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

Asghar Farajollahi¹, Ali Tavili², Bahram Ghilinejad³, Javad Darini¹ and Hossein Pouzesh⁴

¹ Former MSc. Student, Faculty of Natural Resources, Tehran University, Karaj, Iran.
² Associate Professor, Faculty of Natural Resources, Tehran University, Karaj, Iran.
³ Assistant professor, Faculty of Natural Resources, Kurdistan University, Sanandaj, Iran.
⁴ Young Researchers Club, Islamic Azad University, Yasooj Branch, Yasooj, Iran.

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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 *B. 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
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 Cuthberston, 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.
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).

\[
MGT = \frac{\sum D.N}{n}
\]

(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:

\[
IP= 100- \frac{\text{FG percentage in harmal (P.harmala) tissues powder}}{\text{FG percentage in control (without harmal (P.harmala) *100)}}
\]

(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.

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

*, ** 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.

<table>
<thead>
<tr>
<th>Inhibitory percentage</th>
<th>length of root (cm)</th>
<th>length of stem (cm)</th>
<th>Germination rate</th>
<th>Mean germination Time (d)</th>
<th>Germination percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>36.54^b</td>
<td>3.66^a</td>
<td>4.7^a</td>
<td>0.053^bc</td>
<td>18.732^bc</td>
<td>61.33^b</td>
</tr>
<tr>
<td>17.92^a</td>
<td>4.113^a</td>
<td>4.766^a</td>
<td>0.056^a</td>
<td>17.782^c</td>
<td>79.33^a</td>
</tr>
<tr>
<td>26.20^a</td>
<td>4^a</td>
<td>4.473^a</td>
<td>0.055^bc</td>
<td>18.246^bc</td>
<td>71.33^a</td>
</tr>
<tr>
<td>59.99^d</td>
<td>2.7^b</td>
<td>3.226^b</td>
<td>0.046^c</td>
<td>21.780^d</td>
<td>38.67^d</td>
</tr>
<tr>
<td>48.27^c</td>
<td>3.4^b</td>
<td>4.013^ab</td>
<td>0.052^bc</td>
<td>19.216^b</td>
<td>50^</td>
</tr>
</tbody>
</table>

(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.

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

(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).
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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.

<table>
<thead>
<tr>
<th>Inhibitory percentage</th>
<th>Length of root (cm)</th>
<th>Length of stem (cm)</th>
<th>Germination rate (%)</th>
<th>Mean germination Time (d)</th>
<th>Germination percentage (%)</th>
<th>Weight (g)</th>
<th>Tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.441^a</td>
<td>5.166^a</td>
<td>6.066^a</td>
<td>0.073^a</td>
<td>13.574^a</td>
<td>96.6^a</td>
<td>0</td>
<td>root</td>
</tr>
<tr>
<td>6.89^a</td>
<td>4.5^b</td>
<td>5.3^a</td>
<td>0.057^b</td>
<td>17.370^b</td>
<td>90^b</td>
<td>1</td>
<td>stem</td>
</tr>
<tr>
<td>31.02^b</td>
<td>4.2^b</td>
<td>4.8^a</td>
<td>0.055^bc</td>
<td>17.973^bc</td>
<td>66.6^b</td>
<td>3</td>
<td>leaf</td>
</tr>
<tr>
<td>37.92^c</td>
<td>4^b</td>
<td>4.6^c</td>
<td>0.054^b^cd</td>
<td>18.436^bc</td>
<td>60^c</td>
<td>6</td>
<td>capsule</td>
</tr>
<tr>
<td>48.27^ed</td>
<td>2.9^b</td>
<td>4.5^c</td>
<td>0.051^cd^b</td>
<td>19.466^cd</td>
<td>50^d</td>
<td>9</td>
<td>mixture</td>
</tr>
<tr>
<td>58.61^d</td>
<td>2.7^b</td>
<td>4.3^c</td>
<td>0.049^d</td>
<td>20.416^d</td>
<td>40^d</td>
<td>12</td>
<td></td>
</tr>
</tbody>
</table>

(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%.

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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 Harmala 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 & Thompson, 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 koilizolienls 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). Allelochemicals 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 (Naghd Badi et al., 2009; Tavili et al., 2009, 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 allelophatic 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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Investigation the Allelopathic Effects for Different Tissues of Peganum harmala


بررسی و مقایسه اثرات آللوپاتیکی اندازه‌های مختلف استند 

*Bromus tectorum* متغیر بر روی یوگه‌های جوانزئی و رشد

اصغر فرج‌اللهی، علی تجویزی، حسین دری‌نیا، جواد دری‌نیا

1- دانش‌آموخته کارشناسی ارشد، دانشکده منابع طبیعی، دانشگاه تهران، کرج، ایران.
2- دانشیار، دانشکده منابع طبیعی، دانشگاه تهران، کرج، ایران.
3- استادیار، دانشکده منابع طبیعی، دانشگاه کردنی، سنندج، ایران.
4- عضو باشگاه بیوهکاران جوان، دانشگاه آزاد اسلامی واحد پاسیو، پاسیو، ایران.

چکیده در این مطالعه تأثیر آللوپاتی اندازه‌های مختلف گونه *Peganum harmala* بر *Bromus tectorum* به مورد بررسی قرار گرفت. اندازه‌های گیاهی و بذر مورد نیاز از منطقه حفاظت شده بیجار واقع در شمال شهرستان بیجار در استان کردنی جمع‌آوری ویژه یک از اندازه‌های گیاهی پس از انتقال به بیماری‌های خشکشانده، به صورت صحیح آسیب شد که شامل ریشه، ساقه، برگ، گیاه و پوست (ترکیبی از چهار سطح) بود. اندازه‌گیری در شرایط گلخانه‌ای انجام شد. هر آزمایش شامل ۵۰ بذر که درصد ۹۰٪، ۹۴، ۹۸، ۱۰۱ و ۱۲۳ گرم از بوتر اندازه‌های گیاهی در نظر گرفته شد و یک تیمار شامل هم در نظر گرفته شد. آزمایش به مدت ۴ هفته به صورت فاکتوریل در قابل طرح کامل تصادفی انجام شد. شمارش بذرهای جوانزئی به صورت روزانه انجام شد و بیماری‌های چنین در بر جوانزئی سرعت جوانزئی، دوره متوسط جوانزئی و درصد بازدارنده در طول دوره آزمایش مورد بررسی قرار گرفت و در هر آزمایش طول ریشه و ساقه هم بهدست آمد. این پایان آزمایش، پس از انجام تجزیه واریانس بر روی داده‌ها، میانگین تیمارها با آزمون دانکس دسته‌بندی و *Bromus tectorum* مورد مقایسه قرار گرفت. نتایج نشان داد که بوتر اندازه‌های اسلیند جوانزئی بذر و رشد گیاهی به مورد مقایسه قرار گرفت. تاثیر منفی داشت. به طوری که با افزایش مقدار بوتر جوانزئی و رشد گیاهی در آنها به طور معنی‌داری کاهش یافت.


کلمات کلیدی: آللوپاتی، استند (*Peganum harmala*، جوانزئی، علی تجویزی، علی تجویزی)