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Allelopathic effects of *Thymus kotschyanus* on seed germination and initial growth of *Bromus tomentellus* and *Trifolium repens*

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Abstract The allelopathic influence of aqueous extracts of *Thymus kotschyanus* on *Bromus tomentellus* and *Trifolium repens* germination (%), germination speed, and seedling growth (length, fresh and dry weight) was examined. It was noted that aqueous extracts had a considerable inhibitory effect on target plant germination, and the effect at 50%, 75%, and 100% concentration was found to be significantly higher than that at lower concentrations (5% and 25%) and control treatment (distilled water). Seedling length in addition to fresh and dry weights was also reduced significantly over control. The inhibitory effect was increased as the extract concentration was increased. *B. tomentellus* showed a higher sensitivity against *T. kotschyanus* in allelopathic effects compared to *T. repens*, which indicates that *B. tomentellus* planted in rangelands with leaf litter of *T. kotschyanus* will be adversely affected in terms of its germination, growth, and ultimately low forage production.

Keywords allelopathy, germination, *Thymus kotschyanus*, *Trifolium repens*, *Bromus tomentellus*

1 Introduction

The chemical interaction between plants, including stimulatory and inhibitory influences (Molisch, 1937), is one of the most famous definitions of allelopathy.

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Allelopathy plays an important role in both natural and agro-ecosystems. Suitable manipulation of the allelopathy toward the improvement of crop productivity and environmental protection through eco-friendly control of weeds, pests, crop diseases, conservation of nitrogen in crop land, and synthesis of novel agrochemicals based on natural products have gained prominent attention of scientists engaged in allelopathy research.

Although allelopathy has been defined as any direct or indirect useful or harmful effects by one plant or microorganism on another plants or microorganisms (Rice, 1984), allelopathy is generally considered as a harmful effect. Based on this standing point, allelopathy is referred as a phenomenon that toxic organic compounds produced by one plant are released into the environment (Friedman, 1995). Plants produce numerous chemical compounds during the period of growth. These compounds become free in terms of leaching gas from shoots, root discharges, or by decomposing of plants remaining at the environment (Roa, 2000).

Chemicals extracted from plant roots or shoots (allelochemicals) have been shown to directly inhibit or stimulate germination, growth, and development of other plants (Putnam and Weston, 1986). Allelochemicals may also indirectly affect plants through inhibition of microorganisms, including nitrogen-fixing and nitrifying bacteria and ectomycorrhizae. The effects of four lettuce (*Lactuca sativa*) cultivar leaf extracts were examined on brandyard grass. It was reported that leaf residues of lettuce inhibited root and shoot fresh weights by 88% and 79%, respectively (Chon et al., 2005). Inhibitory effects of *Justicia anselliana* (Nees) T. Anderson on *Vigna unguiculata* (L.) was tested by (Kpoviessi et al., 2006). They found that all isolated compounds obtained from *Justicia anselliana* showed an inhibitory effect on the three parameters measured on *Vigna unguiculata* germination (rate of germination, shoot length, and fresh weight).

Allelopathy occurs in every ecosystem, from forests and

grasslands to deserts. Inhibition of germination and retardation of seedlings are the most common characteristics in the natural world, whether it is the inhibition of their own succession or other plants. Given these effects, allelopathic plants have the potential to alter individual plant fitness and thus plant population and community dynamics.

Allelopathy is prevalent in rangelands including all lands on which the potential native vegetation is predominantly grasses, grasslike plants, forbs, or shrubs suitable for grazing and browsing. In this regard, it has been suggested that allelopathy may be an influencing factor on patterning of vegetation in natural grassland ecosystems. For this reason, poor plant composition or lack of plant diversity could be noticed on a regular basis where allelopathy is suspect.

The genus *Thymus* (Lamiaceae) is represented by 14 species in Iran. Among the species grown in Iran, *T. daenensis* and *T. kotschyanus* are widely used as herbal tea, flavoring agents (condiment and spice), carminative, digestive, antispasmodic, antitussive, expectorant, and anti-inflammatory. The mentioned uses of *T. kotschyanus* are because of having different chemical compounds especially thymol and carvacrol (Rustaiyan et al., 1999). It is suggested that little plant individuals could be grown around *T. kotschyanus* due to its chemical effects of compounds (allelochemicals) on other plants. Based on this idea, the current research was conducted to test the allelopathic effects of *T. kotschyanus* on *Bromus tomentellus* and *Trifolium repens*, which are valuable species of Taleghan grasslands. Understanding allelopathic relationships between plants helps managers correctly plan their vegetative projects for the restoration or rehabilitation of disturbed and poor environments when selecting different species that are to be planted together.

2 Materials and methods

This study was conducted to find the allelopathic influence of a medical plant, namely, *Thymus kotschyanus* on seed germination and initial growth properties of *Trifolium repens* and *Bromus tomentellus*. Shoots and roots of *T. kotschyanus* were gathered from Taleghan rangelands located in Tehran Province, Iran, in June 2009.

2.1 Aqueous extracts from *T. kotschyanus* stems, leaves, and roots

The clipped parts were pooled and dried in an oven at 40°C for 48 h. They were ground up to a fine powder using a vegetative grinder. The obtained powder was mixed with distilled water (Escudero et al., 2000) with a proportion of 1:3 (Rezaei and Khajeddin, 2008). The mixture was put in shaker for one hour and then was placed in refrigerator for 24 h with 4 degrees centigrade temperature. The mixture

was placed in shaker again for 2 h. In the next stage, the mixture was transported to a centrifuge at a rate of 3000 r · min⁻¹ for 5 min. In the end, the centrifuged liquid was filtered using Whatman No. 1 filter paper. In this way, pure extract (100%) was prepared (Rezaei and Khajeddin, 2008). Using this extract and adding distilled water, the concentrations 5%, 25%, 50%, and 75% of *T. kotschyanus* extract were prepared.

2.2 Determining the effects of the extracts on germination and growth of *B. tomentellus* and *T. repens*

Seeds of *B. tomentellus* and *T. repens* were collected from their habitats next to *T. kotschyanus* habitat in Taleghan located in Tehran Province, Iran. The seeds were cleaned and prepared for the experiment. To disinfect the seeds, hypo sodium chloride was used for five minutes. Then, the seeds were washed twice with distilled water. The Petri dishes and Whatman filter papers were put in an Autoclave before the experiment.

To do the study, a completely randomized design with six treatments (control, 5%, 25%, 50%, 75%, and 100% extract concentrations of *T. kotschyanus*) in five replications was made. Within each Petri dish, 25 seeds of target plants (separately) were put and submerged in five mL of understudy treatments. Based on daily germinated seeds counting, the number of seeds that germinated during the experiment period was recorded. Then, some parameters including germination percentage, germination speed, shoot and root length, and fresh and dry weight were evaluated. Germination speed was calculated using the following equation (Maguire, 1962):

$$\text{Germination speed} = \sum \frac{\text{number of germinated seeds}}{\text{day of count}}$$

2.3 Statistical analysis of allelopathy effects

A multivariate ANOVA was used to evaluate the effects of extracts on seed germination of *B. tomentellus* and *T. repens*. Data were analyzed using SPSS 13 for Windows. When significant main effects existed, differences were tested by Duncan test.

3 Results

Tables 1 and 2 reveal that all variables responded to the applied aqueous extract application except to fresh (wet) and dry weight of *T. repens*.

3.1 Effects of aqueous extracts on germination percentage

As it is shown in Table 1, the germination percentage of *B.*

Table 1 *F* values of *B. tomentellus* germination properties affected by *T. kotschyanus* extracts

source of variation	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F</i>
germination	44496.6	5	8899.33	59.329**
germination speed	13.238	5	2.648	33.557**
shoot length	250.68	5	50.136	33.525**
root length	389.9	5	77.998	41.067**
fresh weight	1.199	5	0.24	55.829**
dry weight	0.0092	5	0.0018	56.59**

Note: ** means significant difference at 1%.

Table 2 *F* values of *T. repens* germination properties affected by *T. kotschyanus* extracts

source of variation	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F</i>
germination	286.4	5	57.28	3.206*
germination speed	175.95	5	35.192	20.09**
shoot length	26.75	5	0.53	4.28**
root length	31.144	5	6.22	27.18**
fresh weight	0.021	5	0.0043	2.441ns
dry weight	0.000039	5	0.0000079	0.585ns

Note: ** and * mean significant difference at 1% and 5% levels, respectively; ns means nonsignificant difference.

tomentellus is significantly different ($P < 0.01$) under different extract levels influence, while in *T. repens*, the mentioned property shows significant difference at $P < 0.05$. The increase in extracts concentration leads to a significant decrease in germination percentage of *B. tomentellus* (Fig. 1). The most and least germination percentages (96% and 46%) are related to 0 (control) and 50% concentration, respectively. No germination occurred when seeds were put in extracts with 75% and 100% concentrations.

According to Fig. 1, it is revealed that only the extract concentration of 100% shows significant difference with control treatment in the point of view of *T. repens* seed germination percentage. This signifies that the seed

germination percentage of two target plants shows completely different behaviors when facing with allelopathic condition created by *T. kotschyanus*.

3.2 Effects of aqueous extracts on germination speed

Tables 1 and 2 dedicate that the germination speed of both target plants, when affected by aqueous extracts, were significantly different ($P < 0.01$). The germination speed of *B. tomentellus* was slow as compared to *T. repens* (Fig. 2). In the control treatment, *B. tomentellus* germination speed is slower than 4 seeds per day, whereas it is 24 seeds per day for *T. repens*. This shows that normal *T. repens* seeds germinate faster than *B. tomentellus* seeds even when they are not influenced by allelochemicals.

No significant difference is considered between 0% and 5% extracts in for *B. tomentellus* germination speed, while at 25% and 50% concentrations, the germination speed of *B. tomentellus* is significantly different ($P < 0.01$). According to the germination percentage of *B. tomentellus*, it is natural that after 50% concentration, no germination speed has been recorded (Fig. 2).

In *T. repens*, the germination speed of 75% and 100% concentrations are different with each other and also with lower concentrations ($P < 0.05$).

3.3 Effects of aqueous extracts on shoot and root length

Different levels of the aqueous extract of *T. kotschyanus* had huge effects on shoot and root length of *B. tomentellus* and *T. repens* (Tables 1 and 2). As shown in Figs. 3 and 4, root length of target plants is more affected under aqueous

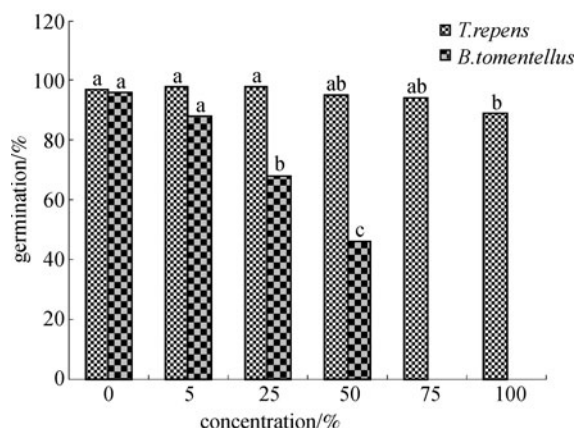


Fig. 1 Comparing allelopathic effect of *T. kotschyanus* extracts on the germination percentage of *B. tomentellus* and *T. repens*

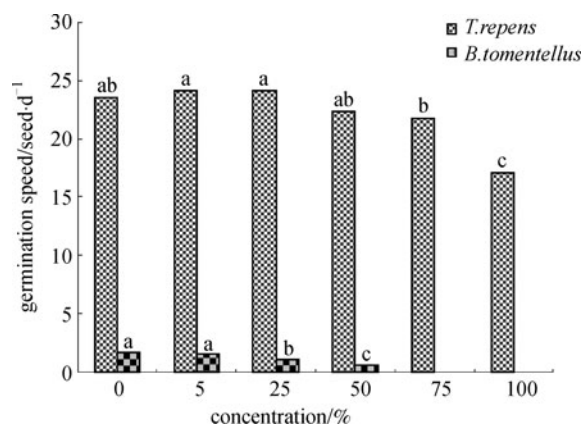


Fig. 2 Comparing inhibitory effect of *T. kotschyana* extracts on germination speed of *B. tomentellus* and *T. repens*

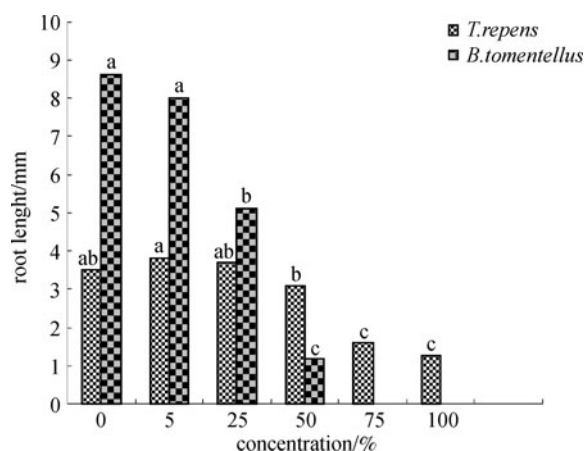


Fig. 4 Effects of different *T. kotschyana* extracts levels on root length of *B. tomentellus* and *T. repens*

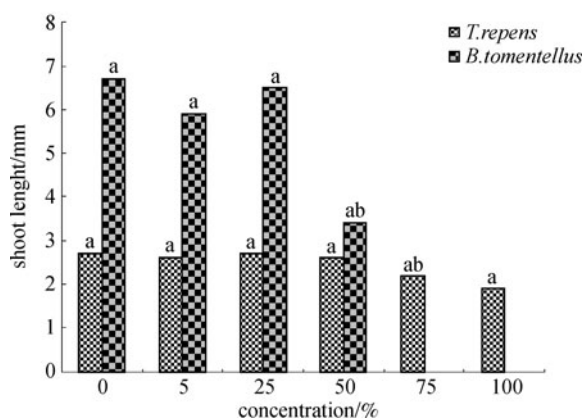


Fig. 3 Effects of different *T. kotschyana* extracts levels on shoot length of *B. tomentellus* and *T. repens*

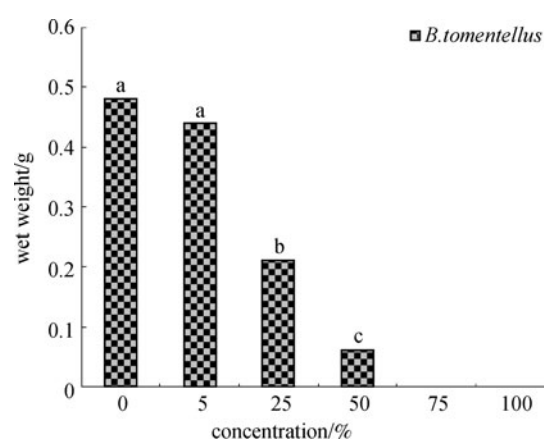


Fig. 5 Fresh (wet) weight of *B. tomentellus* affected by different *T. kotschyana* extracts levels

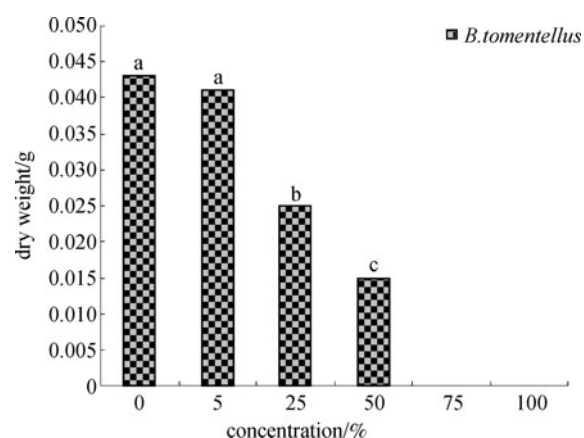


Fig. 6 Dry weight of *B. tomentellus* affected by different *T. kotschyana* extracts levels

extracts compared to shoot length. Also, it can be seen that from control treatment to 50% concentration, shoot and root lengths of *B. tomentellus* are bigger than those obtained for *T. repens*. After 50% concentration, no shoot and root length was observed for *B. tomentellus*, whereas *T. repens* seedlings had both lengths even when affected by 75% and 100% treatments.

3.4 Effects of aqueous extracts on fresh and dry weight

The data in Tables 1 and 2 reveals that fresh and dry weight of *T. repens* have not been influenced by *T. kotschyana* different extract levels, while in *B. tomentellus*, the mentioned properties have been affected strongly ($P < 0.01$). Figures 5 and 6 show that, in 75% and 100% levels, no fresh and dry weight have been recorded for *B. tomentellus*. The biggest ones are related to the control and 5% concentration. In 5% aqueous extract of *T. kotschyana*, the fresh and dry weight of *B. tomentellus* are approximately two times higher than those related to 25% treatment.

4 Discussion and conclusions

Seed germination is considered to be the most critical stage

especially under stress conditions. During germination, biochemical changes take place, which provides the basic framework for subsequent growth and development. The initial metabolic changes that occur immediately after the imbibitions of water are the increase in the hydrolytic enzymes, such as alpha-amylase and protease. Alpha-amylase is an important starch degrading enzyme in the endosperm. The reaction products provide substrate and an energy source for the embryo during germination. The inhibition of seed germination is also due to disturbance in the activities of peroxides, alpha-amylase, and acid phosphates. The results of our study revealed the allelopathic potential of *T. kotschyanus*. This species is a medical plant that includes different chemical compounds. Because of its different chemical compounds, specifically, thymol and carvacrol, this species have various medical uses (Rustaiyan et al., 1999). It seems that these well-known compounds together with some other little known compounds in *T. kotschyanus* are responsible for its allelopathic behaviors. Preventing or decreasing seed germination of associated plants is the markable effect of *T. kotschyanus*, which is observed and confirmed by the results of the current research.

Extracts of *T. kotschyanus* significantly decreased and delayed the seed germination of target plants with increasing extract concentration, that is, from control to 100%, the degree of affecting increased, so that in most of germination properties, no data has been recorded after 50%, especially for *B. tomentellus*. The allelopathic effects of *Scariola orientalis* and *Agropyron elongatum* were studied on *Onobrychis viciaefolia* germination properties by Rezaei and Khajeddin (2008). They found that the extracts of the two allelopath species significantly decreased the seed germination, germination rate, and germination speed of *O. viciaefolia*. The treated seeds with *A. elongatum* extracts germinated only 4.7%, while the control was 78%, which shows 18 times of germination reduction. Decreased and delayed seed germination by an allelopathic extract could be confounded with osmotic effects on rate of inhibition, delayed initiation of germination, and especially cell elongation (Black, 1989); the main factor that affects root growth before and after the tip penetrates the seed coat (Bewley and Black, 1978). Bhawmik and Doll (1982) stated that allelopathy influenced seed germination and seedling development by preventing cell division and inhibiting cell elongation. Avers and Goodwin (1956) reported that fenolic compounds, as main parts of allelochemicals, prevented root cell division. From studies using aqueous alfalfa leaf extract by Chon et al. (2004), they concluded that delayed seed germination and, especially, reduced root elongation were due mainly to toxic factors of the leaf extract. In this research, germination and initial growth properties of *B. tomentellus* were highly influenced as compared to *T. repens* ones when put under different concentrations of *T.*

kotschyanus aqueous extract. In other words, phytotoxic effects of extracts were different between two understudy species. This shows the different sensitivity of two species when facing allelochemicals.

As a whole, it is a useful finding that there is a low allelopathic potential of *T. kotschyanus* on the seed germination properties of *T. repens* as compared to *B. tomentellus*. It may help managers to plan correctly their vegetative projects for restoration or rehabilitation of disturbed and poor environments when selecting different species that would be planted together.

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