:
S_p = Seed Vigor,
G_p = Germination Percent,
MRL = Mean root length,
MHL = Mean shoot length (Abdul-Baki and Anderson, 1973).
The seedling length, fresh weight and dry weight were also measured.

Data analysis

The collected data were subjected to two-way analysis of variance. Means comparisons were made using Duncan method. Prior to analysis of variance, data normalization and the variances homogenization were tested. Statistical analysis was performed using SPSS21 software.

Results

Analysis of variance: Results of analysis of variance are presented in Table 1. The results showed significant differences between species, extract concentrations and species by extract interactions of *Artemisia* on all of traits except germination percent and seedling fresh weight in pot (P<0.05) (Table 1).

Results of means comparisons using LSD method for all of traits are presented in Figs. 1 and 2.

There was a decrease trend for all of traits. Germination percent of *M. polymorpha* was stopped in low concentration of *Artemisia* extracts (25%) whereas there were linear decreasing trends for other specie until irrigation by 100% extraction.

Table 1. Analysis of variance for investigation of allelopathic effects of different *Artemisia sieberi* extraction on seed germination and seedling growth of three alfalfa species

Experiments Condition	Spruce of Variation	DF	MS				
			Germination (%)	Germination Rate (no/day)	Seedling Length (cm)	Seedling Fresh Weight (g)	Seed Vigor Index
Laboratory	Species (S)	2	77.445**	70.945**	8.180**	173.746**	17.440**
	Treatment (T)	4	113.842 **	54.163 **	93.139**	70.057 **	86.530 **
	S x T	4	49.560 **	57.990 **	10.383**	44.135 **	7.031**
Glasshouse	Species	2	14.704**	128.813**	15.049**	6.856**	27.073**
	Treatment	4	114.733**	115.356**	30.611**	3.806*	73.562**
	S x T	4	1.053ns	3.623*	4.610**	0.733ns	4.254*

** ,*,ns: significance at 0.01, 0.05 and probability level

Germination percent: The germination percent of both *M. sativa* and *M. scutellata* was decreased from control to 100% concentrations in both species (Fig. 1). In laboratory, the highest and lowest germination percent of *M. sativa* and the average values of 99% and 85.5% were obtained for control and 100% concentrations of extract, respectively. Similarly, in the glasshouse, the highest and lowest germination percent of *M. scutellata* and the average values of 90 and 20% were obtained in control and 100% concentrations, respectively (Fig. 1).

Germination rate: The germination rate values were decreased from control to higher extraction concentrations in both species. However, the germination rate of *M. sativa* was higher than that for *M. scutellata* in both conditions. In laboratory, the highest and lowest germination rates of *M. sativa* and the average values of 22.5 and 13 (number/day) were obtained in control and 100% concentrations, respectively. Similarly, in glasshouse conditions, the highest and lowest germination rate of *M. sativa* and the average values of 16 and 4 (number/day) were obtained in control and 100% concentrations, respectively (Fig .1).

Seedling length: The seedling length values were decreased from control to higher concentrations in both species. However, the seedling lengths of *M. scutellata* were higher than that for *M. sativa* in both conditions. It seems that higher mean values of seedling lengths of *M. scutellata* were related to its higher 1000 seed weight. In laboratory, the highest and lowest seedling lengths of *M. scutellata* and the average values of 8.4 cm and 4 cm were obtained in control and 75% concentrations, respectively. Similarly, in the glasshouse, the highest and lowest germination rate of *M. scutellata* and the average values of 9.2 and 5 cm were obtained in control and 75% concentrations, respectively (Fig. 1).

Seedling weight: The seedling fresh weight values were not in the same trends for species and conditions. Higher seedling fresh weight was obtained in *M. scutellata* and *M. sativa* in laboratory and glasshouse, respectively. Higher seedling weight of *M. scutellata* in laboratory was related to its higher 1000 seed weight. In laboratory, the highest and lowest seedling weight of *M. scutellata* and the average values of 0.11 g and 0.08 g were obtained in control and 75% concentration, respectively. In contrast, in the glasshouse, the highest and lowest germination rate of *M. sativa* and the average values of 0.32 and 0.05 g were obtained in control and 100% concentrations, respectively (Fig. 2).

Seed vigor index: The seed vigor indices were sharply decreased from control to 100% concentrations in both species. However, the seed vigor indices of *M. scutellata* were higher than those for *M. sativa* in both conditions. It seems that higher vigor index of *M. scutellata* was related to its higher 1000 seed weight. In laboratory, the highest and lowest seed vigor index of *M. scutellata* and the average values of 8.4 and 2.1 were obtained in control and 75% concentrations, respectively. Similarly, in the glasshouse, the highest and lowest seed vigor index of *M. scutellata* and the average values of 7.9 and 1.5 were obtained in control and 75% concentrations, respectively (Fig. 2).

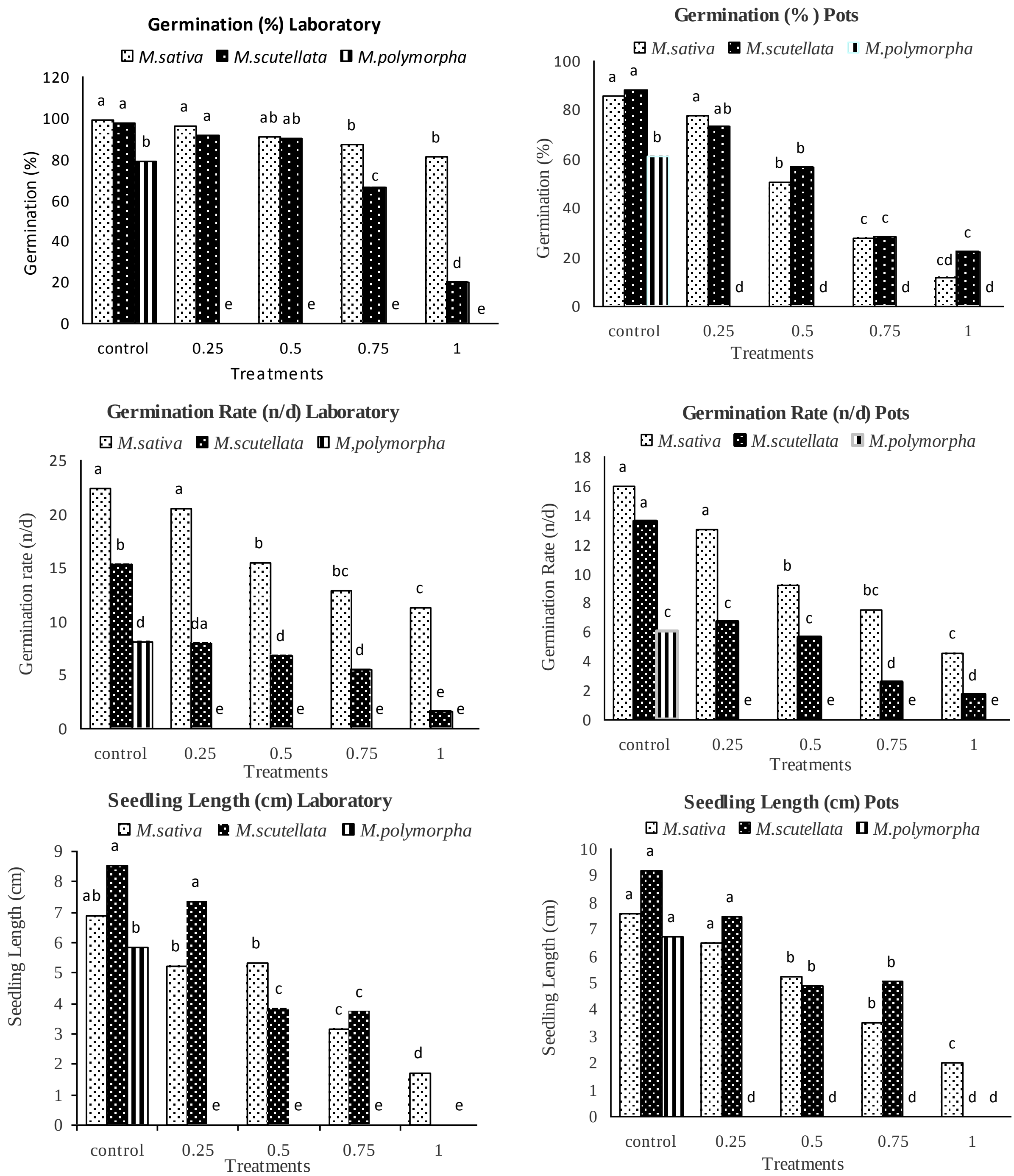

Fig. 1. Means of species by treatments (five concentrations of 0, 25, 50, 75 100% *Artemisia* extract) interaction effects for germination percent, germination rate and seedling length in germination and glasshouse conditions

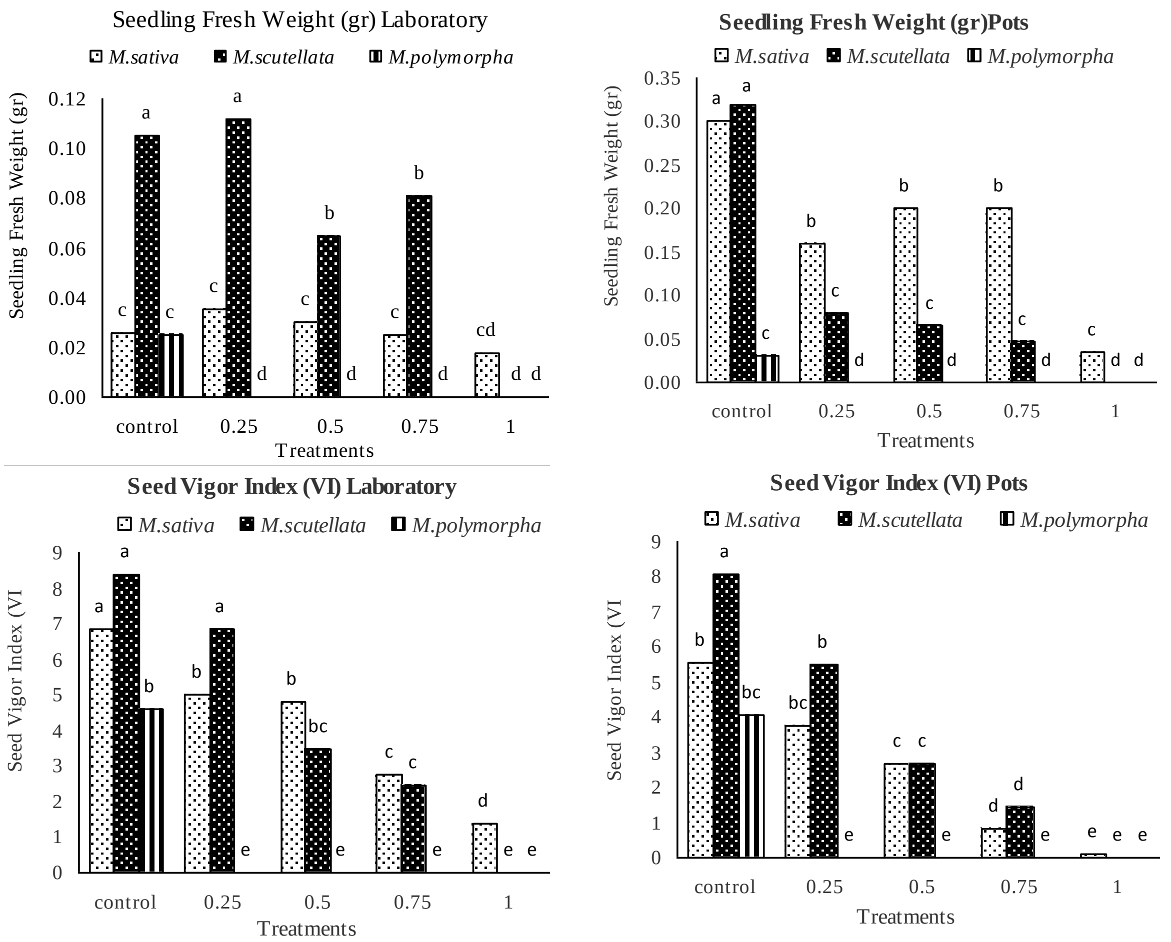

Fig. 2. Means of species by treatments (five concentrations of 0, 25, 50, 75 100% *Artemisia* extract) interaction effects for seedling weight and vigor index in germination and glasshouse conditions

Discussion

Environmental and non-environmental stresses lead to the interactions in plants. Some of environmental stresses are allelopathic compounds by which some plants secrete and cause disturbance in life cycle and activate a series of biochemical reactions (Saberi *et al.*, 2012). The results of this study showed that *Artemisia* extracts had deterrent effects on the germination and growth indices of *M. sativa*, *M. scutellata* and *M. polymorpha* species. The seed germination traits and seedling growth were decreased by increasing the extract concentration. 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), *Avena ludoviciana* (Samdani and Baghestani, 2005), *Artemisia sieberi* (Jabbar Zare and Basiri, 2009), *Salsola rigida* (Tavili *et al.*, 2009), *Atriplex canescens*, *Agropyron elongatum* and *Agropyron desertorum* (Bagheri and Mohammadi, 2011), *Sorghum halpens* L., *Chenopodium album L.*, *Amaranthus retroflexus L.*, *Zea mays L.* (Alipour *et al.*, 2010), *Stipa barbata* (Mohebi *et al.*, (2010), *Festuca arundinacea* and *Dactylis glomerata (*Gholami *et al.*, 2011b), *Medicago Savita L.* and *Onobrychis sativa* (Gholami *et al*., 2011a), *Agropyron elongatum (*Torabi Asl *et al*., 2013), *Chenopodium album*, *Amaranthus retroflexus*, *Setaria viridis*, *Avena ludoviciana* (Makki Zadeh Tafti *et al.*, 2013), *Amaranthus retroflexus L*, and *Convolvulus arvensis L. (*Tabatabayee Zadeh *et al*., 2014).

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 oumarin, 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 mitosis. Flavonoids have been introduced as the first group of mitochondrial 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.

Other species of *Artemisia* genus such as *A. princeps*, *A. annua*, *A. vulgaris*, *A. tridenta*, *A. scoparia* and *A. ordosica* have the same Allelopathic effects (Yun and Han, 1993; Lydon *et al.*, 1997; Inderjit and Foy, 1999; Preston *et al.*, 2002; Kaur *et al.*, 2010; Yang *et al.*, 2012).

Among three tested species, the highest and lowest impact of *Artemisia* aerial organs was on the seedling emergence of *M. polymorpha* and *M. scutellata*, respectively so that the germination of *M. polymorpha* under the influence of *Artemisia* extract in 25% treatment was stopped. Reduction of seedling growth in *M. sativa* under different treatments was significantly lower than *M. scutellata*. The rate of fresh and dry weight reduction in *M. sativa* seedling was higher than that for *M. scutellata*. In other words, low concentrations of *Artemisia* extracts can reduce the germination traits in both *M. sativa* and *M. scutellata*. However, in *M. scutellata*, fresh weight in 75% concentration of extract has increased. The concentration of 75% extract could be considered as the upper threshold of *Artemisia* impact on *M. scutellata* species that will have a low slope after this extent of influence. This mode can be defined according to Rice (1984) who suggested that allelopathy include any beneficial or harmful effects either directly or indirectly that takes place by a plant on other plants through chemical composition production. Thus, these effects may be positive or negative. In the case of *M. polymorpha*, the effectiveness threshold of *Artemisia* is 25% concentration which led to the lack of germination. So if necessary, *M. scutellata* first and then *M. sativa* are preferred as compared to *M. polymorpha*.

The results of this study showed that in both laboratory and pot, the extract of *Artemisia sieberi* could influence the germination and growth traits of three species. In other words, high concentration of *Artemisia sieberi* extract could provide an unsuitable environment for seeds germination by the increased extract concentration. Although all three species were affected in comparison with control; however, *M. scutellata* and *M. sativa* were more resistant against allelopathic substance than that for *M. polymorpha*. So, the latter specie to cultivation in rangeland improvement by *Artemisia sieberi* has to be excluded at least under laboratory and glasshouse studies. Since this study was conducted in laboratory and glasshouse conditions for a more definitive decision about the use of these species as palatable species for livestock feeding in the rangelands improvement by *Artemisia*, more comprehensive tests in field are necessary to find out the allelopathic potentials of *Artemisia sieberi*.

Acknowledgments

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اثر آللوپاتی عصاره اندام هوایی درمنه دشتی بر خصوصیات جوانه¬زنی و رشد گیاهچه سه گونه یونجه

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چکیده. آللوپاتی یکی از مهم‌ترین عوامل محدودکننده رشد گیاهان است. در تحقیق حاضر تاثیر عصاره آبی برگ و میوه *Artemisia sieberi* که یکی از مهم‌ترین گونه‌های مناسب اصلاح مراتع کشور در مناطق استپی به‌شمار می‌رود بر خصوصیات جوانه‌زنی (درصد و سرعت جوانه‌زنی، طول گیاهچه، وزن تر گیاهچه و شاخص بنیه بذر) یونجه زراعی رقم نیک‌شهری (*Medicago sativa*)، یونجه‌های یکساله (*Medicago polymorpha*) و (*Medicago scutellata* cv. *Robinson*) مورد بررسی قرار گرفت. تیمارهای آزمایش شامل عصاره گیاه درمنه‌دشتی در ۴ غلظت ۲۵، ۵۰، ۷۵، ۱۰۰ درصد و آب مقطر (شاهد) بودند. ۵ تیمار مذکور با ۴ تکرار به‌صورت آزمایش فاکتوریل در قالب طرح کاملا تصادفی در ژرمیناتور و محیط کشت گلدانی به‌طور جداگانه به مدت دو هفته (در سال ۱۳۹۳) بررسی شدند. پس از تجزیه واریانس، مقایسه بین میانگین تیمارها با آزمون دانکن انجام گرفت. نتایج نشان داد غلظت‌های مختلف گیاه درمنه دشتی در هر دو محیط کشت، کاهش معنی‌داری در کلیه صفات جوانه زنی یونجه به‌وجود آورد ($P<۰/۰۱$). به‌طوری‌که جوانه‌زنی در بذر (*M. polymorpha*) تحت آبیاری با عصاره ۲۵ درصد درمنه‌دشتی متوقف گردید ولی در دو گونه دیگر میانگین کلیه صفات و رشد گیاهچه با روند کاهشی تا آبیاری با عصاره ۱۰۰ درصد ادامه پیدا کرد. اگرچه عصاره درمنه اثر کاهشی بر همه صفات داشت ولی، میانگین سرعت جوانه زنی در گونه *M. sativa* و میانگین شاخص بنیه بذر گونه *M. scutellata* بیشتر بود. براساس نتایج تحقیق می‌توان گفت درمنه‌دشتی دارای اثرات آللوپاتیکی قوی بوده و از جوانه‌زنی و رشد گیاهچه در یونجه جلوگیری می‌کند.

کلمات کلیدی: آللوپاتی، یونجه، درمنه دشتی، جوانه زنی