The Response of *Hypericum Perforatum* L. to the Application of Selenium and Nano-selenium

Rezaei Nazari Mahmonir

Islamic Azad University

Vahid Abdossi (✉ abdusivahid@gmail.com)

Islamic Azad University

Zamani Hargalani Fariba

Islamic Azad University

Kambiz Larijani

Islamic Azad University

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Abstract

Background: In terms of medicinal and therapeutic properties, *H. perforatum* is the important species. However, Selenium has been applied as an alleviation strategy subjected to producing essential oils and biomass.

Method: For this study, a randomized complete block design with three replications was used so that each experimental unit comprised of 30 number 8 pots. The treatments included the foliar application of selenium (6, 8, 10, and 12 mg/l), nano-selenium (6, 8, 10, and 12 mg/l), and control (distilled water), applied at the rosette stage and harvesting at 50% flowering stage.

Results: The results of the analysis of variance showed that the effect of selenium and nano selenium fertilizers was significant ($p < 0.01$) on plant height, inflorescence length, number of inflorescences, inflorescence weight, shoot weight, root length, the total weight of biomass, essential oil percentage, the content of hypericin and hyperforin, the selenium accumulation in the plant, relative leaf water content, the content of chlorophylls a, b, and total, the content of phenol, the content of proline, production and accumulation of catalase, peroxidase, malondialdehyde, and DPPH enzymes. The highest inflorescence weight (21 g/plant), shoot weight (70 g/plant), and essential oil content (0.14%) were obtained from the control treatment. The highest accumulation of hypericin (3.8 mg/g dry matter) was obtained from the foliar application of 8 mg/l selenium. The maximum accumulation of hyperforin (57 mg/g dry matter) was obtained from the foliar application of 10 mg/l selenium. The highest accumulation of selenium (287.52 $\mu\text{g/g}$ dry matter) was obtained in the foliar application of 12 mg/l nano-selenium.

Conclusion: If the purpose of producing *H. perforatum* is to use the shoots and essential oils of the plant, then the use of selenium and nano-selenium is not recommended at all and should not be used. If the goal is to produce high hypericin, high hyperforin, and also the accumulation of selenium in the plant, the use of treatments of 6 and 8 mg/l of selenium and nano-selenium could be applied.

1. Background

Hypericum perforatum belongs to the Hypericaceae family and has more than 469 species in the world (Crockett, 2010), of which 17 (Mozaffarian, 1996) to 19 species (Zobayed et al., 2005) of this genus have been reported in Iran. *St. John's wort* is a perennial plant and the most important medicinal species of this genus. It is a branched plant with 40–80 cm high. The stems and branches are covered with broad leaves and soft margins, and the leaves are 1 to 3 cm long and 0.3 to 1 cm wide (Couceiro et al., 2006; Singh, 2005).

In the upper parts of *H. perforatum*, several yellow flowers are produced, which are usually 1 to 2 cm wide. There are usually black dots on the edges of the petals. Blood red pigments are generated using crushed flowers. Flowers produced in late summer have capsules that contain tiny brown seeds (Crompton et al., 1988). Although this plant is native to Europe, it is distributed in all temperate regions in Asia, Australia, and North and South America.

In terms of medicinal and therapeutic properties, *H. perforatum* is the most important species of this genus, and today it is widely used in the treatment of mild-to moderate depression. The history of the traditional use of this medicinal plant dates back to over 2000 years ago. Antidepressant, anti-inflammatory, antimicrobial, antiviral, and anti-cancer properties have been reported for *H. perforatum*. Various studies have reported that *H. perforatum* has antiviral (Chen et al., 2019), wound healing (Samadi et al., 2010), antioxidant (Hakimouglu et al., 2007; Saddiqe et al., 2010) antimicrobial (Akhbari et al., 2012), and antibacterial properties (Tozlu et al., 2011).

Clinical trials have been conducted since the early 1990s on the effect of hypericin on the treatment of viral diseases, and experiments in this area show that the plant with low toxicity has significant effects against HIV-like viruses. So far, a wide range of biologically active compounds have been identified and reported in this plant, including hypericin and hyperforin.

Chemical research on the components of *H. perforatum* has led to the identification of several groups of pharmaceutically active compounds. The most important groups include naphthodianthrone (hypericin and pseudo-hypericin), phloroglucinols (hyperforin and adhyperforin), xanthenes and flavonoids (such as phenylpropanes, flavanol glycosides and bioflavonoids) as well as essential oils. Various biological activities including wound healing, anti-anxiety and seizures, antiviral, antifungal, and antioxidant properties have been attributed to the compounds in the extract and essential oil of various species of the genus *Hypericum* (Bertoli et al., 2011). There are various reports on the antimicrobial activity of essential oils of different species of the genus *Hypericum* against bacteria and fungi of human and plant pathogens.

Selenium is one of the essential elements in the human diet. Although selenium has a variety of functions, its antioxidant and anti-cancer properties are of particular importance to humans. Selenium deficiency in the human diet also causes stunted growth, impaired bone metabolism, and thyroid dysfunction (Dumont et al., 2006). Selenium concentrations are usually higher in younger leaves than in older leaves during seedling growth (Galeas et al., 2007). Because selenium is so similar to sulfur, it competes with sulfur and enters plant cells through sulfate carriers in the root plasma membrane (Pilon-Smits et al., 2002).

Nanoparticles react with plants to cause a variety of physical and physiological changes, which significantly depend on the properties of the nanoparticles. The effectiveness of nanoparticles depends on their concentration and varies from plant to plant. The efficiency of nanoparticles depends on the chemical composition, size, surface area, reactivity, and concentration at which they respond positively. Nanoparticles have positive and negative effects on plant growth (Ekinci et al., 2014). Irmak (2017) conducted a study to investigate the effect of selenium application on plant growth and some quality parameters in peanut (*Arachis hypogaea*).

Hence, the main goal of this experiment was to evaluate the different selenium source application for with main focus morphophysiological traits. Furthermore, we pointed to identify the appropriate concentration of Selenium that can develop essential oil and other features.

2. Methods

This study was conducted in 2020 in Tehran, Iran. The study aimed to survey the effect of selenium and nano-selenium on vegetative growth and phytochemical and enzymatic properties of *St. John's wort* in the Tehran Municipality greenhouse. This study was conducted with three replications in a randomized complete block design. Each experimental unit consisted of 30 pots. Treatments included foliar application with different levels of selenium and nano-selenium. For this purpose, selenium at concentrations of 6, 8, 10 and 12 mg/l were foliar applied at the rosette stage. In addition, nano-selenium at concentrations of 6, 8, 10, and 12 mg/l were foliar applied simultaneously with foliar application of sodium selenate. Foliar spraying of distilled water was used as a control (Fig. 1).

One-year-old seedlings of Topaz cultivar were obtained from Pakan Bazr Isfahan Company and planted in pots in March. After seedling establishment, foliar application was performed. Specimen was presented in central herbarium of Tehran University (Herbarium Code: TUH). (6398 No). Samples were authenticated by a botanist. Data collection and plant harvesting were performed at 50% flowering stage. Plant height, inflorescence length, number of inflorescences, inflorescence weight, shoot weight, root length, root weight, total biomass weight, essential oil percentage, hypericin and hyperforin content (Tawaha et al., 2010), selenium content (Singh et al., 1980), Relative Water Content (RWC), total chlorophyll, chlorophyll a and b, phenol content, DPPH, and proline content (Abbas et al., 2014), and the content of catalase (Velikova et al., 2000), peroxidase (Reuveni et al., 1992), and malondialdehyde (Dhindsa and Matowe, 1981), were measured and determined.

3. Results

The results of the analysis of variance showed that the effect of selenium and nano selenium fertilizers on plant height, inflorescence length, number of inflorescences, inflorescence weight, shoot weight, root length, total biomass weight, essential oil percentage, and hypericin and hyperforin content was significant at 1% level, and on the root weight at 5% level (Table 1).

Table 1
Effect of selenium and nano-selenium on morphophysiological characteristics and growth of *St. John's wort* using analysis of variance

S.O.V	df	plant height	inflorescence length	number of inflorescences	inflorescence weight	shoot weight	root length	root weight	total biomass weight	essential oil percentage	hypericin content
Replication	2	75.69*	26.01ns	9ns	57.76**	147.21**	87.84**	2.25ns	36.4*	0.00ns	0.75**
Treatment	8	127.05**	35.45**	54**	45.20**	238.58**	39.45**	37.77*	773.08**	0.00**	3.53**
Error	16	7.22	8.52	5.25	5.58	2.69	0.62	5.46	10.59	0.00	0.06
%CV.		5.52	13.59	22.91	5.35	2.90	3.4	13.03	3.66	23.06	10.82
Ns, * and ** indicate non-significance and significance at the level of 5 and 1%, respectively.											

Continuous of Table (1).

S.O.V	df	selenium	RWC	chlorophylls a	chlorophylls b	chlorophylls total	phenol	proline	CAT	POD	MDA	DPPH
Replication	2	378.56**	272.03**	0.19**	0.006**	0.26**	2.08**	44.44**	0.002**	0.40*	3321.6*	54.76*
Treatment	8	16804.1**	508.77**	0.09**	0.02**	0.17**	3.07**	614.33**	0.004**	0.61**	201147**	350.08**
Error	16	21.81	0.39	0.00	0.00	0.00	0.29	0.98	0.00	0.07	769.53	4.59
%CV.		3.84	3.88	4.00	7.04	3.50	13.06	3.62	13.48	15.14	9.28	3.83
RWC: Relative Water Content, CTA: Catalase, POD: Peroxidase, MDA: Malondialdehyde												
Ns, * and ** indicate non-significance and significance at the level of 5 and 1%, respectively.												

The effect of selenium and nano-selenium on the amount of selenium accumulation in the plant, relative leaf water content, the content of chlorophylls a, b, and total, the phenol and proline content, and production and accumulation of catalase, peroxidase, malondialdehyde, and DPPH enzymes were statistically significant at the 1% level (Table 1).

Comparison of means showed that the highest plant height was obtained from control treatment (58 cm) and foliar application of 6 mg/l selenium (56.4 cm) (Table 2). Inflorescence length was the highest in control treatment and two treatment levels of 6 and 8 mg/l selenium and nano-selenium (Table 2). The number of inflorescences was the highest in the control treatment and the first treatment level (6 mg/l) of selenium and nano-selenium (Table 2).

Table 2
Comparison of means of the effect of selenium and nano-selenium on morphophysiological characteristics and growth of *St. John's wort*

	plant height	inflorescence length	number of inflorescences	inflorescence weight	shoot weight	root length	root weight	total biomass weight	essential oil percentage	hypericin content	hyperforin content
	cm	cm		g/plant	g/plant	cm	g/plant	g/plant	%	mg/g dry matter	mg/g dry matter
co	58a	25.5a	16a	21a	70a	27a	24a	115a	0.14a	2.7cd	44c
si6	56.4a	25ab	14ab	17b	66b	26ab	22ab	105b	0.1b	3.2b	50b
8	50bc	24ab	10cd	15c	60c	25bc	19bc	94cd	0.09bc	3.8a	44c
10	48.5bc	20bcd	8de	12d	55d	22d	17.5c	84.5e	0.07bcde	1.5e	57a
12	41de	18cd	6ef	10e	47e	20ef	16.5c	73.5f	0.05de	1f	33e
nanosi6	51.5b	23.8ab	14ab	17.5b	62c	25bc	18bc	97.5c	0.1b	3bc	46c
8	49b	22.5abc	12bc	15c	57d	23.8c	17.5c	89.5de	0.08bcd	2.5d	41d
10	45.6cd	18.5cd	6ef	12d	48e	21de	15cd	75f	0.06cde	1.2ef	34e
12	38e	16d	4f	9e	44f	18.8f	12d	65g	0.04e	0.8f	26f
The same letters in each column indicate that there is no significant difference between the means											

Continuous of Table (2).

	selenium	RWC	chlorophylls a	chlorophylls b	chlorophylls total	phenol	proline	CAT	POD	MDA	DPPH
	µg/g dw	%	g/100 g FM	g/100 g FM	g/100 g FM	Mg/l FW extract	µmol/g FW	u/g FW/min	U/g FW/min	mM/d FW	%
co	37h	84a	0.77e	0.26d	1.03e	3.5c	12h	0.05e	1.1d	68d	35f
si6	49.5g	76b	0.96d	0.3cd	1.26d	3.23c	15g	0.07de	1.3d	92d	47e
8	79f	75b	1.02d	0.32bc	1.34d	3.31c	25d	0.07de	2ab	103d	56c
10	120d	70c	1.13c	0.35b	1.48c	3.28c	33c	0.1bc	2.3a	185c	68a
12	137c	61f	1.23b	0.28cd	1.51c	4.54b	48b	0.15a	1.5cd	590b	61b
nanosi6	94e	75.5b	1d	0.32bc	1.32d	3.41c	17f	0.08cd	1.4cd	110d	52d
8	122.63d	68.4d	1.19bc	0.36b	1.55c	4.66b	19e	0.09cd	1.8bc	222c	71a
10	167.52b	66e	1.25ab	0.46a	1.71b	5.2ab	26d	0.12b	2.4a	695a	57c
12	287.52a	58g	1.32a	0.49a	1.81a	6a	52a	0.16a	1.5cd	624b	55cd
RWC: Relative Water Content, CTA: Catalas, POD: Proxidase, MDA: Malondialdehyde											
The same letters in each column indicate that there is no significant difference between the means											

The highest inflorescence weight (21 g/plant), shoot weight (70 g/plant), root weight (27 g/plant), total biomass weight (115 g/plant), and essential oil content (0.14%) was obtained from the control treatment. The highest root weight was obtained from the control treatment (24 cm) and foliar application of 6 mg/l selenium (22 cm). The highest accumulation of hypericin (3.8 mg/g dry matter) was obtained from the foliar application of 8 mg/l selenium. The highest accumulation of hyperforin (57 mg/g dry matter) was obtained from the foliar application of 10 mg/l selenium.

It was observed that the highest accumulation of selenium (287.52 µg/g dry matter), the highest content of chlorophyll a (1.32 g), chlorophyll b (0.49 g) total chlorophyll (1.81 g), phenol (6 mg), and proline (52 µmol/g) were obtained in the foliar application of 12 mg/l nano-selenium. The highest catalase content was obtained from the foliar application of 12 mg/l nano-selenium (0.16 u/g FW/min) and foliar application of 12 mg/l selenium (0.15 u/g FW/min). Peroxidase was higher in the foliar application of 8 and 10 mg/l selenium and 10 mg/l nano-selenium than other treatments. The highest malondialdehyde leakage (695 u/g FW/min) was obtained from the 10 mg/l nano-selenium treatment. The highest levels of antioxidant enzyme (71 and 68%) were obtained from the foliar application of 8 mg/l selenium and 10 mg/l nano-selenium, respectively.

The study of simple relationships between traits showed a significant positive correlation between the plant height and inflorescence length, number of inflorescences, inflorescence weight, shoot weight, root length, root weight, total biomass weight, essential oil percentage, the content of hypericin and hyperforin, relative water content, and peroxidase at the level of 1%. The plant height was also significantly correlated with the amount of selenium, chlorophyll a, b, and total chlorophyll, phenol, proline, catalase, malondialdehyde, and DPPH (Table 3). Similar to the plant height, the inflorescence length,

number of inflorescences, inflorescence weight, shoot weight, root length, root weight, essential oil percentage, and amount of hypericin and hyperforin had a significant correlation with other traits (Table 3).

Table 3
Simple correlation of the effects of selenium and nano-selenium on morphophysiological characteristics and growth of *St. John's wort*

	plant height	inflorescence length	number of inflorescences	inflorescence weight	shoot weight	root length	root weight	total biomass weight	essential oil percentage	hypericin content	hyperforin content
	1	2	3	4	5	6	7	8	9	10	11
1	1.00										
2	0.83**	1.00									
3	0.84**	0.77**	1.00								
4	0.55**	0.45*	0.64**	1.00							
5	0.91**	0.85**	0.87**	0.57**	1.00						
6	0.90**	0.81**	0.79**	0.35ns	0.93**	1.00					
7	0.71**	0.57**	0.71**	0.61**	0.82**	0.68**	1.00				
8	0.86**	0.77**	0.87**	0.76**	0.95**	0.81**	0.90**	1.00			
9	0.80**	0.81**	0.79**	0.62**	0.89**	0.78**	0.76**	0.89**	1		
10	0.77**	0.82	0.75**	0.52**	0.83**	0.82**	0.62**	0.79**	0.73**	1.00	
11	0.71**	0.59**	0.54**	0.31ns	0.70**	0.63**	0.56**	0.64**	0.56**	0.58**	1.00
12	-0.78**	-0.68**	-0.75**	-0.74**	-0.79**	-0.66**	-0.81**	-0.87**	-0.74**	-0.73**	-0.68**
13	0.91**	0.78**	0.82**	0.60**	0.96**	0.90**	0.79**	0.93**	0.86**	0.79**	0.70**
14	-0.47*	-0.36ns	-0.50*	-0.94**	-0.49*	-0.25ns	-0.60**	-0.70**	-0.59**	-0.44*	-0.32ns
15	-0.48*	-0.39*	-0.55**	-0.65**	-0.50*	-0.34ns	-0.62**	-0.63**	-0.46*	-0.44*	-0.43*
16	-0.50*	-0.39*	-0.54**	-0.91**	-0.53**	-0.29ns	-0.64**	-0.72**	-0.59**	-0.47*	-0.37*
17	-0.56**	-0.53**	-0.53**	-0.67**	-0.53**	-0.34ns	-0.59**	-0.64**	-0.56**	-0.58**	-0.66**
18	-0.78**	-0.67**	-0.78**	-0.84**	-0.76**	-0.64**	-0.66**	-0.84**	-0.75**	-0.72**	-0.54*
19	-0.71**	-0.54**	-0.71**	-0.87**	-0.68**	-0.52*	-0.67**	-0.81**	-0.72**	-0.71**	-0.56*
20	-0.17ns	-0.24ns	-0.43*	-0.51*	-0.24ns	-0.06ns	-0.19ns	-0.33ns	-0.35ns	-0.15ns	0.16ns
21	-0.73**	-0.72**	-0.76**	-0.72**	-0.82**	-0.67**	-0.69**	-0.86**	-0.73**	-0.81**	-0.76**
22	-0.39*	-0.33ns	-0.42*	-0.63**	-0.45*	-0.28ns	-0.48*	-0.56*	-0.56*	-0.28ns	0.04ns
Ns, * and ** indicate non-significance and significance at the level of 5 and 1%, respectively.											

Continuous of Table (3).

	selenium	RWC	chlorophylls a	chlorophylls b	chlorophylls total	phenol	proline	CAT	POD	MDA	DPPH
	12	13	14	15	16	17	18	19	20	21	22
12	1.00										
13	-0.80**	1.00									
14	0.73**	-0.53**	1								
15	0.83**	-0.49*	0.71**	1							
16	0.8**	-0.55*	0.98**	0.84**	1						
17	0.8**	-0.52*	0.78**	0.73**	0.81**	1					
18	0.81**	-0.77**	0.69**	0.46*	0.66**	0.59**	1				
19	0.83**	-0.71**	0.84**	0.63**	0.83**	0.78**	0.87**	1			
20	0.26ns	-0.15ns	0.52*	0.51*	0.55**	0.25ns	0.19ns	0.28ns	1		
21	0.8**	-0.78**	0.7**	0.64*	0.72**	0.77**	0.71**	0.82**	0.29ns	1	
22	0.38*	-0.49*	0.68*	0.33ns	0.61**	0.31ns	0.41*	0.44*	0.60**	0.29ns	1
RWC: Relative Water Content, CTA: Catalase, POD: Peroxidase, MDA: Malondialdehyde											
Ns, * and ** indicate non-significance and significance at the level of 5 and 1%, respectively.											

It was observed that the content of selenium had a significant negative correlation with morphological traits and yield of different organs and a significant positive correlation with the number of enzymes, chlorophylls, phenol, and proline. In contrast to selenium, relative water content had a significant positive correlation with agronomic traits and yields and a significant negative correlation with the number of enzymes, chlorophylls, phenol, and proline.

As can be seen in Table 3, physiological traits such as chlorophylls, enzymes, phenol, and proline had a significant positive correlation with each other.

4. Discussion

Considering climate change (Peteet, 2000; Prentice et al., 1991), greenhouse gases phenomenon (Avand et al., 2020; Griggs & Noguer, 2002), and drought (Aimar et al., 2014; Aranjuelo et al., 2011; Naumburg et al., 2004) in the regions of the world and especially in Iran, introducing new crops with economic value and high water use efficiencies such as *H. perforatum* and the study of its response to different treatment levels is one of the important research priorities in Iran and the world. The significant difference between replications was because of uneven greenhouse conditions, showing that the random complete blocks design was correctly selected. Moreover, the significant differences between traits (Table 1) show the selection of the hypothesis, the subject of research, and treatments, and the importance of research on *H. perforatum* and the introduced treatments.

Comparison of means showed that the highest plant height was obtained from control treatment and foliar application of 6 mg/l selenium (Table 2), and the plant height decreased with increasing the concentration of treatments. The decreased height with selenium treatments indicates the intolerance of *H. perforatum* to selenium stress. Because the number of inflorescences is affected by environmental and genetic factors and tillers, so by applying environmental treatments, the role of each of the environmental and genetic factors can be revealed. In this study, it was observed that inflorescences are more affected by environmental factors. In this study, the results of the effect of selenium as an environmental factor on the number of tillers are shown in Table 2. These results are consistent with the findings Soleimani et al., (2017) and Uddin et al., (2016) on the effect of environmental and genetic factors on the number of tillers. The highest shoot weight, root weight, and total biomass weight were obtained from the control treatment. Therefore, the results of this part of the research show that *H. perforatum* is intolerant to selenium and nano-selenium.

The studied plant appears to cope with selenium and nano-selenium stress with changes in morphological traits (Thomas and Gausling, 2000), decrease in photosynthetic and transpiration organs, increase in regulatory osmolytes (Kivimäenpää et al., 2003), changes in the synthesis of materials within the plant (Flexas et al., 2009), increased water-absorbing organs (Dambolena et al., 2010) and finally allocation of photosynthetic material for active water absorption by energy (Slattery and Ort, 2015), all of which will reduce plant shoot yield as occurred for *H. perforatum*. The highest essential oil percentage was obtained from the control treatment, while the content of hypericin and hyperforin increased at low levels of selenium and nano-selenium.

Basically, in plants, when they are stressed, the pentose phosphate pathway becomes more active and the production of essential oil increases. It has also been reported that plants under conditions of severe stress increase the percentage of other regulating compounds and osmolytes such as proline instead of essential oils and this is more evident in sensitive plants. The highest accumulation of hypericin and hyperforin was obtained from the foliar application of 8 mg/l and 10 mg/l selenium, respectively, showing that the use of small amounts of these two elements can be useful for producing high-quality plants. Catalase enzyme was highest in the foliar application of 12 mg/l nano selenium and the foliar application of 12 mg/l selenium, which indicates the production of this enzyme at high levels of selenium and nano selenium. However, peroxidase production is faster than catalase in the plant, and it seems that at the same time as peroxidase production, malondialdehyde leakage reaches its maximum.

The results showed that in the foliar application of 12 mg/l nano-selenium, the maximum accumulation of selenium and the highest amount of chlorophyll a, chlorophyll b, total chlorophyll, phenol, and proline were obtained. Therefore, *St. John's wort* was a plant that absorbs selenium and nano-selenium, and despite the increase in chlorophyll content, a decrease in yield and an increase in antioxidant activity were observed. In general, the effect of selenium and

nano-selenium particles depends on the composition, size, surface area, reactivity, and concentration in which they respond positively and have positive and negative effects on plant growth and development (Ekinci et al., 2014). In our study, the same positive and negative results were observed on different traits, and in this regard, the results are consistent with the research of Ekinci et al, (2014). The results of this study are similar to the research on Lemongrass (*Melissa officinalis* L.) in terms of decreasing yield and increasing the concentration of chlorophyll a and b and the activity of catalase and malondialdehyde in the 10 mg/l treatment.

Simple relationships between traits showed a significant positive correlation between morphological traits and yields.

5. Conclusion

Therefore, if the purpose of producing *H. perforatum* is to use the shoots and essential oils of the plant, then the use of selenium and nano-selenium is not recommended at all and should not be used. If the goal is to produce high hypericin, high hyperforin, and also the accumulation of selenium in the plant, the use of treatments of 6 and 8 mg/l of selenium and nano-selenium could be used. A significant negative correlation between selenium content with morphological traits and yield of different organs and a positive correlation with the content of enzymes, chlorophylls, phenol, and proline shows that selenium causes stress in the plant and therefore plant growth is reduced and instead increases the production of antioxidant enzymes and osmolytes. There is a significant negative correlation between selenium content with morphological traits and function of different organs and a positive correlation with the number of enzymes, chlorophylls, phenol and proline indicates that selenium causes stress in the plant and therefore plant growth is reduced and instead production increases antioxidant enzymes and osmolytes.

Abbreviations

S: Selenium, **RWC:** Relative Water Content, **CTA:** Catalase, **POD:** Peroxidase, **MDA:** Malondialdehyde

Declarations

Ethics approval and consent to participate: Not applicable

Consent for publication: Not applicable.

Availability of data and materials: Not available

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Figures

Figure 1

Pictures of the stages of this research