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Allelopathic effect of *Rapistrum rugosum* L. weed on growth, physiological and biochemical parameters of *Hibiscus sabdariffa* L.

Original paper

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Abstract: To study the allelopathic effect of decomposed fresh aerial parts of *Rapistrum rugosum* on growth, physiological and biochemical traits of *Hibiscus sabdariffa*, a pot experiment was conducted as a completely randomized design with three replications under greenhouse condition at the Gonbad Kavous University. Treatments were different amounts of *R. rugosum* residues (0, 10, 20, 40, 80, and 160 g kg^{-1} soil). The results showed that increasing the amount of *R. rugosum* residues significantly reduced the growth traits of *H. sabdariffa*. The lowest amount of stem and root length, fresh and dry weight of the plant was observed in 160 g kg^{-1} of *R. rugosum* residues. The content of chlorophyll *b* and carotenoids, as well as malondialdehyde, decreased with increasing the amount of *R. rugosum*. However, the trend of changes in the content of catalase enzyme, soluble sugars content, proline, total phenol, and flavonoids increased with increasing the amount of *R. rugosum* residues. Consequently, our findings showed that with the increasing in the amount of *R. rugosum* residues, all the studied traits of *H. sabdariffa* decreased significantly. Therefore, due to the negative allelopathic effect of *R. rugosum* on growth and physiological parameters of *H. sabdariffa*, *R. rugosum* density management should be considered during the cultivation of this plant.

Keywords: allelopathic, physiological traits, *Rapistrum rugosum*, total phenol.

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Introduction

Allelopathy is defined as any direct or indirect stimulant and inhibitory effect of a plant on other plants and living organisms by releasing chemical compounds (allelochemicals) into the environment (Al-Watban and Salama, 2012; Rahimzadeh et al., 2012). However, most researches has focused on the adverse effects of allelochemicals. Generally, these compounds released by allelopathic plants affect the germination, growth, development, and establishment of receptor plants and play an essential role in the pattern of vegetation and crop production (Gniazdowska and Bogatek, 2005; Elisante et al., 2013). This harmful effect of allelochemicals occurs by altering various metabolic processes in donor plants. Allelochemicals are usually produced as secondary products or in the main pathways of metabolism in plants. These substances are released in the form of solution due to leaching, decomposition, volatilization and root exudation on the surface of the plant parts (Tigre et al., 2012). Allelochemical compounds of plants, especially weeds, can inhibit physiological and biochemical processes such as germination, cell division, reparation, photosynthesis, changes in cell membrane stability, enzyme activity, *etc* (Cruz - Ortega et al., 2002; Al-Watban and Salama, 2012). Cruz - Ortega et al. (2002) reported that allelochemicals released from allelopathic plants increased reactive oxygen species (ROS) in receptor plants and ultimately stimulate the activity of antioxidant enzymes.

One of the proposed methods to reduce the use of synthetic herbicides and decrease weed population in the field is due to exploitation of the toxic plants as herbicides of natural origin (Bhadoria, 2010; Farooq et al., 2011). Chang et al. (2002) found that allelopathic compounds at low concentrations could negative or positive effects on receptor plants, but the inhibition at higher concentrations. Han et al. (2008) reported that inhibition percentage of allelochemicals is directly dependent on the concentration of the extract.

The presence of allelochemicals in the field is a kind of stress, in which plants grow adjacent to allelopathic plants, and might be exposed to some kind of biological stress (Liu et al., 2003). Asgharipour and Armin (2010) reported that both the root and leaf extracts of *Sorghum halepens* in most cases inhibited the seed germination and early seedling growth of *Plantago ovata*, *Plantago psyllium*, *Foeniculum vulgare* and *Ocimum basilicum*, while the seed germination and the early seedling growth of *Nigella sativa* and *Cuminum cyminum* were simulated by both extracts at lower concentrations. Otusanya et al. (2015) showed that the application of the aqueous extract of *Tithonia diversifolia* and *Chromolaena odorata* at a lower and moderate concentration significantly reduced the seed germination and juvenile seedling growth of *Hibiscus sabdariffa*.

Rapistrum rugosum, belongs to the *Brassicaceae* family, an annual, deciduous, and winter plant that is considered the most important weed of rapeseed fields in the world and included Iran (Baghestani et al., 2005). *Brassicaceae* is one of the important plant families due to its allelopathic activity. Glucosinolates and phenolic compounds are the most important allelochemicals synthesized by the plants in the *Brassicaceae* family. The glucosinolates are converted into several isothiocyanates through enzymatic (myrosinase) activity and express an allelopathic activity (Jabran, 2017). Scientific literature reports allelopathic activities of many *Brassicaceae* plants (Jabran et al., 2015; Cipollini, 2016). The study of the allelopathic effects of *R. rugosum* can be critical in the nature because of containing allelopathic compounds like other *Brassicaceae* plants.

H. sabdariffa L. also known as rosella, belongs to the *Malvaceae* family. It is an ideal crop for developing countries as it is relatively easy to grow, can be grown as part of multi-cropping systems and can be used as food and fiber. In many countries, *H. sabdariffa* is used for its medicinal properties (Da-Costa-Rocha et al., 2014). Therefore, studying the growth barriers of *H. sabdariffa*, including the effect of weed residues left from the previous crop on germination, vegetative growth and nutrient uptake of this plant, can be a practical step in improving and developing the cultivation of this plant. Also, there is no sufficient information about the response of *H. sabdariffa* to the residue of *R. rugosum*, therefore, the present study aimed to evaluate the effect of this weed residue on the growth, physiological and biochemical characteristic of *H. sabdariffa*.

Materials and Methods

Plant material

In the present study, *R. rugosum* aerial part was collected at full flowering stage from Azadshar, which is located East of Golestan province (Iran), with a latitude of 37º 14' North and longitude of 55º 16' East, and 89 m above sea level in 2018. The average annual rainfall of the region was 561 mm and the annual average temperature was 15°C.

Identification and preparation of plant samples of R. rugosum

In the beginning, *R. rugosum* weed specimens were accurately identified with the help of Iranian cormophytes (Ghahreman, 1993). Then, to remove the dust, they were washed with distilled water for one min. Subsequently, the samples of weeds were dried in an oven at 60 °C until reaching a constant weight. The samples after 40 days were first ground into small pieces by a mill and then passed through sieve a 8 mesh.

Preparation of R. rugosum residues and planting seeds of H. sabdariffa.

In order to prepare the soil mixture for 5 kg pots, soil from 0-30 cm depth was prepared and sieved. After measuring physico-chemical soil properties (Table 1), different amounts of *R. rugosum* residues such as 0 (control), 10, 20, 40, 80, and 160 g per kg soil were added in each pot. In order to decompose the decayed plant parts by microorganisms, each pot moistened daily with 300 cc of distilled water. At the end of the 40th day, the pots' soil was exposed to the open air for one week to volatile allelochemicals. 20 disinfected seeds (0.1% sodium hypochlorite) of *H. sabdariffa* were cultivated in each pot.

Table 1. Physico-chemical properties of the soil used in this study.

Sand (%)	Silt (%)	Clay (%)	Soil texture	Potassium (ppm)	Phosphorus (ppm)	Total nitrogen (%)	Organic carbon (%)	TNV (%)	pH	EC (dS /m)
13	56	31	Silty clay loam	340	13	0.08	0.78	10.8	7.6	0.96

This experiment was performed under greenhouse condition at the Gonbad Kavous University, with a relative humidity of 65%, light duration of 16 h light and 8 h dark and temperature between 15-25 ºC. After the emergence of seedling, six uniform seedlings were kept in each pot. The study was conducted as a completely randomized design with three replications.

Measurement of morphological traits

All *H. sabdariffa* seedlings were harvested from each pot after 40 days. Then, some morphological traits such as shoot and root length, fresh and dry weight of seedlings, root volume, number of roots, number of leaves and leaf area (using leaf area meter; Delta-t model) were measured.

Measurement of biochemical traits

At the end of the growing stage, leaf samples of *H. sabdariffa* were collected and then stored at -80º C.

Measurement of chlorophyll and carotenoid content

To measure the chlorophyll and carotenoid content, 0.1 g of fresh leaf tissue of *H. sabdariffa* was ground with 10 ml of 80% acetone. The mix was centrifuged at 1000 rpm for 10 minutes. Then the supernatant solution was transferred into a new tube and the volume was adjusted to 25 ml with 80% acetone. Finally, the absorbance was read at 663, 645 and 470 nm for chlorophyll *a*, *b* and carotenoids, respectively using a Biochrom Libera-S22 spectrophotometer. The values were calculated as mg.g^{-1} of fresh weight using the following formula (Arnon, 1949).

1) Chlorophyll *a*= [(19.3×A663- 0.86× A645) V/100W]
2) Chlorophyll *b*= [(19.3× A645- 3.6× A663) V/100W]
3) Carotenoids= [100(A470)- 3.27 (mg chl *a*) - 104 (mg chl *b*)/ 227]

where V, volume of the filtered solution (centrifuge solution); W, wet weight of the sample in grams; A, light absorption at 663, 645 and 470 nm

Measurement of proline content

Proline was extracted from 500 mg of fresh leaf tissue which was grounded in 10 ml of 3% sulfosalicylic acid. The solution was passed through filter paper, then 2 ml of this solution was transferred into the test tube, followed by the immediate addition of 2 ml of ninhydrin acid, and 4 ml of toluene to each tube and shaken for 20 to 30 seconds until the yellow top layer of toluene appeared. This layer was transferred in a new test tube and the proline content was measured at 520 nm using a spectrophotometer (Biochrom Libera-S22). Proline content was determined using the standard proline curve in µmol/g of fresh weight (Bates et al., 1973).

Measurement of soluble sugars content

Soluble sugars content was measured based on (Kochert, 1978) method, 0.1 g of plant dry matter was mixed in 10 ml of 70% ethanol and kept in the refrigerator for one week. Then 1 ml of the upper solution was separated and their volume was set to 2 ml using distilled water. Next, 1 ml of 5% phenol and 5 ml of concentrated sulfuric acid were added. A yellow solution was produced which changed color. The absorbance of the color at 485 nm which was related to soluble carbohydrate concentrations (mg g^{-1} of dry weight) was read by the spectrophotometer (Biochrom Libera-S22).

Measurement of phenolic content

100 mg of dried leaf tissue was dissolved with 10 ml of 80% ethanol and centrifuged at 1000 rpm for 10 min. The supernatant was kept in a boiling water bath for 5 min. Then volume of the concentrated solution was adjusted to 3 ml with distilled water. 0.5 ml of folic acid Siocalto reagent and 2 ml of sodium carbonate were added to this solution. Again, the reaction mixture was kept in a boiling water bath for 10 min. Finally, absorbance was measured at 650 nm using a spectrophotometer (Biochrom Libera-S22). The standard total phenolic content was expressed as mg gallic acid equivalents (GAE) per gram of dry samples (Malik and Singh, 1980).

Measurement of flavonoid content

10 mg of dry leaf tissue was dissolved in 10 ml of acetone and shaken for 24 hours. The solution was passed through a filter paper. For this purpose, 0.5 ml of each plant extract was taken in test tube, and then 1.5 ml of 95% ethanol, 0.1 ml of 10% aluminium chloride ($AlCl_3.6H_2O$), 0.1 ml of potassium acetate, and 2.8 ml of distilled water were added and mixed well. The solution was kept at room temperature for 3 min, after that the absorbance was read at 415 nm in a spectrophotometer (Chang et al., 2002).

Preparation of enzyme extract

At the beginning, 1 g of fine powder sample was ground in 2.5 ml of potassium phosphate buffer (containing 0.1 ml of ethylene dihydramine, amine, tetra acetic acid (EDTA), 1% (W/V) polyvinyl pyrrolidone (PVP), 0.5% triton X-100 and 20 the percentage of glycerol) by pestle and mortar. Then, the obtained solution was centrifuged at 10,000 rpm for 15 min at 4°C. After that, the upper layer was separated and used as enzyme extract (Kala, 2015).

Estimation of catalase enzyme activity

Catalase enzyme activity measurement was performed by Aebi (1984) method. The kinetic activity of the catalase enzyme was determined using 2.5 ml of 0.1 M phosphate buffer (pH = 7), 100 µl of 10 mM H_2O_2 (V/V) as indicator. In the ice bath, the indicator was mixed with 50 µl of the enzyme extract mixture. Absorbance changes at 240 nm were read by spectrophotometer for 2 minutes. Enzyme activity was calculated according to µmol per g of fresh weight.

Estimation of guaiacol peroxidase enzyme activity

To measure guaiacol peroxidase enzyme activity, 780 µl of 50 mM potassium phosphate buffer (pH = 6.6), 90 µl of 1% guaiacol and 90 µl of 0.3% H_2O_2 were placed in a test tube in the ice bath, followed by immediately addition 20 µl of enzyme extract mixture (Hemeda and Klein, 1990). Absorption changes at 470 nm per min were measured by spectrophotometer (Biochrom Libera-S22).

Estimation of Malondialdehyde (MDA) enzyme activity

Heath and Packer (1968) method was used to measure the activity of the malondialdehyde enzyme. 0.2 g of fresh leaf tissue was homogenized in 5 ml of 0.1% trichloroacetic acid (TCA) with

mortar and pestle, and centrifuged at 10,000 rpm for 5 min. Then 1 ml of the upper layer was suspended in 4 ml of 40% TCA solution along with 0.5% thiobarbituric acid (TBA) on ice. The mixture was evaporated at 95°C for 30 min, and immediately centrifuged for the second time at 10,000 rpm for 10 min at 4°C. The absorption of MDA was read at 532 nm by spectrophotometer (Biochrom Libera-S22).

Statistical analyses

The analysis of variance (ANOVA) was performed using the SAS software, version 9.3, and means were compared with the least significant difference test (LSD) at 0.05 probability level.

Results

The effect of R. rugosum residues on the morphological components of H. sabdariffa

The results showed a significant difference in the allelopathic effect of different amounts of *R. rugosum* residues on stem length, root volume, number of roots, number of leaves, fresh and dry weight of plant and leaf area of *H. sabdariffa* (*Pr* <0.01) (Table 2). The mean comparison showed the stem length of *H. sabdariffa* decreased with increasing the amounts of *R. rugosum* residues, so the lowest stem length (7.16 cm) was recorded at 160 g of *R. rugosum* which was 49.5% lower than the control plants (Table 2). The residuals of *R. rugosum* significantly inhibited the number and volume root of *H. sabdariffa* compared to the control. As, seedling of *H. sabdariffa* grown in control and 160 g of residues had the highest and lowest number and volume of roots, respectively. Both 80 and 160 g kg^{-1} of *R. rugosum* residuals showed the greatest inhibitory effects on the number of leaves (36.6 and 39.7%) and leaf area (63.8 and 69.3%) of *H. sabdariffa*, respectively. In contrast, 10 g of *R. rugosum* residues showed the least reduction effect on the above two parameters (Tablel 2).

The effect of different amount of R. rugosum residues on the pigments content of H. sabdariffa

The chlorophyll b and total chlorophyll content of *H. sabdariffa* seedling unlike chlorophyll a, showed a sharp decline with increasing of residues (Table 2). The maximum decreasing level of these two parameters was observed in 160 g of residues, which did not show a significant difference with 80 g. The 10 g kg^{-1} of *R. rugosum* residues has no significant effect on chlorophyll b and total chlorophyll.

Table 2: Analysis of variance (mean square) effect of different amounts of *R. rugosum* residues on morphological and physiological traits and pigments content of *H. sabdariffa.*

S.O.V	df	Stem length	Root volume	Root No.	Leaves No.	Fresh weight of plant	Dry weight of plant	Leaf erea	Cholorophyll *a*	Cholorophyll *b*	Total cholorophyll
Treat	5	18.8 **	0.35 **	4.88 **	4.14 **	2.45 **	0.052 **	62.9 **	0.0002 **	0.012 **	0.017 **
Error	12	0.38	0.014	0.19	0.087	0.057	0.002	0.6	0.0001	0.0004	0.0002
C.V	-	5.8	12.2	6.9	5.17	8.27	14.2	7.5	13.5	4.43	2.8

Continue of Table 2:

S.O.V	df	Carotenoids	Soluble sugars content	Proline activity	Catalase activity	Peroxidase activity	Malondialdehyde activity	Phenol content	Flavonoids content
Treat	5	0.007 **	1705.64 **	673.06 **	75.43 **	2.81 **	0.07 **	47.95 **	0.0005 **
Error	12	0.0002	0.38	0.65	0.15	0.16	0.0007	0.158	0.00001
C.V	-	7.19	0.64	1.1	1.58	4.79	7.81	5.6	5.56

* and **: significant at 5% and 1% levels of probability, respectively.

Table 3: Means comparison of the different amounts of *R. rugosum* residues on morphological and physiological traits and pigments content of *H. sabdariffa*.

Different amounts of *R. rugosum* residues	Stem length (cm)	Root volume (mm^2)	Root No.	Leaves No.	Fresh weight of plant (gr)	Dry weight of plant (gr)	Leaf erea (cm^2)	Cholorophyll a (mg/g fresh weight)	Cholorophyll b (mg/g fresh weight)	Total cholorophyll (mg/g fresh weight)
0	14.16 ^{a}	1.54 ^{a}	8.44 ^{a}	7.55 ^{a}	4.11^{a}	0.57 ^{a}	16.88 ^{a}	0.09 ^{a}	0.55 ^{a}	0.65 ^{a}
10	12.42 ^{b}	1.23 ^{b}	7.11 ^{b}	6.66 ^{b}	3.59 ^{b}	0.45 ^{b}	14.01 ^{b}	0.08 ^{ab}	0.55 ^{a}	0.63 ^{a}
20	11.05 ^{c}	1.05 ^{bc}	6.55 ^{bc}	5.55 ^{c}	3.01 ^{c}	0.32 ^{c}	11.16 ^{c}	0.073 ^{b}	0.51 ^{b}	0.58 ^{b}
40	10.05 ^{c}	0.89 ^{cd}	5.88 ^{cd}	5.11 ^{cd}	2.82 ^{c}	0.29 ^{c}	8.52 ^{c}	0.066 ^{b}	0.45 ^{c}	0.52 ^{c}
80	8.83 ^{d}	0.75 ^{de}	5.66 ^{d}	4.78 ^{de}	2.12 ^{d}	0.26 ^{c}	6.11 ^{e}	0.073 ^{b}	0.42 ^{cd}	0.47 ^{d}
160	7.16 ^{e}	0.57 ^{e}	4.78 ^{e}	4.55 ^{e}	1.67 ^{e}	0.22 ^{c}	5.17 ^{e}	0.07 ^{b}	0.41 ^{d}	0.47 ^{d}
LSD 5%	1.11	0.22	0.8	0.53	0.43	0.09	1.38	0.02	0.04	0.03

Continue of Table 3:

Different amounts of *R. rugosum* residues	Carotenoids (mg/g fresh weight)	Soluble sugars content (mg/g dry weight)	Proline activity (µmol/g fresh weight)	Catalase activity (µmol/g fresh weight)	Peroxidase activity (µmol/g fresh weight)	Malondialdehyde activity (µmol/g fresh weight)	Phenol content (mg/g dry weight)	Flavonoids content (mg/g fresh weight)
0	0.25 ^{a}	63.09 ^{f}	47.47 ^{f}	18.23 ^{f}	9.73 ^{a}	0.53 ^{a}	2.62 ^{c}	0.06 ^{c}
10	0.24 ^{a}	77.82 ^{e}	63.18 ^{e}	20.47 ^{e}	9.12 ^{a}	0.49 ^{a}	4.25 ^{b}	0.07 ^{b}
20	0.18 ^{b}	90.55 ^{d}	76.54 ^{d}	23.05 ^{d}	8.09 ^{b}	0.38 ^{b}	5.77 ^{b}	0.07 ^{b}
40	0.16 ^{cb}	109.45 ^{c}	78.04 ^{c}	25.81 ^{c}	7.87 ^{b}	0.23 ^{c}	6.36 ^{b}	0.07 ^{b}
80	0.15 ^{c}	114.5 ^{b}	82.84 ^{b}	28.19 ^{b}	7.82 ^{b}	0.22 ^{c}	9.78 ^{b}	0.07 ^{b}
160	0.14 ^{c}	125.66 ^{a}	88.47 ^{a}	31.34 ^{a}	7.07 ^{c}	0.17 ^{d}	13.61 ^{a}	0.13 ^{a}
LSD 5%	0.02	1.10	1.44	0.69	0.71	0.05	0.71	0.01

*= The means followed by the different letters in the same column are significantly different from each other based on LSD test at $P < 0.05$ level

The lowest and highest inhibitory effects on the fresh weight of *H. sabdariffa* were observed in 10 and 160 g (12.6 and 59.3%, respectively) (Figure 1). The same results were observed in the dry weight of *H. sabdariffa*, but the differences were not significant among the treatments of 20, 40, 80 and 160 g kg^{-1} of *R. rugosum* residuals.

Effect of R. rugosum residues on the content of adaptive osmolytes, catalase, peroxidase, and malondialdehyde activity in H. sabdariffa

The lowest and highest content of osmolytes were observed in 10g and 160 g of *R. rugosum* residues (which were about 23.3% and 99.17% higher than those in control plants), respectively (Figure 1). Like the soluble sugar trait, the proline content of *H. sabdariffa* was positively correlated with the quantity of residues. The highest content of proline (+86.3% compared to the control) was observed in the 160 g kg^{-1} of *R. rugosum* residuals. The enzyme changes in *H. sabdariffa* showed that of *R. rugosum* residues had an additive effect on the activity of catalase enzyme compared to the control unlike peroxidase and malondialdehyde enzymes. The maximum catalase amount was recorded in 160 g kg^{-1} of *R. rugosum* residuals (72.2 % compared to control). While, the lowest level of peroxidase and malondialdehyde enzymes was observed in 160 g kg^{-1} of *R. rugosum* residuals (Figure 1). The R. rugosum residues significantly stimulate the phenolic content of *H. sabdariffa*. The minimum and maximum values of phenolic content were recorded in 10 and 160 g kg-1 of *R. rugosum* residuals (+62%

and +419% compared to the control, respectively) (Figure 1). Also, the same results were observed in the content flavonoids of *H. sabdariffa*, and Treatment of 160 g of residues caused a 66.6% increase this parameter in compared to control, but the differences were not significant among of 10, 20, 40 and 80 g of residues (about + 16.6% compared to control).

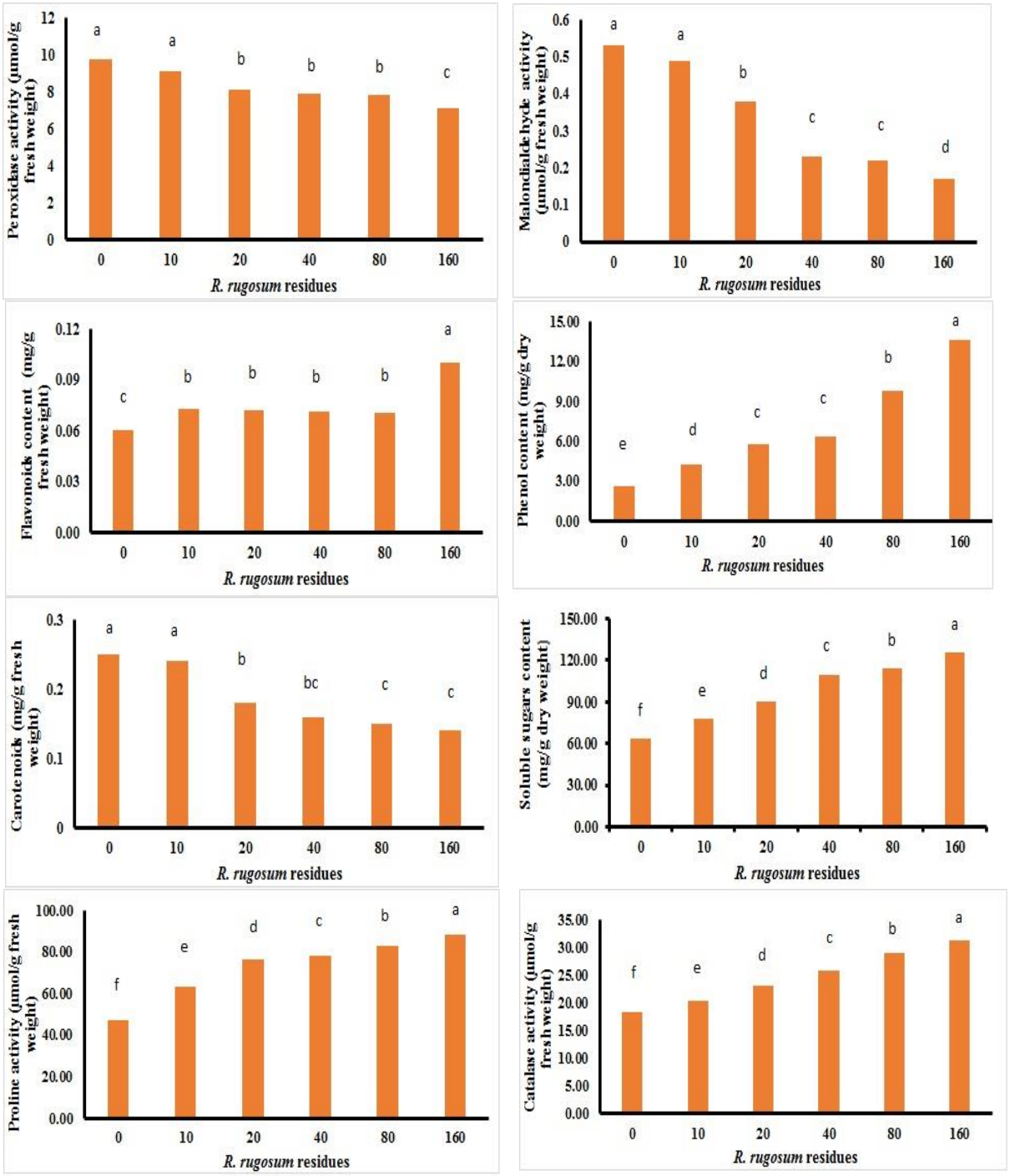

Figure 1. Means comparison of the different amounts of *R. rugosum* residues on physiological traits and pigments content of *H. sabdariffa*.

Discussion

The results of the present study showed that morphological traits of *H. sabdariffa* had a decreasing trend with an increase in *R. rugosum* concentrations. This finding is consistence with the results of Hegab and Ghareib (2010) and Khanh et al. (2008). Poornamazi et al. (2019), who reported that the percentage of inhibition is directly depend on the concentration of the extract. The minimum and maximum number and volume of *H. sabdariffa* roots were recorded at 160 and 10g of residues, respectively. Given that the root is the first organ to be exposed to allelopathic compounds, this is not far-fetched. The negative effect of *R. rugosum* decayed plant parts would be due to the presence of some secondary compounds such as phenolic acid, isothiocyanate, thiocyanate and nitrile Bais et al. (2003). Gulzar et al. (2016) reported that allelopathic compounds target the cell division of growing tissues at the tip of the root and increase the number of abnormal chromosomes. Furthermore, other studies indicated that allelopathic compounds could reduce water uptake and caused stress in plants by affecting root growth and reducing capillary root formation (Feizi et al., 2018). These results showed that the chlorophyll *b*, total chlorophyll and carotenoids in *H. sabdariffa* decreased under different amounts of *R. rugosum* residues. This indicates allelopathic stress due to presence of the harmful compounds (like phenole) in *R. rugosum*. It can also be concluded that chlorophyll *b* and carotenoids, which plays the role of optical protection of chlorophyll *a*, due to its insufficiency in cells, led to the production of chlorophyll triplet and subsequently caused the formation of free radicals and peroxidation of cell membranes, which led to decrease in plant dry weight. The results of Elisante et al. (2013) showed the inhibitory effects of aqueous extract of *Dature stramonium* on chlorophyll content in two wild species of *Cenchrus ciliaris* and *Neonotonia wightii*, which were caused by various factors such as decreased carbon assimilation and increased reactive oxygen species. According to the results, the adaptive osmolytes such as soluble sugars and proline in *H. sabdariffa* leaves were increased under allelopathic stress of various amounts of *R. rugosum*. On the other hand, the activity of peroxidase and malondialdehyde significantly decreased, unlike the content of phenolic compounds and flavonoids. One of the main reasons for the damage of environmental stresses on plants is the production of oxygen free radicals such as singlet oxygen, hydrogen peroxide, superoxide and *etc*, that can target cell organs and cell membrane fats. Studies have shown that flavonoids and phenolic compounds can affect many physiological and biochemical processes, especially plant growth (John and Sarada, 2012). A recent study by (Wang et al., 2018) showed a decreasing trend of CAT, POD and SOD activities on *Amygdalus pedunculata* leaves with an increasing concentration of *Amorpha fruticosa*, *Hedysarum mongolicum*, *Sabina vulgaris*, and *Hippophae rhamnoides* shrubs of aqueous leaf extracts.

Conclusion

Our findings indicated that soluble sugars and proline, the enzymatic antioxidant of catalase and non-enzymatic antioxidants (total phenol and flavonoid) increased under allelopathic stress of *R. rugosum*. While the physiological traits of *H. sabdariffa* such as chlorophyll pigments and malondialdehyde content decreased. This indicates the high stress of *R. rugosum* on *H. sabdariffa* especially at higher concentrations. Due to the fact that *R. rugosum* has a detrimental allelopathic effect on the growth and physiological parameters of *H. sabdariffa*, it is imperative that *R. rugosum* density management be taken into consideration during the cultivation of this plant.

Supplementary Materials

No supplementary material is available for this article.

Author Contributions

Conceptualization, L.A and E.Gh.; methodology, R.K. E. Gh.; software, L.A and E. Gh.; formal analysis, l.A and M.Z.; investigation, L.A, E. Gh and Z.A.; resources, R.K. and L.A.; data curation, L.A.; writing—original draft preparation, R.K., L.A, Z.A and E.Gh.; writing—review and editing, L.A, M.Z and E.Gh.; visualization, E.Gh.; supervision, L.A and M.Z.; project administration, L.A and M.Z.; funding acquisition, L.A.

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Conflict of Interest Statement

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

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مقاله پژوهشی

تاثیر آللوپاتیک علف هرز شلمی (*Rapistrum rugosum* L.) بر پارامترهای رشدی، فیزیولوژیکی و بیوشیمیایی گیاه دارویی چای ترش (*Hibiscus sabdariffa* L.)

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چکیده: به منظور بررسی اثر آللوپاتیک اندام‌های هوایی علف هرز شلمی (*Rapistrum rugosum* L.) بر ویژگی‌های رشدی، فیزیولوژیکی و بیوشیمیایی گیاه دارویی چای ترش، آزمایشی گلدانی در قالب طرح کاملاً تصادفی با سه تکرار در شرایط گلخانه در دانشگاه گنبدکاووس اجرا شد. تیمارها شامل مقادیر مختلف بقایای شلمی شامل ۰، ۱۰، ۲۰، ۴۰، ۸۰ و ۱۶۰ گرم بافت شلمی در کیلوگرم خاک بودند. نتایج نشان داد که افزایش میزان بقایای *R. rugosum* به طور معنی‌داری سبب کاهش صفات رشدی چای ترش گردید. کمترین میزان طول ساقه و ریشه، وزن‌تر و خشک گیاه در تیمار ۱۶۰ گرم بقایا *R. rugosum* مشاهده شد. محتوای کلروفیل bو کاروتنوئیدها و همچنین مالون‌دی‌آلدئید با افزایش مقدار *R. rugosum* کاهش یافت. با این حال، روند تغییرات در محتوای آنزیم کاتالاز، محتوای قندهای محلول، پرولین، فنل کل و فلاونوئیدها با افزایش میزان باقیمانده *R. rugosum* افزایش یافت. در کل، یافته‌های حاصل از این آزمایش نشان داد که با افزایش میزان بقایای *R. rugosum*، تمامی صفات مورد مطالعه چای ترش به‌طور معنی‌داری کاهش یافت. بنابراین با توجه به اثر منفی آللوپاتیک علف هرز شلمی بر پارامترهای رشدی و فیزیولوژیکی گیاه چای ترش در زمان کشت این گیاه باید مدیریت تراکم علف هرز مورد توجه قرار گیرد.

کلمات کلیدی: آللوپاتی، صفات فیزیولوژیکی، *Rapistrum rugosum* ، فنل کل.

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