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Evaluation of antioxidant properties of phenol, flavonoid and allopathy of Achillea wilhelmsii and Salvia officinalis L. extracts

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Abstract

Allelopathy is one of the methods of interspecific communication in plant communities, the study of which can help to understand the interactions of plants. Therefore, this study was conducted to investigate the effect of allelopathy, antioxidant properties, phenolic and flavonoid compounds of different concentrations of Achillea wilhelmsii and Salvia officinalis L. extract on the germination characteristics of two species of Onobrychis viciifolia and Medicago sativa in a completely randomized design with 9 treatments, and 5 repetitions were performed in 2018. Measured parameters included percentage and germination rate, root length, stem and seedling length, mean germination time, allometric coefficient, seed vigor index and seedling fresh and dry weight. The results of one-way analysis of variance showed that different concentrations of the mentioned extracts had a significant inhibitory effect on germination parameters of O.viciifolia and M.sativa. Also, the results of the mean comparison index showed that increasing the concentration of extracts significantly reduced the studied parameters, so that the highest percentage of decrease was observed in the highest concentration of aqueous extract, which can be the reason is the increase in allelopathic substances and consequently the increase in toxicity on the traits. In addition, the results showed that A.wilhelmsii has higher antioxidant properties than S.officinalis. Also, A.wilhelmsii phenols and flavonoids are less than S.officinalis.

Keywords: A.wilhelmsii, Allelopathy, Antioxidant properties, Germination.

1. Introduction

Plants produce a large and diverse group of organic compounds called secondary metabolites that are consumed by humans as medicinal compounds to cure different ailments. Secondary metabolites are used in biotechnology as drugs, biocides, flavorings, natural dyes, poisons, hallucinogens, and fragrances (Mazid et al., 2011). Specific secondary metabolites are often produced in plants during a specific growth period and play an important ecological role in plants to interact with their surrounded environment (Younessi-Hamzekhanlu et al., 2020). These compounds protect plants against various microorganisms and vegetarians, and regulate plant-plant competition and plant-microbial coexistence (Wink, 2010). Plants have special chemical compounds and have a detrimental effect as a competitor to other plants in natural and cultivated lands. It has been shown that a major part of crop yield reduction is related to weed competition with crops, so weed control is essential to obtain optimum yield. Different methods are used to control weeds including, physical, chemical, mechanical, biological and agricultural strategies. Chemical control is very popular as a very suitable method in weed control (Bond and Grundy, 2001).Today, due to the increased resistance of weeds to herbicides, environmental effects and surface and underground water pollution, the use of chemical

toxins is restricted. Given the consequences of overuse of herbicides, researchers and farmers have always been looking for alternative ways to deal with these problems so that they can control weeds with minimal side effects. One of these biological methods is the use of plant allelopathy. Allelopathy, which is part of the knowledge of chemical ecology, refers to biochemical-stimulatory interactions or inhibitions between various plants and microorganisms (Rashed Mohasel et al., 2006). Alochemicals or ecochemicals are responsible for the phenomenon of allelopathy. Physiological and chemical processes of plants such as germination, growth, photosynthesis, respiration, cell division, and growth are induced by plant growth regulators (e.g., gibberellin or auxin) and special secondary. These substances are stored non-toxic in the producer plant or released before reaching the level of toxicity so as not to harm the producer plant.Medicinal plants have an inhibitory role on germination and growth of other plants due to their diverse and abundant secondary metabolites. For example, in a study the allopathic effect of different concentration of essential oils and extract of the Eucalyptus globules Labill. on the germination and growth of weeds (Cynodon dactylon L.) were studied by spraying and foliar mulch methods. Results showed that E.globules mulch had a significant inhibitory effect on rhizome germination, so that in 50% of E.globules mulch treatment, rhizome germination stopped completely (Daneshmandi, and Azizi, 2009). In another study, the allelopathic effects of crocus sativus extract on growth of Amaranthus retroflexus and Chenopodium album seedlings were evaluated. Findings from the study showed that C.sativus extract caused reduction in leaf, stem, and plant weights of the mentioned weeds. In aloe vera extract, 62 allopathic compounds have been identified, the main constituents of which are sabinil acetate, sabinol, cherry santinyl acetate, linalool and cineole (Niazipoor et al., 2013). Therefore, the study of allelopathy deserves special attention for the following purposes: manipulation plant interaction for crop yield improvement, diversity conservation, weed management, plant protection against pests and diseases (Piraste Anoshe et al., 2010). In this regard, allopathic studies can be a unique opportunity for the emergence of natural herbicides and a new generation of growth inhibitors.The antioxidant activity of plant extracts is mainly due to their redox properties, so that as a reducing agents or hydrogen donors are able to eliminate reactive oxygen species and chelate with metallic elements. Plants are potential sources of natural antioxidants and a variety of antioxidant compounds to neutralize the species. Phenol is one of the major groups of phytochemical compounds found in plants. These compounds are potent antioxidants and free radical scavengers that can be used as hydrogen donors, reducing agents, metal chelators and single oxygen extinguishers. It has been documented that phenolic compounds such as catechins and quercetin play an essential role in the stabilization of bilayer phospholipids against peroxidation induced by reactive oxygen species (Yik et al., 2011). Phenolic compounds are also known as plant toxins that are responsible for the allelopathy effects (Li et al., 2010). The genus A.wilhelmsii is one of the most important genera of the Asteraceae family. A. wilhelmsii and S.officinalis plants are allopathic plants, which can affect the growth of important rangeland species such as O.viciifolia and M.sativa. Dokhani et al., (Dokhani et al., 2005) identified various aromatic substances and phenolic compounds in certain species of A.wilhelms ,may contribute in allopathic which interactions. S.officinalis is also an important and native medicinal plant of Iran, which its origin is the northern regions of Mediterranean. Allelopathic effects of this plant have been reported on some plants. Aerial parts of S.officinalis showed allelopathic effects on Cucumis sativus, Lagenaria siceraria and Solanum lycopersicum seeds and reduced the growth of their aerial parts and roots (Qasem, 2001). Therefore, the aim of this study was to investigate the antioxidant properties of phenolic and flavonoid compounds and also to assess the allelopathic effects of S.officinalis and A.wilhelmsii on the germination parameters of O.viciifolia and M.sativa findings of this study would be helpful in production of herbicides of natural origin.

2. Materials and methods

This study was performed to investigate the antioxidant effects of phenolic and flavonoid compounds as well as the inhibitory effect of different concentrations of S.officinalis and A.wilhelmsii extracts on germination and seedling growth characteristics of O.viciifolia and M.sativa seeds.

2.1 Extraction and preparation of different concentrations of extracts

In order to prepare aqueous extracts, the aerial parts of A.wilhelmsii and S.officinalis were collected from the pastures of Ahar, during the growing season and were thoroughly cleaned by hand. The samples were then washed in distilled

water and dried on a paper towel. The dried samples were divided into small pieces and dried in an electric oven at 60°C for 48 hours. The ground plant sample was mixed with distilled water in a ratio of 1 to 10 (w/v) and stirred for one hour with a shaker (160 rpm) and kept in the refrigerator for 24 hours. In order to remove excess material, first pass through filter paper and then use a centrifuge (2000 rpm) for 5 minutes. The prepared extract was considered as main extract (100%) and by adding distilled water to the solution, other treatments (25, 50 and 75%) were prepared and the treatment solution was kept in the refrigerator until the end of the experiment. Pure distilled water was also used as a control treatment.To prepare the planting medium, the sterile disposable Petri dishes (8 cm) with a layer of filter paper on the bottom were used. The filter paper was placed in an oven at 105°C for 2 hours to be disinfected. Also, 5% sodium hypochlorite was used to disinfect the seeds for 30 seconds and to then they were rinsed and washed five times with distilled water to remove the effect of sodium hypochlorite. Then, 20 healthy seeds were placed in petri dishes. Extracts were added to the dishes daily and as needed, and filter paper was changed every three days to keep constant the concentrations of the extracts in the Petri dishes. Petri dishes were placed in the growth room at a temperature of 24±1°C. The emergence of roots and stems was considered as germinated seeds, and the number of germinated seeds was counted daily to determine the percentage and rate of germination. Counting continued until the number of germinated seeds in each sample was constant for three consecutive days. This process lasted for 9 days for M.sativa and O.viciifolia seeds. After this period, several parameters including, root length, shoot length, seedling fresh weight and seedling dry weight (after placing in oven 75°C for 48 hours) were measured with a sensitive scale with an accuracy of 0.001.

2.2 Measurement of antioxidant capacity

In brief, 10 µl of the different extract concentrations of S.officinalis and A.wilhelmsii were added to 1 ml of 0.004% methanol solution of DPPH. After 30 min the remaining DPPH was determined at 517 nm using a UV–visible single-beam spectrophotometer (UK). DPPH inhibition percentage (I%) was computed using the following formula:

$$1\% = \left(\frac{\text{A Control} - \text{A Sample}}{\text{A Control}}\right) \times 100 \qquad (1)$$

Where control is the absorbance of control reaction and sample is the absorbance of the extracts. Half-maximal inhibitory concentration (IC50) was considered as the concentration of the extracts showing 50% of inhibition in DPPH, as calculated from the graph, plotting inhibition against different concentrations of Eos)Dehghan and Khoshkam, 2012).

2.3 Measurement of total flavonoids

Huang et al., (2004) method was used to measure total flavonoids based on the formation of flavonoid-aluminum complex at an absorption wavelength of 415 nm. First, one gram of aluminum chloride was weighed to 50 ml with methanol. Concentrations of 10, 20, 40, 80 and 100 µg/ml quercetin were used to draw the standard curve. The reaction mixture consisted of 1.5 ml of methanol, 100 µl of aluminum chloride, 100 µl of sodium acetate, 500 µl of extract and 2.8 ml of distilled water. The control solution contained all of the above compounds except the extract. The samples were then kept at room temperature for 30 minutes and their absorbance was measured at 415 nm. The content of flavonoids was expressed in micrograms of quercetin per gram of extract.

2.4 Phenol measurement

The amount of phenol was determined using folin reagent. To prepare this reagent, the normal solution of Folin Siocalto 2 was diluted with an equal volume of distilled water. 20 g of sodium carbonate was weighed and diluted in 100 ml distilled water. Then 1.5 ml methanol, 2 ml of folate (diluted), 500 µl of extract were mixed and after 5 minutes 3.75 ml of the prepared sodium carbonate solution was added. The resulting reaction mixture was kept at room temperature for 90 minutes and their absorbance was measured at 725 nm. The control solution contained all of the above ingredients except the extract. The total phenol content of each sample was expressed in terms of gallic acid equivalent (GAE) according to the standard sample (Duet ,.al2009).

2.5 Calculation of different germination indices

In this experiment, germination percentage, germination rate, mean germination time, seed vigor index and allometric coefficient were calculated using following formula (Ranal and Santana, 2006). Germination percentage was obtained from Equation (2) where GP is germination percentage, N is the total number of seeds and n is the number of germinated seeds)Ranaland and Santana, 2006).

$$\text{GP} = \frac{\text{n}}{\text{N}} \times 100 \qquad (2)$$

Germination rate is calculated by Equation (3) that GR germination rate, ni number of seeds

germinated up to day i and di time after planting associated with ni It is by day (Ranal and Santana, 2006)

$$GR = \sum_{i=1}^{n} \frac{ni}{di} \quad (3)$$

The average germination time is obtained from Equation (3) where Fi is the day of counting, Xi is the number of germinated seeds per day, F is the total number of germinated seeds and AGT is the average germination time (Matthews and Khajeh Hosseini, 2007).

$$AGT = \frac{\sum_{i=1}^{n} FiXi}{N} \quad (4)$$

Equation (5) was used to calculate the seed vigor index. SVI is the seed vigor index, ASL is the average seedling length in cm and GS is the germination rate (Agrawal, 2003).

$$SVI = \frac{GS}{ASL} \quad (5)$$

The allometric coefficient (Equation 6) is obtained by dividing the dry weight of the stem in grams by the dry weight of the root in grams.

$$AC = \frac{\text{Dry weight of the stem}}{\text{Dry weight of the root}} \quad (6)$$

2.6 Statistical calculations

Data obtained from different measurements were statistically analyzed using SAS software version 9. The means were compared using Duncan's multiple range test at a 5% significantly level. EXCEL software version 2013 was also used to draw the graphs. Experiments related to the allelopathic effect of A.wilhelmsii and S.officinalis plant extracts on O.viciifolia and M.sativa were performed separately in a completely randomized design (CRD) with 9 treatments and 5 replications. Treatments included control, S.officinalis extract containing concentrations of 25% (S1), 50% (S2), 75% (S3), 100% (S4) and extract A. wilhelmsii containing concentrations of 25% (A1), 50% (A2), 75% (A3) and 100% (A4).

3. Results

The results of analysis of variance of the effect different concentrations of S.officinalis and A.wilhelmsii extracts on germination traits of O.viciifolia (Table 1) showed that the use of these treatments had a significant effect on all the studied traits.

Table 1. Analysis of variance of allelopathic effect of S.officinalis and A.wilhelmsii extracts on germination characteristics of O.viciifolia

		Mean of squares							
Source of changes	Degrees of freedom	Germination percentage	Germination rate	Average germination time	Root length	Stem length	Seedling length	Seed vigor index	Allometric coefficient
Treatment	8	182.5**	0.6**	8.63*	2.95**	2.83**	10.65**	1.19**	0.23**
Error	36	16.38	0.08	3.03	0.48	0.29	1.32	0.08	0.05
Coefficient of variation		34.69	53.22	32.35	29.93	24.67	25.35	49.38	24.47

**** and * are significant at 1% and 5% probability levels, respectively**

Mean comparison results (Figure 1) showed that with increasing the concentration of S.officinalis and A.wilhelmsii extract, value of the majority of traits were decreased significantly. For example, in case of germination percentage of O.viciifolia, its highest amount was obtained in control and S1 treatments (S.officinalis extract with a concentration of 25%) with 21% and its lowest amount was observed in A4 treatment (A.wilhelmsii extract with 100% concentration) with 5% (Figure 1A). Also, the highest (1.13 and 3.67, respectively) and lowest (0.116 and 1, respectively) germination rate coefficient (Figure 1B) and root length (Figure 1D) were obtained in S1 and A4 (maximum concentration) treatments, respectively. As shown in the figure 1 the same results can be concluded from the data for other traits including, stem and seedling length and seed vigor index (Figure 1E, Figure 1F. Figure 1G). However, as expected the maximum value for the average germination time was achieved in the A4 treatment (100% A.wilhelmsii concentration) with 8.6% (Figure 1C). Moreover the highest (1.3%) and lowest (0.65%) allometric coefficient obtained in treatments S2 (S.officinalis extract with a concentration of 50%) and A2 (A.wilhelmsii extract with a concentration of 50%), respectively (Figure 1H).

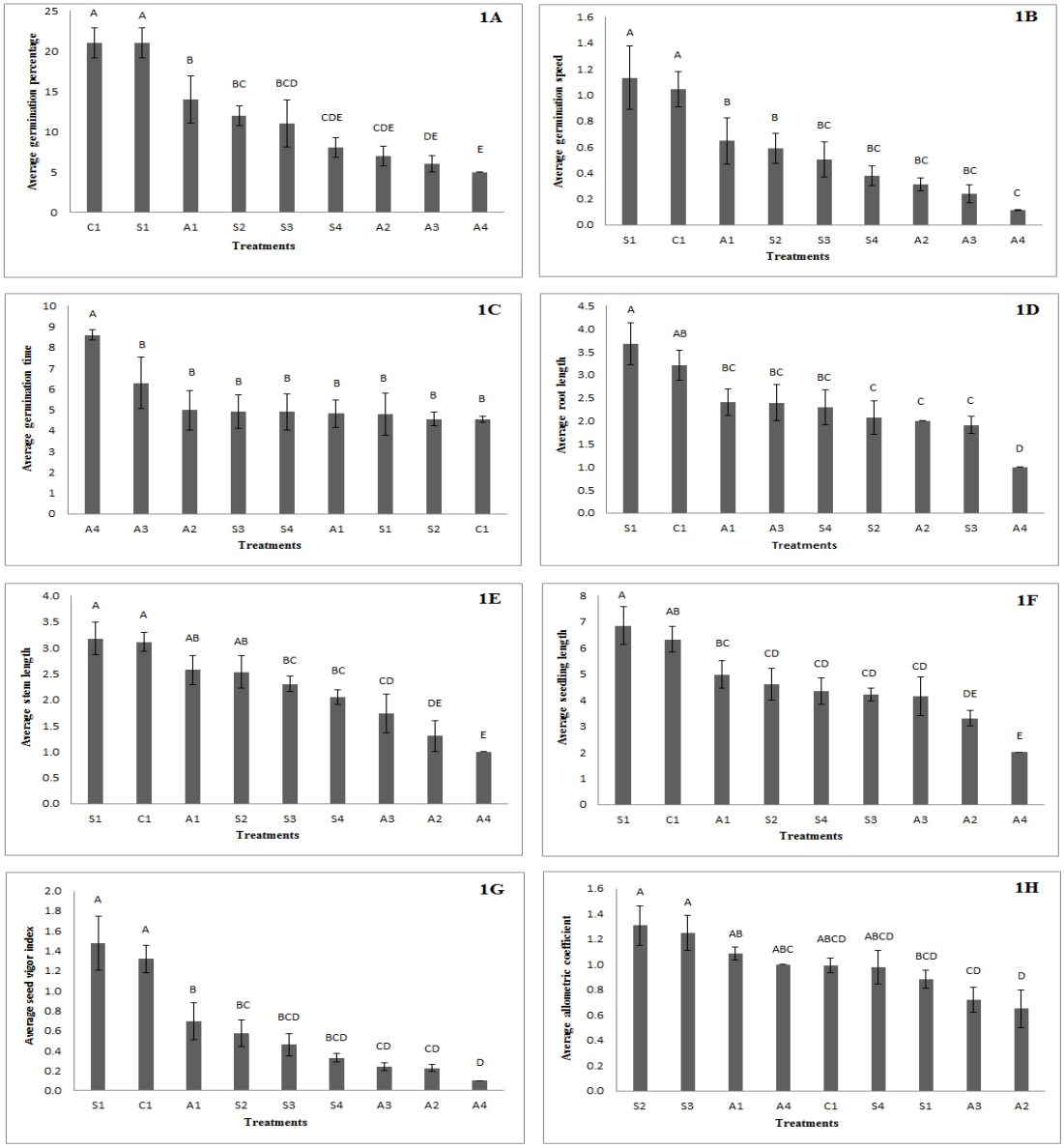

Figure 1: Comparison of the mean of different treatments of S.officinalis and A.wilhelmsii extracts on germination characteristics of O.viciifolia. Treatments with at least one common letter do not differ significantly from each other at the 5% significantly level.

The results of analysis of variance of the effect of different concentrations of S.officinalis and A.wilhelmsii extracts on the germination characteristics of M.sativa (Table 2) also showed that the use of these treatments had a significant effect on all measurement parameters.

Table 2. Analysis of variance of allelopathic effect of S.officinalis and A.wilhelmsii extracts on germination characteristics of M.sativa

		Mean of squares							
Source of changes	**Degrees of freedom**	**Germination percentage**	**Germination rate**	**Average germination time**	**Root length**	**Stem length**	**Seedling length**	**Seed vigor index**	**Allometric coefficient**
Treatment	8	3092.5**	11.1**	15..72**	29.17**	10.54**	69.94**	51.54**	1.75**
Error	36	224.44	1.31	2.66	0.42	0.5	1.21	1.88	0.31
Coefficient of variation		52.26	76.65	28.58	22.18	22.75	18.20	54.44	40.79

**** and * are significant at 1% and 5% probability levels, respectively**

In accordance to effect of the extract effect on germination parameters of S.officinalis, similar results with a slight difference were obtained in the M.sativa crop germination. Maximum (68%) and minimum (5%) germination percentage was related to control treatment and A4 treatments, respectively (Figure 2A). In the case of other traits, the higher the concentration of extracts, the more severely the traits decreased. The opposite association was true for the average germination time trait, so that the highest and lowest average germination time was observed in A4 and C1 treatments, respectively (Figure 2B, Figure 2C). Concentrations of 100% (A4) and 75% (A3) yarrow and 100% (S4) sage extracts were not significantly different from each other in terms of their effect on majority of the traits.The results of mean comparisons indicated that the lowest values of the root (Figure 2D), stem (Figure 2E) and seedling length (Figure 2F) traits are related to A4 treatment. Conversely, their highest amount was observed in the C1 treatment. However, in the case of stem length there was no significant difference between C1, S1, and S2 treatments. In addition, A4, A3, A2, and S3 treatments caused significant decrease in the seed vigor index in compare with other treatments (Figure 2G). In terms of allometric coefficient of S3 and A2 treatments, showed significantly higher amounts than other treatments (Figure 2H).

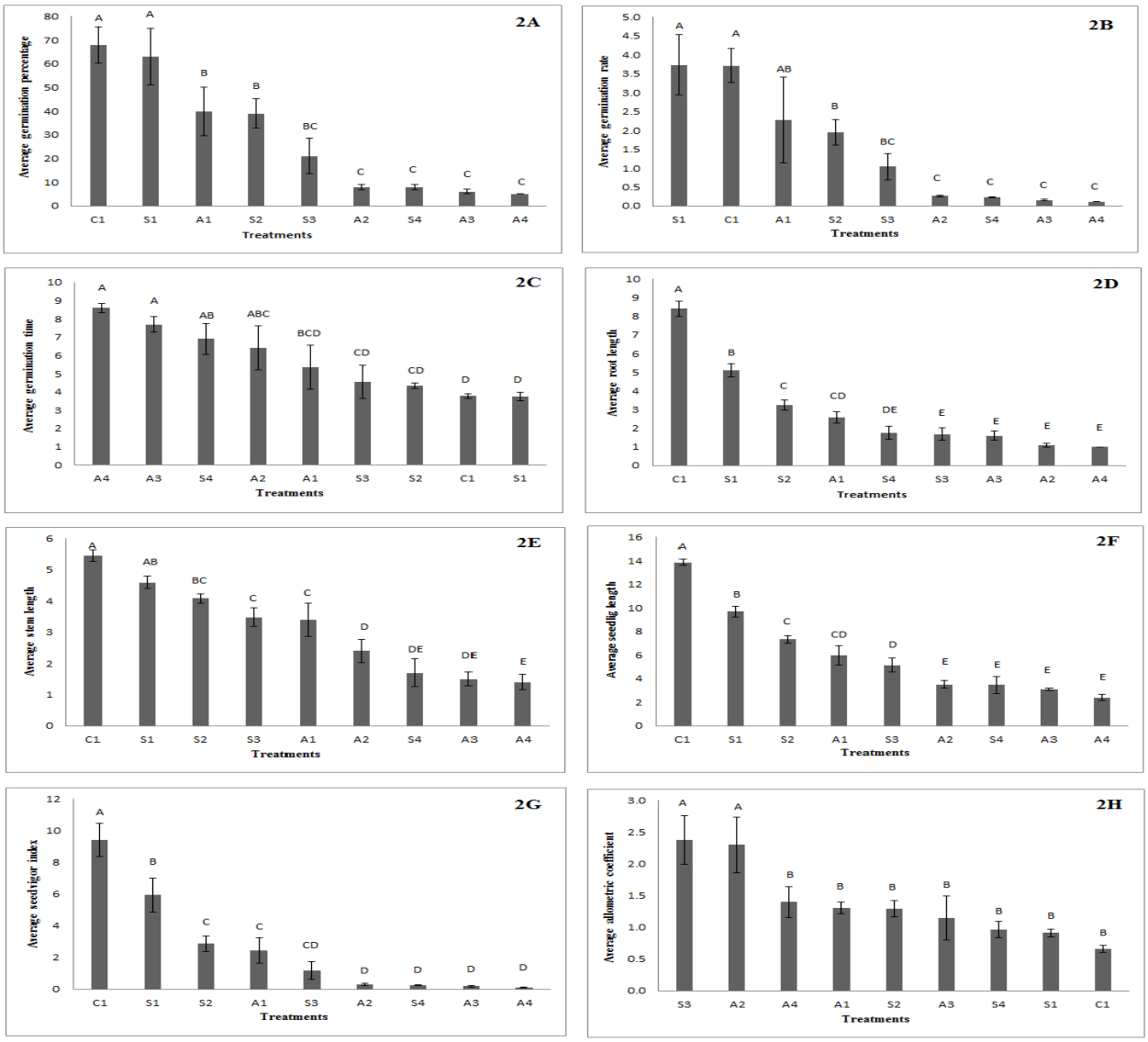

Figure 2: Comparison of the mean of different treatments of S.officinalis and A.wilhelmsii extracts on germination characteristics of M.sativa.Treatments with at least one letter in common do not differ significantly from each other at the 5% probability level

Also in this study, the antioxidant, phenolic and flavonoid activity of S.officinalis and A. wilhelmsii extracts were investigated and their association with germination characteristics evaluated. The results showed that S.officinalis extract has a higher antioxidant capacity than A. wilhelmsii extract. In the other hand, the amount of phenols and flavonoids in S.officinalis extract was significantly higher than A.wilhelmsii extract (Figure 3).

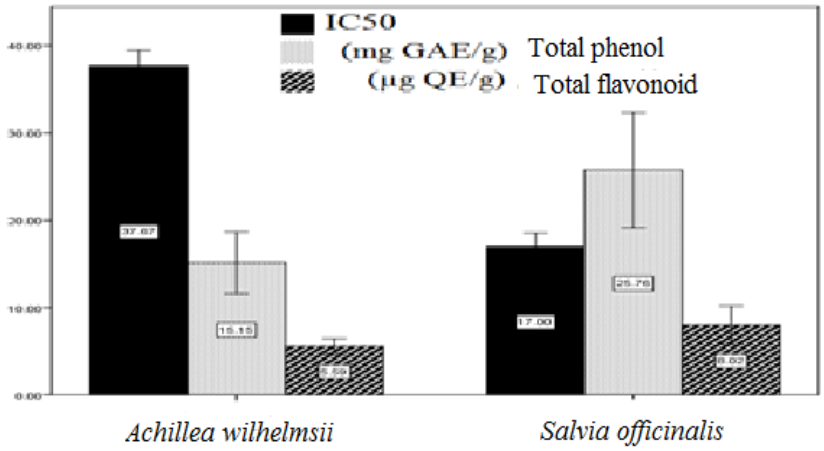

Figure 3. Comparison of antioxidant power, phenolic and flavonoid compounds of S.officinalis and A.wilhelmsii. ** and * are significant at 1% and 5% probability levels, respectively

Also, the correlation coefficients between phenolic and flavonoid compounds with germination indices for *O.viciifolia* (Figure 4) and *M.sativa* (Figure 5) are given separately in the form of a heat map. As shown in the Figure 4 Phenolics and flavonoids showed significantly negative correlation with germination percentage and rate, and stem length of *O.viciifolia* crop. In the other side, these two phytochemicals exhibited significantly opposite correlation with all the germination related parameters excepted to root length and allometric index.

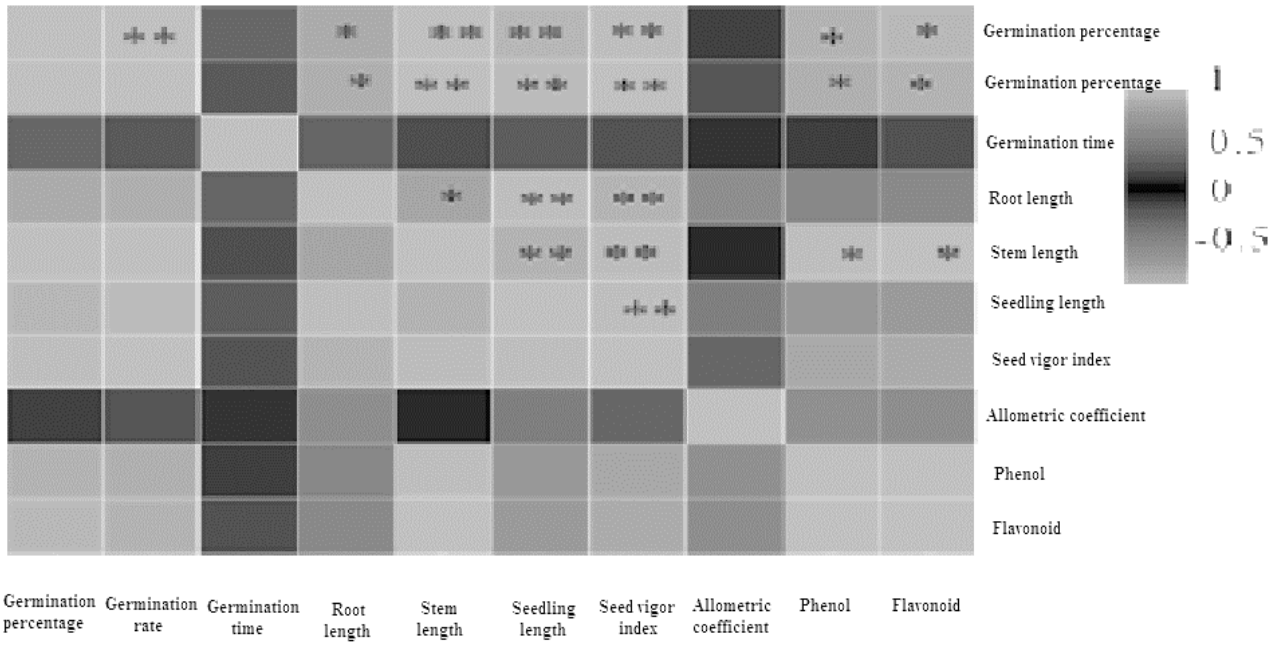

Figure 4. Correlation coefficients between phenolic and flavonoid compounds with germination indices for *O. viciifolia*. ** and * are significant at 1% and 5% probability levels, respectively

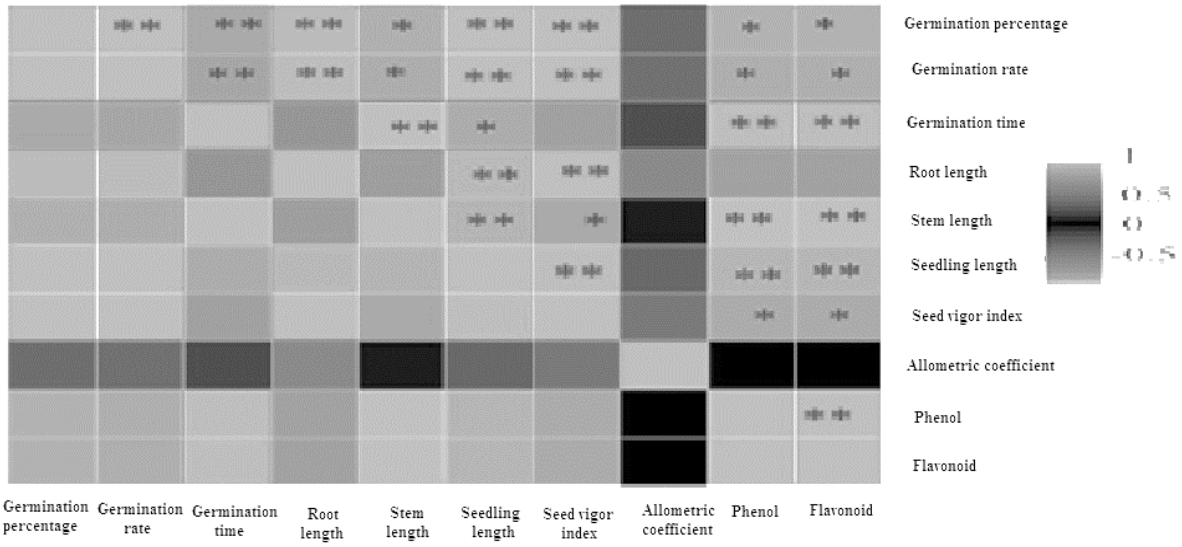

Figure 5. Correlation coefficients between phenolic and flavonoid compounds with germination indices for *M.sativa*. ** and * are significant at 1% and 5% probability levels, respectively

4. Discussion

The use of plant allelopathy, especially medicinal plants, plays an important role in the management and control of weeds and can be a good opportunity for the emergence of natural herbicides and a new generation of growth inhibitors. For this purpose, in this study, the effect of allelopathy of A.wilhelmsii and S.officinalis extracts on germination characteristics of M.sativa and O.viciifolia forage seeds was investigated. The results showed that these treatments have significant effects on germination and growth traits of O.viciifolia and M.sativa seeds that can be due to phytochemical compounds with inhibitory properties of germination and growth in these extracts.The results our study showed that the tested weed extract effects on germination percentage and rate of O.viciifolia and M.sativa seeds has significant inhibitory effects. These inhibitory effects were observed in all concentrations used in the experiment. However, the mean comparison showed that with increasing the concentration of the extract, the percentage and germination rate had a significant decrease so that the highest and lowest percentages and germination rates were observed in control and A.wilhelmsii treatments with 100% concentration, respectively. Cessation of germination may be attributed to changes in the activity of enzymes that affect the transfer of stored compounds during germination, which can lead to a shortage of respiratory products and ultimately to a persistent ATP deficiency in seeds that have been exposed to alchemicals. The effects of allelopathy not only reduce germination, but also delay germination, which can have a profound effect on the competitive outcome of plants and seedlings. Larger sizes may compete better with their neighbors under adverse conditions such as low soil moisture or nutritional elements. Slowing down the vital processes of plants due to the reduction in respiration in the seeds due to the presence of alchemicals also reduces the germination rate (Rezaie et al., 2008). Similar to previous studies, increasing the concentration of S.officinalis and A.wilhelmsii extracts reduced the root and stem length of the studied species. This phenomena, may be due to decreased mitotic divisions and reduced respiration. In the other hand, Allelopathic compounds reduce water uptake in plants and thus reduce seedling length by affecting root growth (Chon et al., 2005).Another germination characteristic is the seed vigor index, which decreased with increasing the concentration of S.officinalis and A.wilhelmsii extracts. Among the germination treatments, the highest seed vigor index belonged to the control and S.officinalis treatments. This index is a function of germination percentage and seedling length. The mechanism that reduced seed germination and vigor may be related to a decrease in the activity of enzymes such as alpha-amylase, which are involved in seed germination (Rezaie et al., 2008).Plants are potential sources

of natural antioxidants and a variety of antioxidant compounds to neutralize species. They produce reactive oxygen, so there are several ways to measure antioxidant activity, in addition to examining the properties of allelopathy. The level of antioxidant activity was measured by DPPH free radical scavenging capacity. Plant-derived secondary metabolites such as phenols and flavonoids have strong potential for scavenging free radicals. (Santana et al., 2009). It has been shown that with increasing phenolic compounds, the antioxidant properties increase. Phenolic compounds with a high molecular weight, have great ability to scavenge free radicals and antioxidant activity. Jamshidi et al., (2010) have studied the methanolic extract of some native plants of Mazandaran in terms of flavonoid and phenolic content. Their results showed that there is a good relationship between antioxidant activity and polyphenolic compounds of the plant.In the current study, it was shown that aqueous extracts of .wilhelmsii and S.officinalis have high phenolic and flavonoid content. Therefore, for the practical use of these compounds in industry, it is recommended to do more research to identify the compounds (components) and precisely evaluate the antioxidant power of the plant extract. As shown in the result section, strong negative correlation was observed between phenolics/flavonoids content with germination characteristics. Therefore, considering the allopathic inhibitory effect of aqueous extract of medicinal plants, these plants can be considered as an important competitor for germination of the plants. Also A.wilhelmsii and S.officinalis can be used as a useful and beneficial allelopathy plant in weed management and control. With further research in this field, we can understand the mechanism of inhibitory action of compounds in these plants.

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