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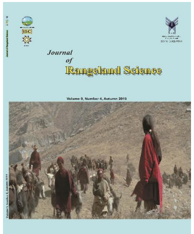

Research and Full Length Article:

Growth, Phytoremediation Potency and Essential Oil Component of *Ferula haussknechtii* H.Wolff ex Rech.f. Grown Under Lead Stress Condition

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Abstract. *Ferula haussknechtii* H. Wolff ex Rech.F. is an important medicinal plant belonging to the Apiaceae, family. In order to investigate the effect of Pb on the growth, chemical composition of essential oil and accumulation of metals in this species, a pot study was conducted under glasshouse condition. Seeds were collected from natural rangelands of Lorestan province, Iran in summer 2016. Seeds were tested for dormancy breaking and were sown in total 12 of 5 kg plastic pots. After 120 days, when the plants grew to about 10 cm, the plants were exposed to three different lead nitrate [$Pb(NO_3)_2$] concentrations of control (0), 2 and 4 mM (as Pb1, Pb2 and Pb3), respectively. The results revealed that the shoot length decreased by 5.2 and 28.9% with the application of 2 and 4 mM lead respectively as compared to the control. The maximum value for the survival rate was obtained in Pb1 treatment. The Root Concentration Factor (RCF) and Translocation Factor (TF) values were all less than the desired unit. The essential oil percent decreased by 21 and 39% with the application of 2 and 4 mM lead nitrate, respectively, as compared to the control. According to the results of GC-MS, a total of 36 compounds were detected in the essential oils of *F. haussknechtii*. β-Eudesmol (10.8%), camphor (13.8%), γ-eudesmol (8.7%), α-pinene (5.9%), myrcene (8.9%) and caryophyllene oxide (8.2%) were the main constituents of the essential oil of *F. haussknechtii*. It was concluded that addition of lead nitrate affected the constituents of the essential oil of *F. haussknechtii* and this plant is not appropriate for phytoremediation or phytoextraction purposes.

Key words: *Ferula haussknechtii*, Essential oil, GC-MS, Lead

Introduction

Environmental stresses such as heavy metals affect the growth and quality of essential oil component of plants. Lead is a very immobile, toxic and highly persistent element in the environment (Finster *et al*., 2004) which accumulates in the topsoil (Traunfeld and Clement, 2001) and does not have much downward movement (Haiyan and Stuanes, 2003). Heavy metal pollutants influence the growth of plants (Schmidt, 2003) and may lead to their death (Guala *et al*., 2010) at higher concentrations. In general, lead pollution has shown negative impact on plant characteristics (Moameri *et al*., 2017).

Total biomass productions of *Vetiver zizanioides* (Chantachon *et al*., 2004) and *Plantago major* (Kosobrukhov *et al*., 2004) remarkably decreased with the increase in Pb concentrations. However, the increased root dry matter and root shoot weight ratio (RS) after exposing plants to different concentrations of Pb has been reported by other researchers (Larbi *et al*., 2002). The literature review indicates that RS decreased significantly (Lu *et al*., 2013) or not significantly altered (Subin and Francis, 2013) when plants were exposed to different concentrations of heavy metals.

Some researches indicated that Pb contamination is capable of plant growth reduction by reducing biomass and leads to reduce the quality of essential oil components (Sabra *et al*., 2018). At low concentrations, lead pollution could cause instability in ion absorption by roots of plants, significant metabolic changes in photosynthetic capacity and ultimately plant growth inhibition (Amin *et al*., 2018). In contrast to organic compounds, heavy metals are not naturally decomposed (Jadia and Fulekar, 2008).

Phytoremediation is a green and environmentally friendly technology (Paz-Alberto and Sigua, 2013) which can be used economically and effectively to remediate metal-contaminated sites (Yoon *et al*., 2006). Phytoextraction is recognized as a helpful phytoremediation technique for removing heavy metals from contaminated soils (Milic *et al*., 2012). Exclusion and accumulation of heavy metals in plants are two chief tolerance mechanisms and have been reported by other researchers (Reeves and Baker, 2000). Translocation Factor (TF) in hyper-accumulators is more than one (Macnair, 2003) which shows the ability of heavy metals to move from roots to shoots (Zhao *et al*., 2006). Plants are classified as excluder if this ratio is less than one (Baker, 1981). Both root concentration factor (RCF) and TF can be used to assess the efficiency of a plant species for phytoremediation application (Yoon *et al*., 2006).

Various environmental stresses affect the essential oil production of aromatic plants (Burbott and Loomis, 1969). Heavy metals have the potential to change the generation of secondary metabolites in plants, but the severity depends on the plant species, heavy metal type and concentration of pollution (Maleki *et al*., 2017).

As an important environmental factor (Ebrahimi *et al*., 2014), heavy metals have a significant effect on the essential oil composition of plants (Figueiredo *et al*., 2008). The application of heavy metals changes the growth, metal accumulation and chemical composition of essential oils of *Ocimum basilicum* and mint species (Ghorbanpour *et al*., 2016; Prasad *et al*., 2010, 2008).

Ferula haussknechtii H.Wolff ex Rech.f. is a species of perennial herbs belonging to the Apiaceae family. The literature review showed that studies on *F. haussknechtii* are very limited. So far, few scientific reports have been published on the essential oil composition or phytoremediation potency of this plant. Considering the fact that heavy metals are increasing in plant growth environments, studies on the effect of heavy metal stress on the characteristics of plant species are necessary. Thus, the aim of this study is to investigate the effect of Pb on the growth, chemical composition of essential oil and accumulation of metals in *F. haussknechtii*.

Materials and Methods

Experiment layout and data collection

This study was conducted under greenhouse conditions in Malayer University, Iran. Seeds were sown in pots in greenhouse at 15-27°C under a photoperiod of 16 h. Soil samples were provided from the field (where the greenhouse experiment was conducted) in depth of 10 cm. Soil samples were air-dried and sieved at 2 mm mesh to remove the stones and litter residues.

The seeds were collected from Chal-e Kabod mount rangeland (33°52'41" N, 48°28'09" E) of Aleshtar county, Lorestan province, Iran. Seeds were homogenized and assessed for seed dormancy by the germination test. After the detection of seed dormancy, for seed dormancy breaking, the seeds were immersed in 30 ml GA_3 solution according to the doses and immersion times of the standard method (Fernandez *et al*., 2002). Then, 100 seeds were sown in pots at depth of about 2 cm in 5 kg plastic pots. Since *F. haussknechtii* is a slow-growing plant, to obtain an adequate volume of plant biomass, after the emergence of the seeds and their growth to about 10 cm, the plants were exposed to three different lead nitrate [$Pb(NO_3)_2$] concentrations including 0, 2 and 4 mM. Lead exposure treatments were developed with separately measured amount of dissolved lead nitrate (Merck) in distilled water. Each pot was irrigated with a different concentration of lead nitrate solution. The soil moisture content of the pots was kept in field capacity using the corresponding lead nitrate solutions as irrigation water. The growth parameters of the plants were measured after 8 months (October 2017 to May 2018). At this time, plants were in vegetative growth stage and shoot length and root length were separately measured in cm.

The dry weight was determined after the plants were oven-dried at 70°C for 48 h. In addition, the dry weight of the roots and shoots was separately measured in g.

Root Shoot weight ratio (RS) was calculated according to root to shoot dry weight.

Survival rate were determined as the ratio of survived seeds to total seeds number per pot as percent. The plants were assumed dead if necrosis of all leaves, stems and roots (Chelli-Chaabouni *et al*., 2010).

Lead determination

Lead was determined in plant and soil for assessment of the potential of plant species for phytoremediation. The concentrations of lead in plant tissues and soil samples were measured using atomic absorption spectrometry (Contra 700 flame). In order to assess the potential of phytoremediation of Pb-contaminated soil, the concentration factor (RCF) and translocation factor (TF) were determined. TF was measured by dividing metal concentration at shoot by its concentration at root (Marchiol *et al*., 2004). RCF was calculated as the ratio of root Pb concentration to soil Pb concentration (Yoon *et al*., 2006).

Essential oil analysis

For essential oil extraction, 200 g dried sample (aerial parts) from each replication were triturated and steam-hydro distilled for 3 h to measure essential oil using Kelvenger Instrument (Clevenger, 1928). The essential oil content was measured as percentage of the plant dry weight (Khalid, 2012) as follows.

$$\text{Essential oil content } \% = \frac{\text{Essential oil weight g}}{\text{Aerial biomass yield g}} \times 100$$

The essential oil compositions were detected using GC/MS a Varian 3400

GC-MS system equipped with a db-5 fused silica column (Adams, 2007).

Statistical analysis

The experiment was carried out according to completely randomized design using four replications. The Kolmogorov-Smirnov test was applied to test a normal distribution and the achieved means were compared using Duncan's test.

Results

The effect of Pb concentrations on seedling growth and essential content

Based on ANOVA and means comparison between treatments, results showed significant differences between treatments for shoot and root length traits. Both traits were decreased significantly with increase in lead concentration level. The highest and lowest shoot length values with average values of 9.3 and 4.6 cm were observed in Pb1 and Pb3 treatments, respectively. The decrease in the shoot length was 5.2 and 28.9% with the application of 2 and 4 mM lead, respectively, as compared to the control. The highest and lowest root length values were obtained in Pb1 and Pb3 treatments, respectively. As compared with the control, significant decreases were observed in the root length (19.44 and 50% in Pb2 and Pb3 treatments, respectively) (Table 1). For root shoot weight ratio (RS), results showed significant differences between lead treatments ($P<0.05$) (Table 1). The plants exposed to higher levels of lead had a higher RS.

Survival rate was significantly different among various lead application treatments in the studied plant ($P<0.05$) (Table 1). Based on the ANOVA results, the survival rate was significantly reduced with increased lead concentrations. The higher and lower values of the survival rate with average values 87.5 and 55% were observed in Pb1 and Pb3, respectively (Table 1).

The Translocation Factor (TF) values of Pb in *F. haussknechtii* were all less than unit in different lead levels (Table 1). The TF of *F. haussknechtii* varied significantly ($p\leq 0.05$) under different levels of lead exposure (Table 1). Generally, TF increased significantly as lead application rate increased (Table 1). The lowest value of TF was obtained in Pb1 treatment while the highest value (0.32) was observed in Pb3 treatment (Table 1).

The Root Concentration factor (RCF) values differed under different Pb concentrations. There were significant differences ($p \leq 0.05$) between RCF values for various lead exposure levels. The highest RCF of Pb was found as 0.42 in Pb2 as compared to the other treatments while Pb1 exhibited the lowest RCF values (Table 1).

Table 1. The effect of different Pb concentration rates on shoot length, root length, RS, survival rate, and phytoremediation potency of *F. haussknechtii*

Pb-stress levels	Shoot Length (cm)	Root length (cm)	Root shoot weight ratio (RS)	Survival Rate (%)	Translocation Factor (TF)	Root Concentration factor (RCF)
Pb 1	9.3 a	3.6 a	0.39 a	87.5 a	0.00 c	0.00 c
Pb 2	8.2 a	2.9 a	0.35 b	70.0 b	0.21 b	0.42 a
Pb 3	4.6 b	1.8 b	0.39 a	55.0 c	0.32 a	0.21 b

(Different letters in the same column represent significant differences at the 0.05 probability level)

The results of analysis of variance (ANOVA) showed that the essential oil content (%) of *F. haussknechtii* differed significantly at different lead nitrate concentrations ($P<0.05$) (Fig. 1). The essential oil percent reduced significantly with increased lead concentrations. The decreases in essential oil percent of *F. haussknechtii* were 21 and 39% with the concentration of 2 and 4 mM lead nitrate, respectively, as compared to the control (Fig. 1).

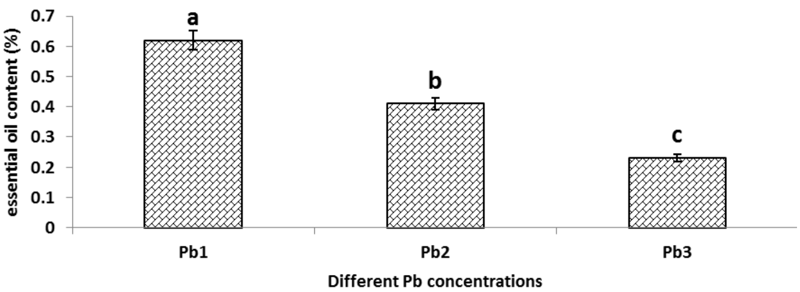

Fig. 1. The effect of different Pb concentrations on essential oil percent of *F. haussknechtii* (Different letters on each bar represent significant differences at the 0.05 probability level)

The effect of Pb on essential oil composition

Based on GC-MS results, a total of 36 compounds were identified in the essential oils of *F. haussknechtii*. β-Eudesmol (10.8%), camphor (13.8%), γ-eudesmol (8.7%), myrcene (8.9%), caryophyllene oxide (8.2%) and 1 α-pinene (5.9%) were the main constituents of the essential oil (Table 2). The constituents of the essential oil of the plant were affected by the addition of lead nitrate (Table 2).

The amount of α-pinene, β-pinene and myrcene decreased with the concentration of 2 mM lead nitrate as compared to the control. The significant decreases in the values were 72.8% in α-pinene, 55.2% in β-pinene and 60.6% in myrcene with the application of 2 mM lead nitrate as compared to the control. A further increase in the level of Pb (4 mM of lead nitrate) had no significant effect on α-pinene, β-pinene and myrcene content (Table 2). The contents of limonene in *F. haussknechtii* oil significantly increased with the application of 2 mM lead nitrate as compared to the control treatment. The application of 4 mM lead nitrate led to no significant decrease in limonene content (Table 2). The amount of 1,8-cineol and linalool decreased with the concentration of 2 and 4 mM lead nitrate as compared to the control treatment.

Table 2. The effects of Pb concentration on the chemical composition (%) of the essential oil of *F. haussknechtii* (Values are given as mean ± SD (standard deviation)

Compounds	RT(min)[#]	RI*	Pic. No	Pb 1	Pb 2	Pb 3
α-Thujene	8.47	925	1	1.0±0.02	1.1±0.02	0.9±0.03
α-Tinene	8.64	938	2	5.9±0.08	4.3±0.08	5.8±0.06
Camphene	8.90	950	3	1.7±0.17	1.9±0.17	1.9±0.15
β-Pinene	9.16	974	4	3.8±0.11	2.1±0.11	3.6±0.06
1,8-dehydro-Cineole	9.39	988	5	0.8±0.63	0.7±0.18	0.2±0.01
Myrcene	9.47	997	6	8.9±1.03	5.4±1.07	8.7±1.96
δ-۳-Carene	9.92	1015	7	0.7±0.18	1.0±0.11	0.6±0.13
Limonene	10.22	1029	8	2.8±0.62	3.4±0.54	2.7±0.15
1,8-Cineol	10.43	1040	9	1.5±0.11	0.9±0.07	0.9±0.11
Linalool	10.71	1098	10	2.9±0.05	2.3±0.05	1.9±0.06
6-Compheol	10.73	1115	11	0.5±0.01	0.6±0.01	0.4±0.01
β-Trans-Ocimene	10.75	1133	12	0.3±0.03	0.3±0.14	1.6±0.03
Camphor	11.00	1145	13	13.8±1.31	14.0±1.14	14.5±1.03
Pinocarvone	11.22	1164	14	1.8±0.62	1.8±0.26	1.4±0.22
p-Cymen-8-ol	11.44	1180	15	1.2±0.64	1.3±0.23	1.1±0.25
Myrtenol	11.57	1195	16	1.4±0.27	1.5±0.41	2.3±0.25
1-Verbenone	12.05	1211	17	0.5±0.08	0.5±0.02	0.8±0.03
Geraniol	12.09	1233	18	1.9±0.14	2.0±0.12	1.7±0.13
Bornyl acetate	12.45	1268	19	0.8±0.01	0.6±0.02	0.9±0.02
Carvacrol	12.52	1340	20	1.5±0.02	1.4±0.02	1.3±0.03
α- Cubebene	12.54	1361	21	1.3±0.12	1.3±0.10	1.1±0.12
β-Elemene	12.89	1388	22	0.7±0.02	0.8±0.05	1.8±0.04
Cedrene	13.05	1409	23	1.0±0.04	0.9±0.06	1.2±0.03
(E)-Caryophyllene	13.39	1420	24	1.4±0.05	1.5±0.25	1.6±0.06
Aristolene	13.66	1432	25	2.3±0.43	2.4±0.40	2.2±0.21

α-Amorphene	13.84	1490	26	1.0±0.16	1.0±0.05	0.9±0.09
cis-c-Cadinene	14.10	1511	27	0.9±0.03	0.8±0.03	1.3±0.06
trans-cadina-1(2)-4-diene	14.19	1537	28	0.4±0.01	0.3±0.02	0.9±0.03
Caryophyllene oxide	14.25	1578	29	8.2±0.11	7.9±0.13	9.3±0.11
Carotol	14.37	1596	30	0.4±0.05	0.5±0.05	0.6±0.04
10-epi-c-Eudesmol	14.69	1608	31	1.4±0.04	1.3±0.05	1.5±0.02
γ-Eudesmol	14.72	1619	32	8.7±1.10	9.0±1.37	9.3±1.83
β-Eudesmol	15.01	1629	33	10.8±1.18	10.0±1.80	13.2±1.92
α-Eudesmol	15.50	1640	34	4.2±0.81	4.5±0.70	3.9±0.63
Cadalene	15.96	1678	35	0.4±1.08	0.5±0.86	0.4±0.72
(Z)-(E)- feranesol	16.29	1705	36	0.5±0.03	0.4±0.04	0.4±0.01

RT: Retention Time, RI: Retention Indices

Discussion

Reduction in shoot and root lengths were observed in *F. haussknechtii* after lead exposure in this study. The survival rate of the plants reduced significantly with increased lead concentration rates (Table 1). Similar results have been reported by other researchers (Tandy *et al*., 2005; Abu Zinada *et al*., 2011). Based on the literature review, as an abiotic environmental stress, Pb-stress may affect the growth of parameters and physiological traits of plant species. The shoot and root length of *Spinacia oleracea* (Zinada *et al*., 2011) decreased with increasing concentrations of Pb. Other researchers (Tandy *et al*., 2005) have also reported a decrease in the shoot length and root length of *Helianthus annuus* under Pb pollution conditions. However, an increase in the shoot length and root length of *Sesbania exaltata* was detected after exposure of the plants to higher concentrations of Pb (Miller *et al*., 2011). Based on another research, the shoot characteristics of sugar beet were not significantly affected by Pb exposure (Miller *et al*., 2011). Therefore, the impact of heavy metals on plant growth depends on the species, soil characteristics and concentration of toxic metals in the growth environment (Prasad *et al*., 2010).

Based on the ANOVA results (Table 1), all values of the RCF and TF of *F. haussknechtii* were less than unit. Yoon *et al*. (2006) indicated that plants with a high RCF >1 and TF <1 are suitable to be applied in phytostabilization, and plants with both TF and RCF >1 are appropriate to be used in phytoextraction. Thus, the studied plant in this research (*F. haussknechtii)* is not suitable for phytoremediation or phytoextraction purposes.

This research indicated that the essential oil percent of *F. haussknechtii* significantly reduced as a result of the decrease in the herbage growth with increased lead concentrations (Fig. 1). Similar results have been reported by other authors (Prasad *et al*., 2010). The reasons for the changes in the essential oil percent of plants under heavy metals concentration are not well-known but could be related to the effects of metallic elements on enzymatic activity and carbon metabolism of plants (Prakash and Kardage, 1980).

Based on the results, the constituents of the essential oil of *F. haussknechtii* were affected by the addition of lead nitrate (Table 2). A similar result was previously reported by Prasad *et al*. (2010). Study on the chemical contents of the essential oil of the studied plant indicated that the quality of the produced essential oil was significantly affected by lead addition. Enzyme activity reduction (Van Assche and Clijsters, 1990), photosynthesis disruption (Vassilev and Yordanov, 1997), closing of the stomata (Barcelo and Poschenrieder, 1990), and prevention of the absorption of nutrients (Sanita di Toppi and Gabbrielli, 1999) were due to heavy metals stress.

The impact of metallic cations on the enzymatic activity and carbon metabolism under heavy metals stress (Prakash and Karadge, 1980) may be due to changes in the various constituents of the essential oil of *F. haussknechtii*. Heavy metals perform their catalytic function by forming an enzyme–substrate metal complex in some enzymes that produce several compounds of essential oils (Harrewijin *et al*., 2001).

Conclusion

So far, no research reports have been published on the essential oil composition or phytoremediation potency of *F. haussknechtii*. Considering the increase of heavy metals in plant growth environments, studies on the effect of heavy metal stress on the characteristics of plant species are necessary. The results of this investigation indicated that the growth of *F. haussknechtii* was significantly affected by the addition of lead stress. Based on TF and RCF values obtained in this research, *F. haussknechtii* is not suitable for phytoremediation or phytoextraction purposes. However, this plant produces essential oil in which 36 compounds were identified. The results indicated that the compositions of the essential oil of this plant were affected by lead stress.

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رشد، توان گیاه پالایی و ترکیبات اسانس *Ferula haussknechtii* H.Wolff ex Rech.f. تحت تاثیر تنش سرب

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چکیده. *Ferula haussknechtii* H.Wolff ex Rech.f. به عنوان یک گونه گیاهی تولید کننده اسانس به خانواده Apiaceae تعلق دارد. تاکنون تحقیقات کمی در مورد ترکیبات اسانس و توان گیاه پالایی این گونه گیاهی انجام شده است. بذر این گونه از مراتع طبیعی استان لرستان در تابستان سال ۱۳۹۵ تهیه شد. بعد از اعمال تیمارهای شکست خواب بذر، بذرهای تیمار شده در گلدان‌های پلاستیکی ۵ کیلوگرمی کاشته شدند. بعد از جوانه زنی بذرها، گیاهان در معرض تیمارهای مختلف آلودگی سرب قرار گرفتند. سطوح بدون آلودگی بعنوان تیمار شاهد (Pb1)، ۲mM سرب (Pb2) و ۴mM سرب (Pb3) برای انجام آزمایش در نظر گرفته شدند. نتایج نشان داد که طول ساقه در اثر کاربرد سطوح ۲ و ۴ mM سرب به میزان ۵/۲ و ۲۸/۹ درصد در مقایسه با تیمار شاهد کاهش داشت. بیشترین میزان عددی درصد زنده‌مانی در تیمار شاهد (Pb1) دیده شد. صفات میزان تجمع ریشه (RCF) و فاکتور انتقال (TF) کمتر از ۱ به دست آمد. بنابراین این گونه برای گیاه پالایی و تثبیت فلزات سنگین مناسب نیست. همچنین نتایج نشان داد که در مقایسه با تیمار شاهد، میزان تولید اسانس در تیمارهای Pb2 و Pb3 به ترتیب به میزان ۲۱ و ۳۹ درصد کاهش نشان داد. بر اساس نتایج به دست آمده از دستگاه GC-MS، در کل ۳۶ ترکیب شیمیایی در اسانس *F. haussknechtii* شناخته شدند که β-Eudesmol، camphor، γ-eudesmol، α-pinene، myrcene و caryophyllene oxide به ترتیب با ۱۰/۸، ۱۳/۸، ۸/۷، ۵/۹، ۸/۹ و ۸/۲ درصد ترکیبات اصلی اسانس *F. haussknechtii* بودند. اعمال تنش سرب سبب تغییر ترکیبات شیمیایی اسانس *F. haussknechtii* گردید.

کلمات کلیدی: *Ferula haussknechtii*، اسانس، GC-MS، سرب