

Investigation and Compare the Allelopathic Effects for Different Tissues of *Peganum harmala* in Different Amounts on the *Bromus tectorum* Germination and Growth Characteristics

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ABSTRACT This research was carried out to investigate allelopathic effect of *Peganum harmala* on seed germination of *Bromus tectorum*. Aboveground and underground tissues of *Peganum harmala* in addition to its seeds were collected from Bijar rangelands. Collected materials of root, leaves, stem, seeds and a complex of mentioned parts were dried and powder. The research was performed at the greenhouse condition. The treatments included 1, 3, 6, 9 and 12 weight percentage of above mentioned powders. Also a control treatment was used, too. A completely randomized design (CRD) was applied for 4 weeks. Germination seeds were counted and recorded daily. Germination properties included: germination percentage, mean germination time, germination speed, inhibitory percentage. At the end of study period, radicle and stem length were measured. Data were analyzed by ANOVA. The results revealed that allelopathic effect of *P. harmala* resulted in negative effects on germination properties of *Bromus tectorum*. The high concentration of *P. harmala* has strong Allelochemicals inhibitory effects on germination and initial growth characteristics of *B. tectorum*. It was found that the materials obtained from different tissues of *P. harmala* had different levels of inhibition on germination properties of *B. tectorum*, so that seeds were the most inhibitor tissues. The lowest germination percentage and seedling growth resulted from 12g powder of seeds.

Key words: Allelopathy, *Bromus tectorum*, Germination, *Peganum harmala*

1 INTRODUCTION

Allelopathy is a phenomenon of direct or indirect, beneficial or adverse effects of a plant on its own or another plant via release of chemicals into the environment. It affects plant distribution, community formation, intercrops evolution and biodiversity conservation and is now arousing further international interest (Zhang *et al.*, 2004)

and mentioned phenomenon is toxic organic compounds produced by one plant that released into the environment (Friedman, 1995). Plants produce numerous chemical compounds during growth season. These compounds become free in terms of leaching gas from shoots, root discharges, or by decomposing of plants remaining at the environment (Roa, 2000). Inhibitory effects

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of *Justicia anselliana* (Nees) on *Vigna unguiculata* (L.) was tested by T. Anderson 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). Harmal (*Peganum harmala*) is a plant of the family Zygophyllaceae, native of the eastern Mediterranean region. It is a perennial plant which can grow to about 0.8 m high (Parsons and Cuthbertson, 1992) but normally it is about 0.3 m high. The roots of the plant can reach a depth of up to 6.1 m. The round seed capsules measure about 1–1.5 cm in diameter. Smoke from the seeds kills algae, bacteria, intestinal parasites and molds. *P. harmala* is used as an analgesic and anti-inflammatory agent (Monsefi *et al.*, 2004). According to characteristics of *P. harmala* medicinal plants, low intake by livestock and especially not to be used by animal in summer and Harmal inhibitory effect on plant species and very few studies is done regarding to effects of *P. harmala* allelopathic in Iran. *Bromus tectorum* is an annual grass, usually germinating in the autumn, overwintering as a seedling, then flowering in the spring or early summer. It is an abundant seed producer, with a potential in excess of 300 seeds per plant; seed production per plant is dependent on plant density. Seeds can withstand high soil temperatures. This study was done to investigate the effects of different tissues of *P. harmala* in different amounts on the *B. tectorum* germination and seedling growth characteristics. It is suggested that little plant individuals could be grown around *P. harmala* 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 *P. harmala* on *Bromus tectorum*, which is valuable species of Bijar protected region rangelands. 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

The tissues of *P. harmala* and seeds of *B. tectorum* were collected from Bijar protected region at the end of growing period and seeding time respectively. The allelopathic effect of different tissues of *P. harmala* as an herbal plant on the germination characteristics of *B. tectorum* were evaluated in a completely randomized factorial experiment with three replications. CRD statistical method was used because the effect of replication was not significant and was considered the effect of treatment only. The plant tissues were obtained in four parts includes root, stem, leaf, and capsule. In addition, another experiment was performed to study the effect of composition of all parts altogether with equal ratio. The plant tissues were dried naturally out of reach sunshine and grinded. The experiments were performed in isolated and controlled conditions and there were 6 treatments (0, 1, 3, 6, 9, and 12g plant powder from *P. harmala*) with 3 replications in each treatment. Mentioned six treatments were considered for each plant organ. Plant powder of *P. harmala* organs were mixed with the soil inside the pots and then the seeds of *B. tectorum* were cultivated into pots and the number of 15 seeds was used in each pot in the uniformity form. Therefore, 78 pots were used. The experiment was lasted for four weeks and the number of germinated saplings was registered every day. Data collecting performed during 4 weeks because of after 4 week, measured characteristic not have any change and germination properties of *B. tectorum* after this time was fixed. The plants were pulled out, cleaned, stems and roots were separated, and the length of each plant was measured at the end of experiment.

Table 1 Physicochemical properties of soil in spots.

Factor	Sand (percent)	Clay (percent)	Silt (percent)	pH	EC (ds/m)	Lime (percent)	Organic matter (percent)	Nitrogen (percent)	Phosphor (mgkg ⁻¹)	Potassiu m (ppm)
Value	28	38	34	7.5	0.22	14.04	1.67	0.14	16	362

Rate of germination was estimated using modified Timpson's index of germination velocity (Khan and Ungar, 1984). Mean Germination Time (MGT) was calculated to assess the rate of germination (Ellis and Roberts, 1981).

$$MGT = \frac{\sum D.N}{n} \quad (1)$$

Where N is the number of seeds which in D day grow, n is the total number of seeds grown and D is the number of days from the date of germination and the germination rate index was obtained by reversing MGT at the end of this period, final germination percentage was recorded. Inhibitory percentage (IP) was calculated by this formula:

$$IP = 100 - \left(\frac{\text{FG percentage in harmal } (P.harmala) \text{ tissues powder}}{\text{FG percentage in control (without harmal } (P.harmala))} \right) * 100 \quad (2)$$

Where FG is final germination percentage. After collecting data, Experimental data was analyzed by SAS 9.2 program. The difference between the means was compared using Duncan's multiple range tests at level of 5% probability.

3 RESULTS

3.1 Germination percentage

The germination percentage was decreased from 96.6% to 48.6% for control at 12 g weight although the effect of different tissues of *P.*

harmala on germination percentage was different significantly ($P < 0.01$) (Table 2). The maximum and minimum of germination percentage was related to stem and capsule respectively (Table 3) whereas there was a significant interaction effect of weight percentage and organ powder. The germination of *B. tectorum* was decreased significantly due to increasing of tissues weight of *P.harmala* as it has shown in Table 4. Table 5 represents that while the highest germination percentage was observed in control (without *B. tectorum*) as 96.6 percent, the lowest was for 12g (26.6 %).

3.2 Mean germination time

Mean germination time for *B. tectorum* seed was significantly different ($P < 0.01$) at various weight of *P. harmala* (Table 2). There was not a significant effect for interaction of weight percentage (amount of powder according to gram) and tissues powder of *P.harmala* (Table 2). In comparison of various tissues of herbal plant, the lowest mean germination time was seen for stem as 17.782 days while the highest was seen for capsule as 21.780 days (Table 3). As Table 3 indicates, the lowest mean germination time was for control and the highest belongs to 12g powder. The highest and lowest mean germination time was observed for 12g capsule (23.666 days) and for control 13.574 days respectively (Table 5).

Table 2 Variance effect analysis of *P. harmala* organs powder on measured characteristics of *B. tectorum*.

Mean of squares						DF	Source of Changes
Inhibitory percentage	length of root (cm)	length of stem(cm)	Germination rate	Mean germination Time(d)	Germination percentage		
3542.105**	398.094**	506.738**	0.0008**	30.620**	0.330**	4	Organ powder (p)
4594.212**	1200.320**	1300.624**	0.006**	100.763**	0.429**	5	Weight percentage (w)
264.840**	52.647 ns	63.385 ns	0.0007ns	1.670ns	0.024**	70	PW
80.662	102.058	116.670	0.000009	0.987	0.007	58	Error

*, ** and ns refer to 0.1%, 0.5% and no significant, respectively.

Table 3 comparison of germination various parameters of *B. tectorum* under effect various treatments of *P. harmala* Organs.

Inhibitory percentage	length of root (cm)	length of stem (cm)	Germination rate	Mean germination Time(d)	Germination percentage	Tissue of <i>Peganum harmala</i>
36.54 ^b	3.66 ^a	4.7 ^a	0.053 ^{bc}	18.732 ^{bc}	61.33 ^b	root
17.92 ^a	4.113 ^a	4.766 ^a	0.056 ^a	17.782 ^c	79.33 ^a	stem
26.20 ^a	4 ^a	4.473 ^a	0.055 ^{bc}	18.246 ^{bc}	71.33 ^a	leaf
59.99 ^d	2.7 ^b	3.226 ^b	0.046 ^c	21.780 ^a	38.67 ^d	capsule
48.27 ^c	3.4 ^{ab}	4.013 ^{ab}	0.052 ^b	19.216 ^b	50 ^c	mixture

(a, b, c and d): The Means with same letters in each column does not have significant difference according to Duncan multiple range tests at level of 5%.

Table 4 comparison of germination various parameters of *B. tectorum* under effect various Weight percentages (Powder weight (gr)) of *P. harmala*.

Inhibitory percentage	length of root (cm)	length of stem (cm)	Germination rate	Mean germination Time(d)	Germination percentage	Powder weight(g)
3.441 ^a	5.166 ^a	6.066 ^a	0.073 ^a	13.574 ^a	96.6 ^a	0
24.82 ^b	4.506 ^{ab}	5.093 ^{ab}	0.057 ^b	17.603 ^b	72.6 ^b	1
35.85 ^{bc}	3.913 ^{bc}	4.546 ^{bc}	0.055 ^b	18.107 ^b	62 ^b ^c	3
35.85 ^{bc}	3.613 ^{bcd}	4.166 ^{bc}	0.053 ^{bc}	19.004 ^{bc}	62 ^b ^c	6
42.75 ^{bc}	3.073 ^{cd}	3.913 ^{bc}	0.050 ^{cd}	20.105 ^{cd}	55.3 ^{bc}	9
49.65 ^c	2.766 ^d	3.46 ^c	0.048 ^d	20.947 ^d	48.6 ^c	12

(a, b, c and d): The Means with same letters in each column does not have significant difference according to Duncan multiple range tests at level of 5%.

3.3 Germination rate

Various weight values (g) of *P.harmala* have a significant effect on germination speed of *B.tectorum*, moreover, the highest speed of germination was for control and the lowest for

12g (Table 4). In addition, the highest and lowest speed of germination was for stem and capsule powder respectively (Table 2). Table 5 shows that the maximum germination rate was for control while the 12g capsule powder had the lowest (0.073 in contrast 0.042).

3.4 The length of root and stem

There was no significant effect of interaction between weight values and organs powders on length of stem and root properties (Table 2). When contrasting various tissues as shown in Table 3, the longest and shortest mini stem and mini root was affected by capsule powder (Table 3). With increasing weight value of *P.harmala* from 0g

(control) to 12g, the length of stem and root decreased in *B.tectorum*. Therefore the lowest stem and root length observed in 12g value of *P.harmala* powder (Table 4). Table 5 represents that the shortest length of stem and root was for 12g capsule powder, in another words, the most powerful preventive effect resulted from 12 g capsules.

Table 5 comparison of germination mutual parameters of *B. tectorum* under effect weight values for different organs powder of *P. harmala*.

Inhibitory percentage	length of root (cm)	length of stem (cm)	Germination rate	Mean germination Time(d)	Germination percentage	Weight(g)	Tissue
3.441 ^a	5.166 ^a	6.066 ^a	0.073 ^a	13.574 ^a	96.6 ^a	0	root
6.89 ^a	4.5 ^{ab}	5.3 ^a	0.057 ^b	17.370 ^b	90 ^a	1	
31.02 ^b	4.2 ^{ab}	4.8 ^a	0.055 ^{bc}	17.973 ^{bc}	66.6 ^b	3	
37.92 ^b	4 ^{ab}	4.6 ^a	0.054 ^{bcd}	18.436 ^{bc}	60 ^b	6	
48.27 ^{cd}	2.9 ^b	4.5 ^a	0.051 ^{cd}	19.466 ^{cd}	50 ^{cd}	9	
58.61 ^d	2.7 ^b	4.3 ^a	0.049 ^d	20.416 ^d	40 ^d	12	
3.441 ^a	5.166 ^a	6.066 ^a	0.073 ^a	13.574 ^a	96.6 ^a	0	stem
13.78 ^{ab}	4.733 ^a	5.2 ^a	0.063 ^b	15.895 ^b	83.3 ^{ab}	1	
17.23 ^{ab}	4.2 ^a	4.933 ^a	0.058 ^{bc}	17.058 ^{bc}	80 ^{ab}	3	
17.23 ^{ab}	4.1 ^a	4.8 ^a	0.056 ^c	17.632 ^{cd}	80 ^{ab}	6	
17.23 ^{ab}	3.833 ^a	4.6 ^a	0.053 ^{cd}	18.611 ^{de}	80 ^{ab}	9	
24.13 ^b	3.7 ^a	4.3 ^a	0.053 ^d	19.738 ^e	73.3 ^b	12	
3.441 ^a	5.166 ^a	6.066 ^a	0.073 ^a	13.574 ^a	96.6 ^a	0	leaf
17.23 ^{ab}	4.3 ^a	4.833 ^a	0.060 ^b	16.720 ^b	80 ^{ab}	1	
24.13 ^b	4.133 ^a	4.666 ^a	0.058 ^b	17.148 ^b	73.3 ^b	3	
27.58 ^b	4.1 ^a	4.5 ^a	0.056 ^b	17.848 ^b	70 ^b	6	
27.58 ^b	3.866 ^a	4.3 ^a	0.051 ^c	19.680 ^c	70 ^b	9	
34.47 ^b	3.6 ^a	4.066 ^a	0.050 ^c	19.833 ^c	63.3 ^b	12	
3.441 ^a	5.166 ^a	6.066 ^a	0.073 ^a	13.574 ^a	96.6 ^a	0	capsule
44.82 ^b	4.3 ^b	4.933 ^b	0.051 ^b	19.588 ^b	53.3 ^b	1	
58.61 ^c	3.333 ^c	3.733 ^c	0.049 ^b	20.5 ^b	40 ^c	3	
58.61 ^c	2.566 ^d	3.166 ^c	0.045 ^{bcd}	21.838 ^{bc}	40 ^c	6	
65.51 ^{cd}	1.966 ^e	2.466 ^d	0.043 ^{cd}	23.333 ^c	33.3 ^{cd}	9	
72.41 ^d	1.333 ^f	1.833 ^e	0.042 ^d	23.666 ^c	26.6 ^d	12	
3.441 ^a	5.166 ^a	6.066 ^a	0.073 ^a	13.574 ^a	96.6 ^a	0	mixture
34.47 ^b	4.7 ^{ab}	5.2 ^{ab}	0.054 ^b	17.855 ^b	63.3 ^b	1	
44.82 ^c	3.7 ^{abc}	4.6 ^{ab}	0.056 ^b	18.444 ^{bc}	53.3 ^c	3	
48.27 ^{cd}	3.3 ^{abc}	3.966 ^{bc}	0.052 ^b	19.266 ^{bc}	50 ^{cd}	6	
55.16 ^{de}	2.8 ^{bc}	3.5 ^{bc}	0.051 ^b	19.433 ^c	43 ^{de}	9	
58.61 ^e	2.5 ^c	2.8 ^c	0.047 ^c	21.083 ^d	40 ^e	12	

(a, b, c and d): The Means with same letters in each column does not have significant difference according to Duncan multiple range tests at level of 5%.

In comparison between effects of different tissue of *P.harmala* on germination properties of *B.tectorum*, the highest germination percentage and speed germination observed in stem powder treatment of *P.harmala*. More length of stem and root of *B.tectorum* and the lowest mean germination time with little inhibitory effect observed in stem powder also. Root and leaf powder of *P.harmala* have effects similar to stem powder for more germination properties. Whereas, the lowest percentage and speed of germination, the shortest stem and root, the longest time of germination, and the most powerful preventive effect was for capsule powder which may be resulted from strong negative effect of capsule on germination characteristics of *B. tectorum* (Table 2). While powder weight increased from 0 to 12 gr, the preventive effect also increased from 3.441% to 49.65% for control and 12g respectively (Table 4). In addition, the capsule and stem had the highest (59.99%) and lowest (17.92%) effect respectively as it has shown in Table 2. The 12g capsule powder had the highest preventive effect (72.41%) among different treatments.

4 DISCUSSION AND CONCLUSION

In all treatments, the negative allelopathic effect of *P. harmala* on germination characteristics of *B. tectorum* was increased as the powder weight was increased so that the 12g powder caused the strongest negative effects. The most and less important negative effect among different organs was related to capsule and for stem respectively, however, the second important negative effect on the germination and growing characteristics was came out by a mixture of all plant organs. The root and leaf almost had the same effects but the root effects were more apparent than leaf. The results showed that the materials of upper and underground parts of plant especially capsule had a preventive effect on germination characteristics of *B. tectorum* seed. In the other words, *B. tectorum* has been

sensitive to allelochemical material during germination time thus, its germination and growth suppressed. Several alkaloids that function as monoamine oxidase inhibitors (MAOIs) are found in the seeds of *P. harmala* (also known as *Harmal* or *Syrian Rue*), including harmine, harmaline, and harmalol, which are members of a group of substances with a similar chemical structure collectively known as *harmala alkaloids*. The harmala alkaloids occur in *P. harmala* in concentrations of roughly 3%, though tests have documented anywhere from 2-7% or even higher as natural sources tend to vary widely in chemical makeup (Herraiz et al., 2010). One of the compounds found in *P. harmala*, vasicine (peganine) has been found to be safe and effective against *Leishmania donovani*, a protozoan parasite that can cause potentially "fatal visceral leishmaniasis. Peganine hydrochloride dihydrate, besides being safe, was found to induce apoptosis in both the stages of *L. donovani* via loss of mitochondrial transmembrane potential (Misra et al., 2008). The other studies have been stated that essence and extract of most herbal plants like *P.harmala* affect the mitochondrial activity and fat oxidation as they can use as biological herbicides (Robles et al., 1999; Ehlers & Thampson, 2004). *P.harmala* has more harmalin, harmalol, and harmin alkaloids which are poisonous and they may affect plant seed germination negatively (Mikdad et al., 1991; Berlin et al., 1993; Giampietro et al., 2008; Kartal et al., 2003). The results of this study stated that the powder acquired from different parts of herbal plant affected mini stem and mini root significantly. Also represented that the strongest preventive effect of plant growth was resulted from capsule. Therefore, it can conclude that the different organs of *P.harmala* have preventive compositions for growth. In addition, the amount or type of composition is different for each plant organ (Tawaha et al., 2007;

Giampietro *et al.*, 2008) and have various preventive effects. In addition compounds, the tissue of *P.harmala* contain some koilizoliens such as vasizin and vasizinon which are abundant in capsule (Mahmoudian *et al.*, 2002; Giampietro *et al.*, 2008; Kartal *et al.*, 2003). These findings are consistent with those of Kartal *et al.* (2003). The Harmala alkaloid almost is found in capsule, seed, and root respectively (Giampietro *et al.*, 2008). The length of stem and root of *B.tectorum* decreased with the same ration that this result agreed with the result of Omidi *et al.* (2005) and Naghdi Badi *et al.* (2009). Low growth of roots and stems of *B.tectorum* maybe derived from decreasing of cellololytic division (Anaya, 1999). Allelochemichals reduce oxin values (Tawaha *et al.*, 2007). These compounds decrease plant growth by preventing of nutrients absorption or direct interference into respiration or phosphorilation oxidative. At the present study, while weight of *P.harmala* powder increased, germination percentage and germination speed decreased whereas mean germination time besides inhibitory percentage increased. These results agree with some research findings (Naghdi Badi *et al.*, 2009; Tavili *et al.*, 2009, Ghorbanali *et al.*, 2008; Adryan *et al.*, 2000). It seems that allelopathic activity which usually is seen as delaying or germination preventing effect is resulted from primitive effects of these materials on metabolic process. The reactions and processes like cellololytic division, hormone production, resistance and penetrability of membrane, photosynthesis, and respiration can be introduced as effect goal for allelopathic materials (Menges, 1988). In this study, the 12g capsule powder had the strongest preventive effect in the germination environment which caused more preventive effect in mini stem and mini root. In the other hand, as there was more negative osmotic potential in the germination environment, the

water absorption in *B. tectorum* seeds reduced and due to this fact metabolic process such as catabolism decreased that resulted in emerge delaying of root and stem (Ghaderi *et al.*, 2008; Spollen *et al.*, 1998). Considering to pharmaceutical useful properties of *P.harmala* and rangeland properties of *B.tectorum*, the result of this study can be use in rangeland management program.

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بررسی و مقایسه اثرات آللوپاتیکی اندام‌های مختلف اسپند (*Peganum harmala L*) در مقادیر**متفاوت بر روی ویژگی‌های جوانه‌زنی و رشد *Bromus tectorum***

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چکیده در این مطالعه تأثیر آللوپاتی اندام‌های مختلف گونه *Peganum harmala* بر جوانه‌زنی بذر *Bromus tectorum* مورد بررسی قرار گرفت. اندام‌های گیاهی و بذر مورد نیاز از منطقه حفاظت شده بیجار واقع در شمال شهرستان بیجار در استان کردستان جمع‌آوری شد. اندام نمونه‌های گیاهی پس از انتقال به آزمایشگاه و خشک شدن، به صورت مجزا آسیاب شد که شامل ریشه، ساقه، برگ، کپسول و مخلوط (ترکیبی از قطعات اسپند) بود. آزمایشات در شرایط گلخانه‌ای انجام شد. هر آزمایش شامل ۵ تیمار بود که درصدهای وزنی ۱، ۳، ۶، ۹ و ۱۲ گرم از پودر اندام‌های گیاهی در نظر گرفته شد و یک تیمار شاهد هم در نظر گرفته شد. آزمایش به مدت ۴ هفته به صورت فاکتوریل در قالب طرح کاملاً تصادفی انجام شد. شمارش بذرهای جوانه‌زده به صورت روزانه انجام شد و ویژگی‌هایی چون درصد جوانه‌زنی، سرعت جوانه‌زنی، دوره متوسط جوانه‌زنی و درصد بازدارندگی در طول دوره آزمایش مورد بررسی قرار گرفت و در آخر آزمایشات طول ریشه و ساقه هم بدست آمد. در پایان آزمایش، پس از انجام تجزیه واریانس بر روی داده‌ها، میانگین تیمارها با آزمون دانکن دسته‌بندی و مورد مقایسه قرار گرفت. نتایج نشان داد که پودر اندام‌های اسپند بر جوانه‌زنی بذر و رشد گیاهچه *Bromus tectorum* تأثیر منفی داشت به طوری که با افزایش مقدار پودر، جوانه‌زنی و رشد گیاهچه در آنها به طور معنی‌داری کاهش یافت. اندام‌های مختلف اسپند، اثرات بازدارندگی متفاوتی بر جوانه‌زنی و رشد گیاهچه گیاه مذکور نشان دادند و اندام کپسول دارای بیشترین اثر بازدارندگی بود. کمترین میزان جوانه‌زنی بذر و رشد گیاهچه در پودر کپسول با مقدار ۱۲ گرم مشاهده شد.

کلمات کلیدی: آللوپاتی، اسپند (*Peganum harmala*)، جوانه‌زنی، علف پشمکی (*Bromus tectorum*)