

Research Article

Allelopathic effect of herbal formulation containing *Ferula assa-foetida* L. essential oil and castor oil (*Ricinus communis* L.) as an herbicide on *Amaranthus retroflexus* L. seed germination

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ARTICLE INFO	ABSTRACT
<p>Keywords: Herbicide Essential oil Germination Biocompatible Safe</p>	<p>Background: Medicinal plant contain phytochemicals which have inhibitory effects on plant pathogens. Weeds compete with the main crops and reduce the growth, yield, and quality of agricultural products to some extent. One of the most common methods of reducing weed damage is the use of chemical herbicides. Due to the side effects that chemical herbicides on human health and the environment, there is a need to replace biocompatible and low-risk herbicides. Objective: In this study, the allelopathic properties of the <i>Ferula assa-foetida</i> L. essential oil and castor oil (<i>Ricinus communis</i> L.) have been investigated as a botanical herbicide to prevent germination of redroot pigweed seeds (<i>Amaranthus retroflexus</i> L.). Methods: In this regard, an herbal formulation based on <i>Ferula assa-foetida</i> essential oil and castor oil was prepared and its effect on the germination of redroot pigweed seeds was studied in laboratory conditions. The chemical composition of the herbal formulation was analyzed by GC/MS. Results: The results showed that this herbal formulation in concentration 0.75 % and 1 % inhibits the germination of weed seeds about 70 %. The main constituents of <i>Ferula assa-foetida</i> and <i>Ricinus communis</i> were (E)-1-propenyl sec-butyl disulfide (43.9 %) and ricinoleic acid methyl ester (58.1 %), respectively. Conclusion: It was found that the studied botanical formulation has herbicidal properties. Therefore, more research is needed to achieve promising results in order to replace chemical herbicides with botanical herbicides.</p>

1. Introduction

In recent years, medicinal plants with biologically active compounds have been widely studied because their compounds inhibit or delay the growth of microorganisms. [1]. Essential oils

(EOs) are naturally produced by aromatic plants and contain a wide variety of volatile molecules, mainly secondary metabolites that have multiple biological activities. The properties of essential oils such as antioxidant, antimicrobial and anti-

Abbreviations: GC/MS, Gas Chromatography/Mass Spectrometry; 2,4-D: 2,4-Dichlorophenoxyacetic Acid; IKS, Indigenous Knowledge System

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inflammatory effects have been known for a long time and are therefore widely used in traditional medicine, cosmetics and the food industry. Despite its action against many phytopathogenic fungi, oomycetes and bacteria, as well as weeds, its use in agriculture remains surprisingly rare [2]. Nowadays, the demand for healthy food and sustainable agriculture is increasing, and the interest in using oil and medicinal plant extracts to replace traditional chemical pesticides to control pests and diseases is also increasing. Bioactive compounds from plants are usually extracted in aqueous form, using ethanol or other organic solvents, or when present in essential oils by steam extraction [1]. The ability of plants to produce various secondary metabolites can significantly reduce pest population dynamics through new and efficient plant pesticides based on plant extracts, thus providing natural protection for agricultural pests [3].

A variety of phytochemicals found in most medicinal plants, such as alkaloids, cyanogenic glycosides, phenylpropanoids, polyketides, anthocyanins, carbohydrates, amino acids, lipids, nucleic acids, terpenes, flavonoids, phenols, saponins, and tannins are essential materials in production. Various pesticides and fungicides that can be used to protect crops and horticultural crops. In all agricultural systems, weeds are the most costly pest to control, accounting for 30% of potential losses [4]. Weeds are harmful plants that interfere with the management of agricultural production systems, compete with main crops for available nutrient resources and space, and to a certain extent reduce the growth, yield, and quality of agricultural products [5]. Herbicides are designed to kill plants, so if they are applied directly to these plants, or if they drift or volatilize on the plants, it is not surprising that they will harm or kill the desired species. Many

ester herbicides have been shown to evaporate enough vapor from the treated plants to cause serious damage to other plants. In addition to killing target plants, exposure to pesticides can also cause sublethal effects on plants. Phenoxy herbicides, including 2,4-D, can damage nearby trees and shrubs if they drift or volatilize on the leaves. Exposure to the herbicide glyphosate can severely reduce seed quality. It can also increase the susceptibility of certain plants to diseases. This poses a special threat to endangered plant species. The US Fish and Wildlife Service has identified 74 endangered plants that may be threatened only by glyphosate. Exposure to the herbicide chlorpyrazone can reduce the yield of potato plants [6].

Glyphosate is the most widely used herbicide in the world [7]. Although people are increasingly concerned about the consequences of the use of large amounts of glyphosate-based herbicides in agricultural ecosystems, their potential effects on non-target soil organisms and soil functions are largely unknown [8]. The use of ecological methods to control weeds is gaining more and more attention in agricultural practices. On the one hand, the widespread use of herbicides of synthetic origin has led to many adverse effects, such as the high persistence of herbicides in the environment and the food chain, and the development of highly resistant weed populations. Second, there are some special sowing systems, such as those for organic production, where the use of synthetic chemicals is prohibited. In this general framework, more and more farmers are looking for alternative technological options for weed management [9]. Among natural plant products, volatile essential oils and their components have attracted a lot of attention because of their phytotoxicity and their strong activity against weeds. Terpenoids,

especially monoterpenoids and sesquiterpenoids, are the main components of essential oils and are usually responsible for their inhibitory activity in plants. Plant essential oil herbicides have been shown to be effective against a variety of weeds and may be a natural substitute for non-selective herbicides. They can be used as fumigants, granular preparations, or direct sprays [10]. *Ferula assa-foetida* is an herb, hermaphrodite and perennial plant of the Umbelliferae family. About more than 170 species, sixty species of *Ferula* are widely distributed in Central Asia, especially in West Afghanistan, Iraq, Turkey and Eastern Iran, Europe and North Africa [11]. *Asafoetida* originated in Afghanistan from Central Asia and eastern Iran and is now cultivated primarily in Iran and Afghanistan, where it is exported to other parts of the world. Although it is not of Indian origin, it has long been used in Indian medicine and cooking [12]. *Ferula assa-foetida* essential oils exhibit a variety of activities including antibacterial, insecticidal, antioxidant, germination, cytotoxicity, antitumor and antidiabetic [13]. The resin of *Ferula assa-foetida* fraction contains ferulic acid and its esters, coumarin, sesquiterpene coumarin, and other terpenoids. Gum contains glucose, galactose, arabinose, rhamnose, glucuronic acid, polysaccharides and glycoproteins, and the volatile fraction contains sulfur-containing compounds, monoterpenes and other volatile terpenoids [11].

Ricinus communis is an annual oil seed plant commonly known as castor oil. Sometimes called castor beans, they are not real beans. *Ricinus communis*, which belongs to the family Euphorbiaceae, has the potential to grow in various geographic regions [14]. Therefore, it is used in multiple fields such as agriculture, pharmaceuticals, and industry. Castor oil products include: Ointments, nylons, lacquers,

lubricants for aircraft engines, hydraulic fluids, dyes, detergents, plastics, artificial leathers, cosmetics, perfumes [14]. Castor oil contains the monounsaturated hydroxy fatty acid ricinoleic acid as the main component of its fatty acid profile. This acid is 12-hydroxy-9-octadecenoic acid and has an unusual polarity due to the position of the hydroxyl group. Castor oil, sometimes referred to as ricinoleic acid triglyceride, is the only commercially available vegetable oil rich in the hydroxy functional groups of fatty acids (70-90 %) [15].

Castor is native to the southeastern Mediterranean basin, East Africa and India, but it is widely distributed in tropical regions and is widely grown as an ornamental plant in other places. For centuries, people have been cultivating castor to get the oil stored in its seeds. The hulled seeds contain 35-55 % oil. Plant leaves, stems, and especially seeds contain ricin and ricin, which are toxic to humans and animals if ingested. However, ricin is not present in the oil [16]. In the world, the redroot pigweed is considered the most dangerous weed [17]. Redroot pigweed weeds are an annual C4 species that will produce up to 100,000 seeds per plant, although this number can be much higher under optimal growing conditions. Seeds are spread primarily by wind, water, and animals, but they are also spread by human disturbance, for example when trapped in the ground by agricultural machinery. Although some researchers report that seeds can still survive for a long time, others have concluded that the viability of buried seeds is significantly reduced after two years [18]. The objectives of this research were to determine the phytotoxic effects of botanical formulation of *Ferula assa-foetida* essential oil and castor oil (*Ricinus communis*) on the germination and primary root and primary pedicle length of redroot pigweed.

2. Materials and Methods

Redroot pigweed seed with herbarium code 724 (IMPH) was used in this study. *Ferula assa-foetida* essential oil and castor oil were obtained from Samanik Pharmaceutical Co. (Tehran, Iran).

2.1. Extraction of essential oils

We used an all-glass Clevenger-type apparatus to conduct 2.5 h of hydro-distillation on the gum of *Ferula assa-foetida* (100 gr) of each target plants and the pale yellow essential oil produced. This method for the extraction of oils is recommended by the European Pharmacopoeia [19]. The oils were dehydration over anhydrous sodium sulphate (Merck, Darmstadt, Germany) and stored in sealed vials at 2 °C before analysis.

2.2. Preparation botanical formulation

The formulation was based on Jokar and coworkers method with some modifications and changes [20]. Briefly *Ferula assa-foetida* essential oil and castor oil was used in a ratio of 1:1 to prepare botanical formulation. Suitable solvents and cosolvents were used.

2.3. Gas Chromatography/Mass Spectroscopy (GC/MS) of *Ferula assa-foetida*

Analysis of *Ferula assa-foetida* volatile component was performed on Agilent 6890 system (Agilent, Littleton, Colorado, USA) coupled with Agilent 5973 N mass selective detector equipped with a BPX5 fused silica column (30 m × 0.25 mm i.d., film thickness 0.25 µm). Following injection, 5 min after injection, oven temperature was increased from 50 to 240 °C at a rate of 3 °C/min and then reached to

300 °C at a rate of 15 °C/min and hold 3 min in this temperature. Other operating conditions were as follows: carrier gas, He (99.999 %), with a flow rate of 0.5 ml/min; injector temperature, 250 °C; and split ratio, 1:35. Mass spectra were taken at 70 eV a scan time of 1 s and a mass range of 40-500 amu [21].

2.4. Preparation of castor oil methyl ester for GC analysis

2 mg of sample was weighted in 2 ml tube and 2 ml of heptane was added to it and vortex. Then 1.2 ml potassium hydroxide (2 mM) in methanol was added, fast stirred and 1 min vortex. After 5 min, the heptane phase was separated and added to the vial containing 2 g of sodium hydrogen sulfate. After mixing the sample was ready for injection to gas chromatography.

2.5. Gas Chromatography/Mass Spectroscopy (GC/MS) of castor oil

Analysis of castor oil was performed on Agilent 6890 system (Agilent, Littleton, Colorado, USA) coupled with Agilent 5973 N mass selective detector equipped with a BPX5 fused silica column (30 m × 0.25 mm i.d., film thickness 0.25 µm). Following injection, 5 min after injection, oven temperature was increased from 70 to 280 °C at a rate of 10 °C/min and hold 3 min in this temperature and then reached to 300 °C at a rate of 25 °C/min and hold 5 min in this temperature. Other operating conditions were as follows: carrier gas, He (99.999 %), with a flow rate of 1 ml/min; injector temperature, 290 °C; and split ratio, 1:20. Mass spectra were taken at 70 eV a scan time of 1 s and a mass range of 40-700 amu.

Table 1. Chemical composition of the essential oil of *Ferula assa-foetida*

No.	RT ^a	Compound	KI ^b	%
1	23.40	<i>sec</i> -Butyl propyl disulfide	1173	0.68
2	23.66	(<i>Z</i>)-1-Propenyl <i>sec</i> -butyl disulfide	1178	23.30
3	23.97	(<i>E</i>)-1-Propenyl <i>sec</i>-butyl disulfide	1184	43.90
4	25.73	<i>sec</i> -Butyl Disulfide	1221	1.30
5	35.81	Thiazolidine	1445	8.19
6	38.49	α -Farnesene	1509	0.20
7	38.80	β -Dihydroagarofuran	1517	1.09
8	42.41	Guaiol	1609	0.37
9	42.74	Carotol	1618	0.24
10	43.50	γ -Eudesmol	1638	1.92
11	44.00	Hinesol	1651	0.35
	Total			81.54

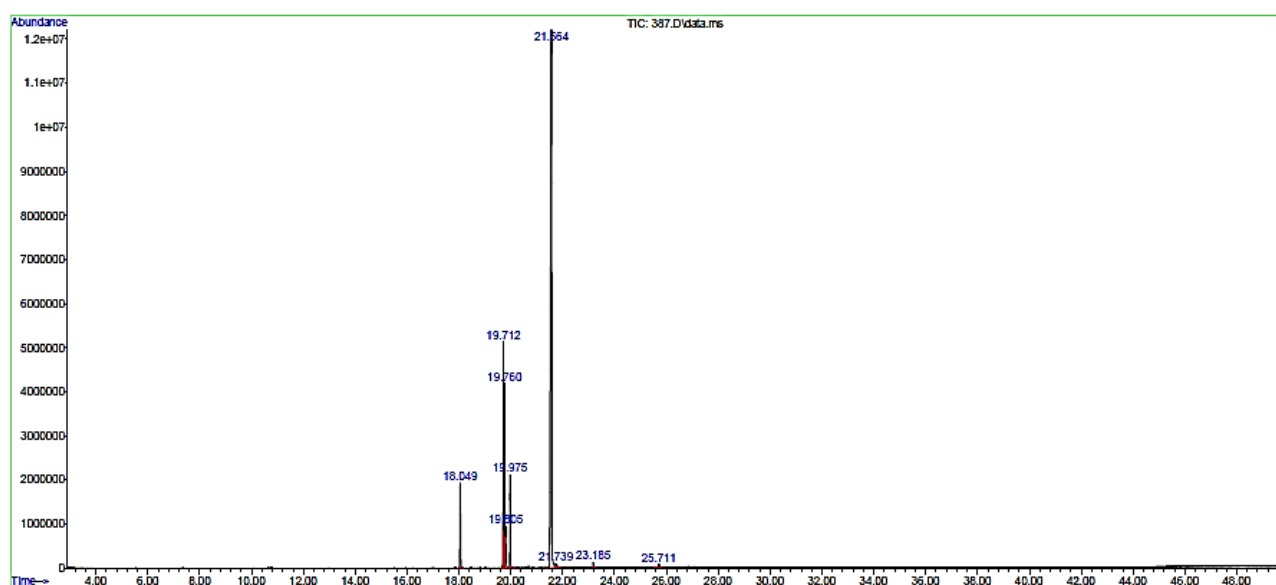
^a RT: Retention time^b KI: Kovats index**Fig. 2.** The GC Chromatogram of castor oil

Table 2. Chemical composition of methylated castor oil through GC/MS

No.	RT ^a	Compound	KI ^b	%
1	18.05	Palmitic acid methyl ester	1932	4.98
2	19.71	Linoleic acid methyl ester	2054	14.91
3	19.76	Oleic acid methyl ester	2056	12.68
4	19.80	9-Octadecenoic acid methyl ester	2059	2.47
5	19.98	Stearic acid methyl ester	2068	5.64
6	21.57	Ricinoleic acid methyl ester	2258	58.14
7	21.74	Arachidic acid methyl ester	2268	0.50
8	25.71	Squalene	2808	0.28
Total				99.60

^a RT: Retention time

^b KI: Kovats index

The three important sulfur components identified include 2 butyl disulfide 1 propyl 1 (methylthio) propyl 1 propenyl disulfide and 2 butyl 3 (methylthio) 2 propenyl disulfide [11].

Based on the results, the herbal formulation was significant on the lack of germination of weed seeds at the level of one percent. It was also found that with increasing concentration, this effectiveness increased. By measuring the primary roots and primary pedicles, it was found that the herbal formulation was also effective on the length; so that with increasing concentration, the primary root and primary pedicles' length decreased. The lowest germination percentages were observed at concentrations of 0.75 and 1 %, 32.5 and 30 %, respectively. Comparison of means based on the LSD test showed that there was no significant difference between concentrations of 0.75 and 1 and both were in group C. Concentrations of 0.5 and 0.25 were also in the same group. Negative control was

grouped in a with the highest germination rate of 85 % (Fig. 3).

The primary root lengths at 0.75 and 1 were 0.75 and 0.55 cm, respectively, both in group C. Negative control was grouped in a with the longest germination length (4.5 cm) (Fig. 4).

The primary pedicle lengths at 0.5, 0.75 and 1 were 0.625, 0.3 and 0.2 cm, respectively. All three were placed in group C. Negative control was grouped in a with the longest pedicle length (2.5 cm) (Fig. 5).

The lowest germination rate in Fig. 6 was observed in the herbal formulation with a concentration of 1 % and the highest in negative control treatment. In mean germination time (MGT), the lowest percentage was related to negative control, and the highest was related to 1% of herbal formulation (Fig. 7). In germination index (Fig. 8) and vigor index (Fig. 9), the highest percentage was related to negative control, and the lowest percentage was seen in 1 % of herbal formulation.

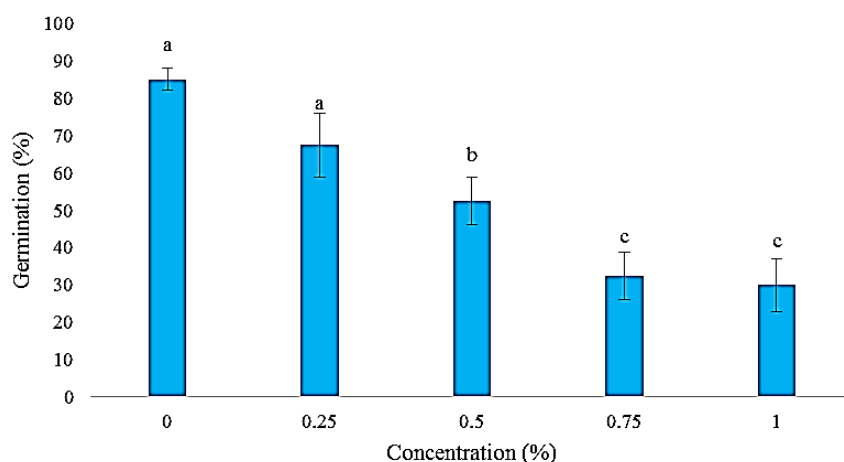


Fig. 3. The percent of germination pigweed seeds treatment with negative control and four concentrations of botanical formulation (0.25, 0.5, 0.75 and 1 %). The bars on the columns represent the standard error.

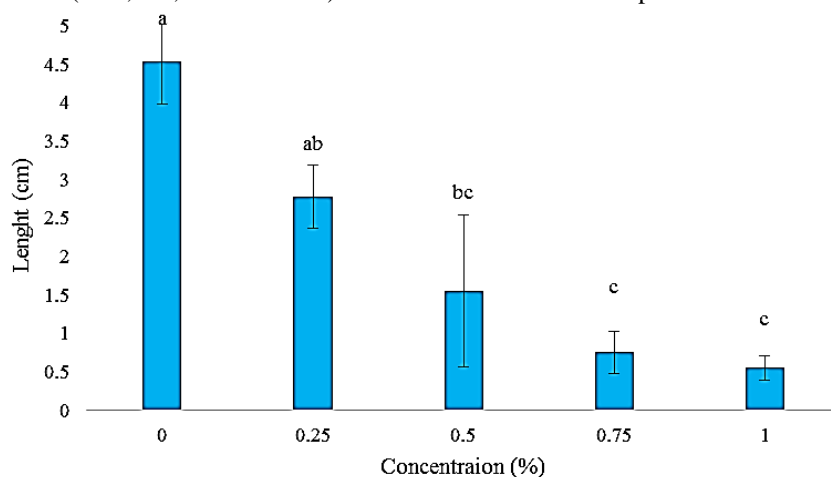


Fig. 4. Primary root of red pigweed after treatment with negative control and four concentrations of botanical formulation (0.25, 0.5, 0.75 and 1 %) based on centimeter. The bars on the columns represent the standard error.

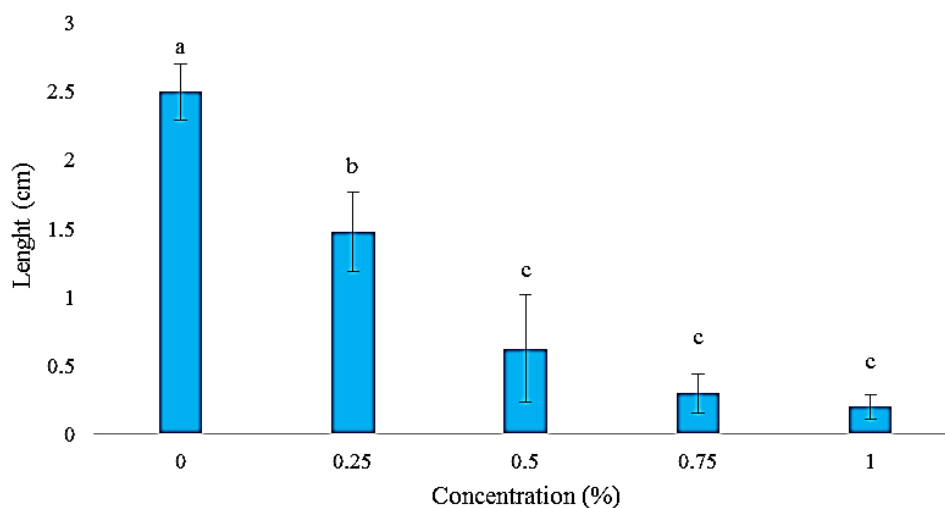


Fig. 5. Primary pedicle of red pigweed after treatment with negative control and four concentrations of botanical formulation (0.25, 0.5, 0.75 and 1 %) based on centimeter. The bars on the columns represent the standard error.

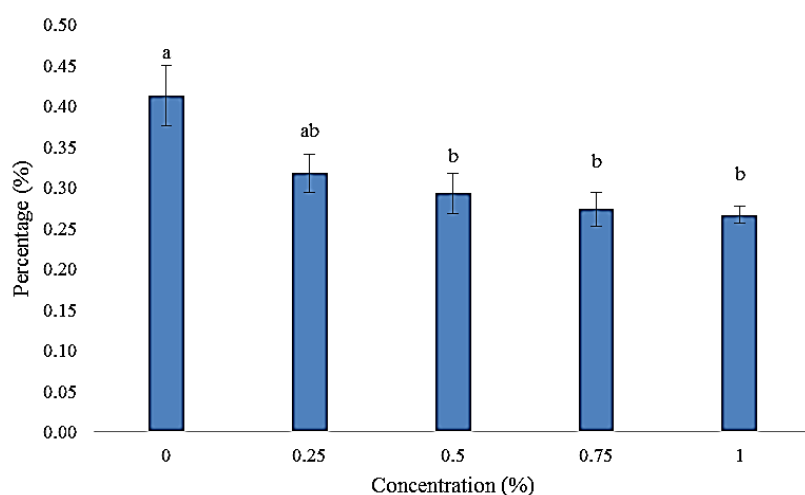


Fig. 6. Germination rate of red pigweed after treatment with negative control and four concentrations of botanical formulation (0.25, 0.5, 0.75 and 1%) based on centimeter. The bars on the columns represent the standard error.

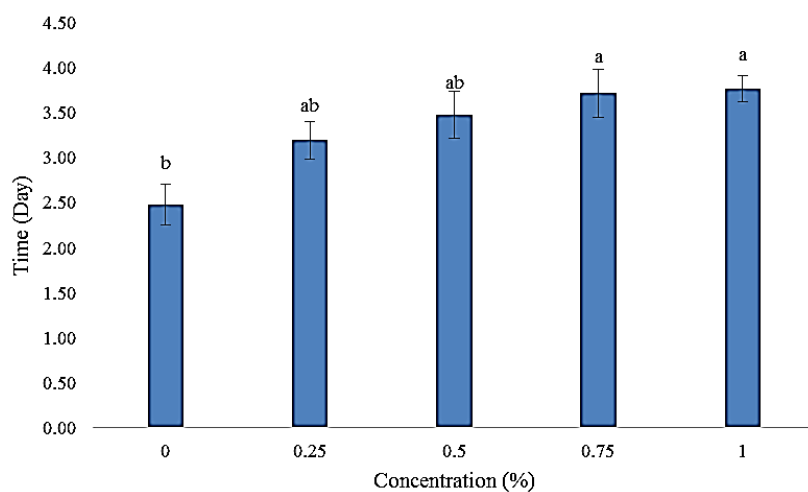


Fig. 7. MGT of red pigweed after treatment with negative control and four concentrations of botanical formulation (0.25, 0.5, 0.75 and 1%) based on centimeter. The bars on the columns represent the standard error.

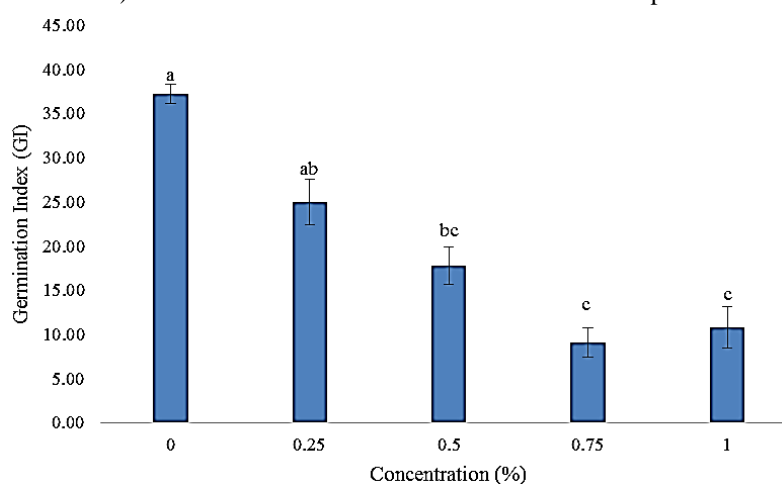


Fig. 8. Germination Index of red pigweed after treatment with negative control and four concentrations of botanical formulation (0.25, 0.5, 0.75 and 1%) based on centimeter. The bars on the columns represent the standard error.

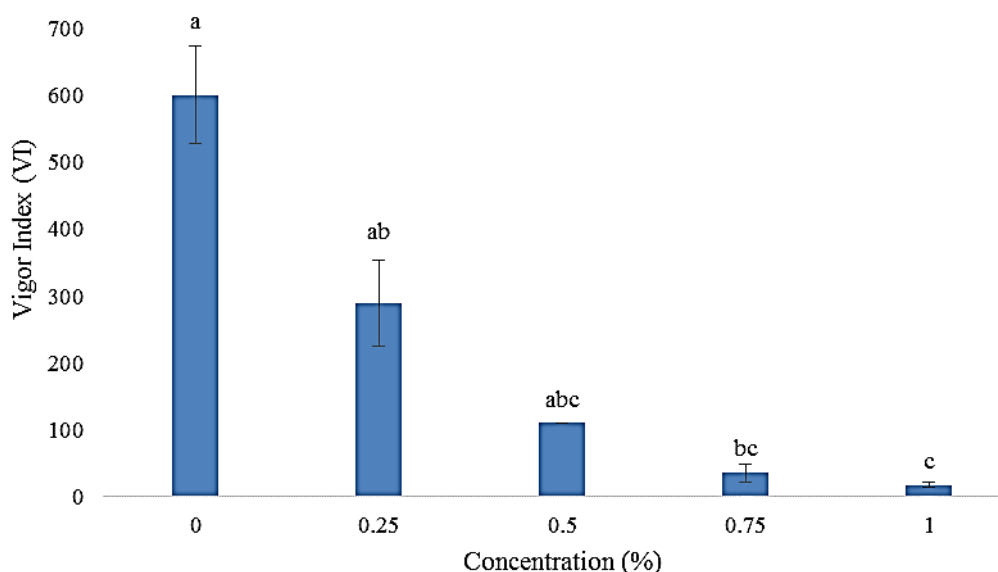


Fig. 9. Vigor Index of red pigweed after treatment with negative control and four concentrations of botanical formulation (0.25, 0.5, 0.75 and 1 %) based on centimeter. The bars on the columns represent the standard error.

4. Discussion

Increasing weed control problems such as herbicide-resistant species and chemical residues in soil and environment, increase the demand for alternative weed control methods with growing concerns about environment. One possibility is to identify natural substances that have an allelopathic effect. In this regard, many researchers have emphasized the use of medicinal plants containing aromatic compounds [22]. Allelopathy is a complex process involving interactions between different groups of chemicals such as flavonoids, alkaloids, steroids, terpenoids, phenolic compounds and amino acids [23]. This process is important in two respects: minimizing the negative effects of allelopathy on plant growth and yield, and using allelopathy for weed control [24]. In general, research in the field of allelopathy has received a lot of attention over the last few decades to achieve these goals: Manipulating allelopathy to increase yields and protect biodiversity and weed management. Protect the environment by controlling weeds, pests and plant diseases with eco-friendly

allelochemicals [25]. There are a few reviews concerning the mode of action of plant allelochemicals in controlling or suppression of weeds [26]. Assessment of allelopathic properties of plants is a new ecological and biological approach that may lead to the discovery of biological herbicides and growth inhibitors. A study was conducted by Razavi and his colleagues in 2013 to investigate the potential of *Ferula assa-foetida*'s allelopathy for germination and seedling growth of two weeds, *Cardaria draba* and *Plantago major*. *Ferula assa-foetida* extract seems to possess allelopathic effect on *Cardaria draba* and *Plantago major* at germination stage, the property which can be considered in developing bio herbicides [27]. In 2014, the essential oil composition and the allelopathic properties of Anghuzeh (*Ferula assa-foetida* L.) were investigated by Bagheri and et al. A large number of growth parameters, such as germination, radical and coverage of seeds, and a large number of growth parameters, such as their dry and fresh weight, and an abnormal percentage of seedlings that was

evaluated. The results of this survey showed an essential oil of Anghuzeh significant ($P \leq 0.05$), and the shrill effect on the weeds and crops studied. In addition, the results of essential oil analysis showed that a total of 13 components in α -pinene (21.3 %), β -pinene, (47.1 %), and 1,2-tineolane oil (18.6 %) have been identified. The effect of observed allelopathy of essential oils is related to the components of oil and their synergistic effects. The findings of this research was the first step of using Anghuzeh essential oil as a natural herbicide [23].

The result of mentioned study in above was same to our research and they also found Anghuzeh have an herbicidal properties. There are some reasearch that demonstrated the *Ricinus communis* was effective as an herbicide and had allelopathic effect. The results of the research in 2015 by Saadaoui and et L. showed a high allelopathic effect of aqueous extract of *Ricinus communis* in Tunisian agricultural land (*H. vulgare*, *M. Sativa*, *M. Sativa*, *T. foenum-graecum*, *L. Culinaris*, and *C. arietinum*) [24].

In other research, the effects of *Crocus sativus* L., *Ricinus community* L., *Nicotiana tabacum* L., *Datura inoxia* Mill., *Nerium oleander* L. and *Sorghum vulgare* L. were determined on redroot pigweed. Powders and water extracts from these plants are used for experiments under laboratory and greenhouse conditions. In the laboratory, all water extracts showed significant inhibitory effects on germination, seedling length, and weight of red-rooted weed plants. The most allelopathic effect on redroot pigweed were *R. communis*, common tobacco, and *D. inoxia*. In the greenhouse test, the extracts and powders of these plants also showed a significant inhibitory effect on dry weight, height, leaf area, number of viable plants and chlorophyll content of the weeds. In germination bioassays and powder application, the inhibitory effect depends on the

dose, the higher the concentration, and the stronger the inhibitory effect. From the results obtained, it can be concluded that the powders and extracts of the tested species have herbicidal potential for red root weeds and can be used as natural herbicides and mulches [17].

In general, allelopathy is the end result of the simultaneous motion of numerous compounds and frequently consists of compounds, which their chemistry is divergent [25]. It has been suggested that a few allelochemicals can purpose root molecular demise not directly through facilitating the manufacturing of reactive oxygen species (ROS), which may additionally act as signaling molecules main to modifications in hormonal stability in the course of seed germination [26].

5. Conclusion

Research on medicinal plant extracts has revealed thousands of phytochemicals, which have inhibitory effects on plant pathogens. Therefore, it is necessary to study the medicinal plants used in the Indigenous Knowledge System (IKS), which are known to have high antimicrobial and/or antioxidant abilities. Different extraction procedures can be used to determine the composition of different medicinal plants, and the identified molecules must be safe before they can be used for crop protection and preservation of horticultural crops [27]. Based on the results, it was found that the combination of castor oil and Anghuzeh oil has a good potential to prevent the germination of rooster weeds in laboratory conditions. Given the harms and dangers of chemical toxins, there is a need to look for a suitable alternative. Chemical pesticides designed to kill organisms are serious threats to human health. According to the Declaration of

the National Cancer Institute, 30 % of the insecticide, 60 % of the herbicide, and 90 % of the fungicide are carcinogenic. This is one of these substances of the negative side effects of these substances because chemicals can also damage the nervous system and hormonal systems. Among these, children are more vulnerable than adults for agriculture [14]. Therefore, more research is needed to use natural compounds to develop herbicides in this field and other plant compounds and weeds should also be studied.

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Author contributions

F. JS.: Project administration, Data analysis, Writing original draft; Z. T.: Chemical analysis; MR. L.: Editing.

Conflict of interest

The authors declare that there is no conflict of interest.

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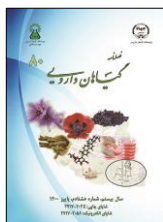
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مقاله تحقیقاتی

بررسی اثر آللوپاتیک فرمولاسیون گیاهی حاوی اسانس آنقوزه و روغن کرچک به عنوان علف‌کش بر جوانه‌زنی بذر گیاه تاج خروس

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اطلاعات مقاله	چکیده
گل‌واژگان:	مقدمه: گیاهان دارویی حاوی ترکیبات فیتوشیمیایی که اثرات مہاری بر عوامل بیماری‌زای گیاهی دارند. علف‌های
علف‌کش	هرز با محصولات اصلی رقابت می‌کنند و رشد، عملکرد و کیفیت محصولات کشاورزی را تا حدی کاهش می‌دهند.
اسانس	یکی از رایج‌ترین روش‌های کاهش آسیب علف‌های هرز استفاده از علف‌کش‌های شیمیایی است. با توجه به عوارض
جوانه‌زنی	جانبی که علف‌کش‌های شیمیایی روی سلامت انسان و محیط زیست دارند، نیاز به جایگزینی علف‌کش‌های زیست
زیست سازگار	سازگار و کم‌خطر وجود دارد. هدف: در این مطالعه، خواص آللوپاتیک اسانس آنقوزه (<i>Ferula assa-foetida</i> L.)
ایمن	و روغن کرچک (<i>Ricinus communis</i> L.) به عنوان علف‌کش گیاهی برای جلوگیری از جوانه‌زنی بذرهای علف
	هرز تاج خروس (<i>Amaranthus retroflexus</i> L.) مورد بررسی قرار گرفته است. روش بررسی: در این راستا، یک
	فرمولاسیون گیاهی بر پایه اسانس آنقوزه و روغن کرچک تهیه شد و تأثیر آن بر جوانه‌زنی بذر علف هرز تاج خروس
	در شرایط آزمایشگاهی مورد بررسی قرار گرفت. ترکیب شیمیایی فرمولاسیون گیاهی توسط GC/MS آنالیز شد.
	نتایج: نتایج نشان داد که این ترکیب گیاهی در غلظت ۰/۷۵ و ۱ درصد از جوانه‌زنی بذر علف‌های هرز حدود ۷۰
	درصد جلوگیری می‌کند. ترکیبات اصلی اسانس آنقوزه و روغن کرچک به ترتیب (E)-۱- پروپنیل سکو بوتیل دی
	سولفید (۴۳/۹ درصد) و ریسینولنیک اسید متیل استر (۵۸/۱ درصد) بودند. نتیجه‌گیری: مشخص شد که فرمولاسیون
	گیاهی مورد مطالعه دارای خاصیت علف‌کشی است. بنابراین، برای دستیابی به نتایج امیدوارکننده به منظور جایگزینی
	علف‌کش‌های شیمیایی با علف‌کش‌های گیاهی، تحقیقات بیشتری مورد نیاز است.

مخفف‌ها: GC/MS، کروماتوگرافی متصل به طیف‌سنج جرمی؛ 2,4-D، ۴،۲- دی کلرو فنوکسی استیک اسید؛ IKS، سیستم دانش بومی

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