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Differential Effects of Four Abiotic Factors on Seed Germination and Seedling Growth of Rangeland-Medical Plant, Wild Mustard (Sinapis arvensis L.)

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ABSTRACT

Aims The aim of this study was to determine the effect of drought stress induced by using polyethylene glycol (PEG), heavy metals (Cd and Ni), and salinity (NaCl) on germination and seedling growth of Sinapis arvensis, an important medicinal plant in the Brassicaceae.

Materials & Methods The Sinapis arvensis seeds treatments contained i), control ii), PEG (5%, 10%, 15%), iii) NaCl (50, 100, 150 mM), iv) Cd+2 (50, 100, 150µM), and v) Ni+2 (50, 100, 150µM). The experiment used a randomized complete block design with 4 replicates per treatment. The experiments were performed in a programmed incubator at 25±2oC. Seed germination was recorded every day for 16 days. The root and shoot lengths of seedlings were measured after 16 days of incubation. Then, the seedlings were dried and root and shoot dry weights were measured.

Findings The results showed that germination percentage reduced with increasing concentrations of the tested factors. The maximum germination (97%) was observed in PEG (5%) and the minimum germination rate was recorded in NaCl (150 mM) with 41%. The maximum of root and shoot lengths were recorded in PEG (5%) and Ni (100µM) with 59 and 73 mm, respectively. Results showed that the maximum root and shoot fresh and dry weights were recorded at Ni (100µM) treatment.

Conclusion Understanding plant responses to environmental stresses can help select suitable plants in order to obtain sustainable products. Overall, we can conclude that under aforementioned treatments, the root growth of S. arvensis was more affected than the shoot growth.

Keywords Germination; Seedling Growth; Abiotic Stress; Sinapis Arvensis; Medical Plant

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Introduction

Germination of Seeds is one of the most important phases in the plant life cycle and it is very sensitive to its environment [1]. The abiotic factors such as drought, salt, and heavy metals stresses are the 3 most important abiotic stresses that restrict germination of seeds and seedling growth [2-5].

Abiotic stress conditions negatively affect plants' survival, agglomeration of biomass, and yield [6]. Drought stress significantly affects seed germination. Seed germination is adversely affected by undesirable conditions of soil moisture caused by lack of rainfall and irrigation [7, 8]. Insufficient moisture leads to erratic germination and seedling emergence, which in turn affects the establishment and performance of crops and medical plants [7-9].

Osmotic adjusting has a very important role in sustaining plant growth under water deficiency conditions. The plants' response varies significantly at different organizational levels, depending on the intensity and duration of stress and also plant species and its life stage [10]. In the arid and semi-arid area, water is generally one of the most important parameters restricting plant production.

Seed germination and emergence are crucial stages for the establishment of plants and plant growth in arid and semi-arid areas and assist in determining the standing density of crop and yields [11]. High concentrations of salt in the soil can create restrictions for plant production and development. The main parameters that make this problem are the arid and semi-arid climate and the addition of salt by water that is utilized for irrigation [8].

Soil salinity stress can lead to harmful effects on plant growth and development, and biochemical and physiological in plants [12]. These effects may be as a result of low osmotic potential of the soil solution, specific ion influences, and imbalances of nutritional or a complex of these factors [13, 14]. High concentrations of salt in the germination media lead to a highly osmotic pressure that impedes necessary water absorption for germination stage. High levels of salts have toxicity effects on the embryo and can decrease or delay the seed germination [15].

The salinity stress reduces seed germination, root length, coleoptile length, callus size, and seedling growth [16-19]. Over the last years, the heavy metals level are increasing in the agricultural soils as a result of increasing environmental pollution from agricultural, energy, municipal, and industrial wastes. Scientists have reported a decrease in plants' growth as a result of the presence of high levels of heavy metals such as arsenic, cadmium (Cd), nickel (Ni), mercury, and lead [20].

Cd is the most common heavy metal found in the soil surface layer, which inhibits nutrients uptake by plants and also its growth [21]. The plant growth inhibition can be caused by the cadmium phytotoxic effects on various processes in plants including photosynthesis, respiration, water relation, and metabolism of carbohydrates [22].

Ni has been recognized as a component of a number of enzymes containing peptide deformylases, methyl-CoM reductase, glyoxalases, ureases, and a few hydrogenases, and superoxide dismutases [23, 24]. Hence, Ni plays an important role in different metabolic processes including acetogenesis, methane biogenesis, hydrogen metabolism, and ureolysis [25]. Ni is necessary for plants [26], but the Ni concentration in the majority of plants is very low (0.05–10 mg/kg dry weight) [27].

Toxicity effects of high concentrations of Ni in plant species have often been reported. These effects cause an incline in plant growth [28], inhibition of mitotic activities [29], and negative effects on quality and fruit yield [30].

Salt, drought, and heavy metal stress are injurious during early stages of seed germination and seedling growth. With increasing health hazards realization and toxicity related to the undiscerning use of antibiotics and synthetic drugs, people are interested in the use of plants and plant-based drugs being resuscitated throughout the world. Thus, the utilization of medicinal plants has become more and more common.

In the past several years, various scales of plant physiology have been used to investigate responses to drought, salt, and heavy metals stress tolerance methods and mechanisms to overpower these stresses in crops [31]. It seems essential to do research about the correlation between medicinal plants and drought, salt, and heavy metals stress for the increasing need of medicinal plants. To meet the ever-increasing medicinal plants' requirement for the native systems of medicine and also for the pharmaceutical industry, some medicinal plants are required to be commercially cultivated, but the soil pollutions pose critical threats to production of plants [32]. Thus, it seems important to test these medicinal plants for their tolerance capacity.

Sinapis arvensis L. is usually known as field mustard, charlock, or wild mustard. Brassicaceae includes 3,700 species distributed over 338 genera [33]. This family has a worldwide distribution. In many countries, *Sinapis arvensis* L. is used as fodder to livestock, folklore medicine, and food [34, 35]. This species is native to Europe and grows wildly in North Africa and western Asia [36].

Plants are continuously exposed to several biotic and abiotic stresses such as salinity, drought, and exposure to heavy metals in their life that limit their productivity and growth.

The aim of this study was to determine the effect of drought stress induced by polyethylene glycol (PEG), heavy metals (Cd and Ni), and salinity (NaCl) on germination and seedling growth of *Sinapis arvensis*, an important medicinal plant in the Brassicaceae.

Materials and Methods

The seeds of *Sinapis arvensis* were obtained from the Seed Gene Bank, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran. To demolish seed-borne microorganisms, the seeds were sterilized by 5% solution of sodium hypochlorite for 5 minutes and washed 3 times with sterilized distilled water. The seeds were placed in Petri dishes on 2 layers of filter paper. Each Petri dish included 50 seeds. The experiment used a randomized complete block design with 4 replicates per treatment. The treatments contained i), control ii), PEG (5%, 10%, 15%), iii) NaCl (50, 100, 150mM), iv) Cd^{+2} (50, 100, 150μM), and v) Ni^{+2} (50, 100, 150μM). The experiments were performed in a programmed incubator at 25±2°C. The criterion for germination of seed was the radicle emergence to a length of 2-5mM [37]. Seed germination was recorded every day for 16 days, where each time the germination percentage was calculated. The seedlings were harvested after 16 days of incubation and root and shoot lengths were measured. Then, the seedlings were dried in an oven at 80°C for 24 hours and root and shoot dry weights were measured.

Statistical analysis: Statistical analyses were performed, using SPSS 11.5. All data were analyzed by one-way analysis of variance (ANOVA) to determine the effect of treatments, and LSD multiple means comparison test at p≤0.05 was carried out to determine the statistical significance of the differences between means of treatments.

Findings

Effects of PEG, Cd, Ni, and NaCl on Seed Germination: Effect of different concentration of treatments were presented in Figure 1 and Table 1. Seed germination rate was different at different treatment levels. The results showed that seed germination reduced with increasing of treatment concentrations (F=312.59; p=0.000). The first germination was recorded in PEG (5%), Cd (50μM), Ni (50μM), NaCl (50mM) treatments, and control on the second day, after which germination rates increased. The maximum germination (97%) was observed in PEG (5%) and the minimum germination (41%) was observed in NaCl (150mM).

Table 1) Overall mean values for various traits of *S. arvensis* germination percentage and seedlings grown under different concentration of polyethylene glycol (PEG), heavy metals (Cd and Ni), and salinity (NaCl)

Treatments	Concentration	Germination (%)	Root fresh weight (g)	Shoot fresh weight (g)	Root dry weight (g)	Shoot dry weight (g)
Control	0	96±0.91^{ab}	2.21±0.01^{d}	3.25±0.00^{c}	0.73±0.00^{cd}	1.08±0.00^{b}
PEG	5 %	97±0.91^{a}	2.26±0.00^{c}	2.29±0.00^{g}	0.75±0.00^{bc}	0.76±0.01^{f}
	10 %	93±2.1^{bc}	2.34±0.01^{b}	1.54±0.01^{j}	0.78±0.00^{ab}	0.51±0.01^{i}
	15 %	61±1.95^{h}	2.4±0.00^{a}	0.87±0.01^{k}	0.8±0.00^{a}	0.29±0.01^{j}
Cd	50 μM	92±2.04^{c}	2.26±0.01^{c}	3.04±0.01^{d}	0.75±0.01^{bc}	1.01±0.00^{c}
	100 μM	74±2.27^{f}	1.73±0.01^{g}	2.7±0.00^{e}	0.57±0.01^{f}	0.9±0.01^{d}
	150 μM	41±2.08^{j}	1.22±0.01^{h}	1.87±0.01^{h}	0.4±0.01^{g}	0.62±0.01^{g}
Ni	50 μM	83±2.61^{e}	2.31±0.01^{b}	3.29±0.01^{b}	0.77±0.01^{abc}	1.09±0.00^{ab}
	100 μM	81±2.48^{e}	2.42±0.01^{a}	3.37±0.00^{a}	0.81±0.00^{a}	1.12±0.01^{a}
	150 μM	53±2.34^{i}	2.11±0.00^{e}	2.54±0.00^{f}	0.7±0.01^{d}	0.84±0.01^{e}
NaCl	50 mM	88±1.82^{d}	1.92±0.01^{f}	1.7±0.01^{i}	0.64±0.01^{e}	0.56±0.01^{h}
	100 mM	66±2.67^{g}	1.02±0.00^{i}	0.75±0.01^{l}	0.34±0.02^{h}	0.25±0.00^{k}
	150 mM	42±1.68^{j}	0.68±0.01^{j}	0.45±0.00^{m}	0.22±0.01^{i}	0.15±0.01^{l}

* Means followed by the same letter are not significantly different at the 0.05 level of probability by LSD test. Data are the mean ±S.E. n=4

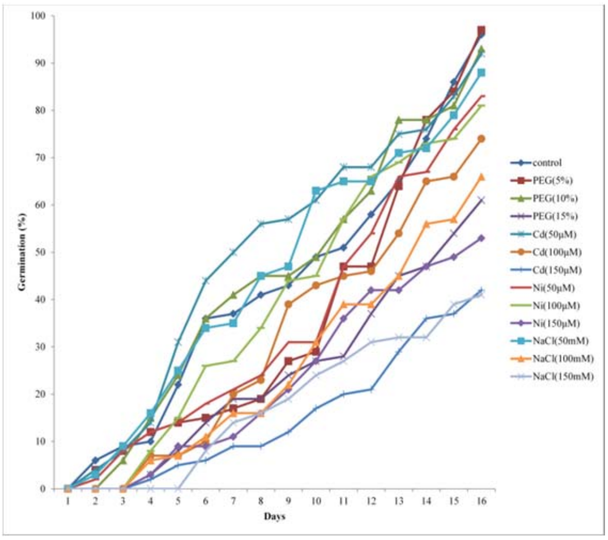

Figure 1) Time course of seed germination of *S. arvensis* as affected by PEG, NaCl, Ni, and Cd

Effect of PEG, Cd, Ni, and NaCl on Root and Shoot Growth: The results of root and shoot growth were presented in Diagram 1. A one-way ANOVA showed that the effects of PEG, CD, Ni, and NaCl concentrations on root growth differed significantly (F=141.653; p=0.000). All PEG and Ni treatments (50 and 100µM) significantly promoted the root growth as compared to the root growth of the control condition (p≤0.05). With increasing concentrations of Cd and NaCl, the root length significantly decreased (p≤0.05). We observed the maximum root length (59mM) in PEG (15 %) and the minimum root length in NaCl (150mM) with 11mM. In addition, the results showed that with increasing of PEG, Cd, and NaCl concentrations, the shoot length significantly decreased (F=241.127; p=0.000). Ni did not have an inhibitory effect on shoot length. The maximum inhibitory effect was in NaCl (150mM). The minimum (12mm) and maximum (73mM) shoot length was related to NaCl (150mM) and Ni (100µM), respectively.

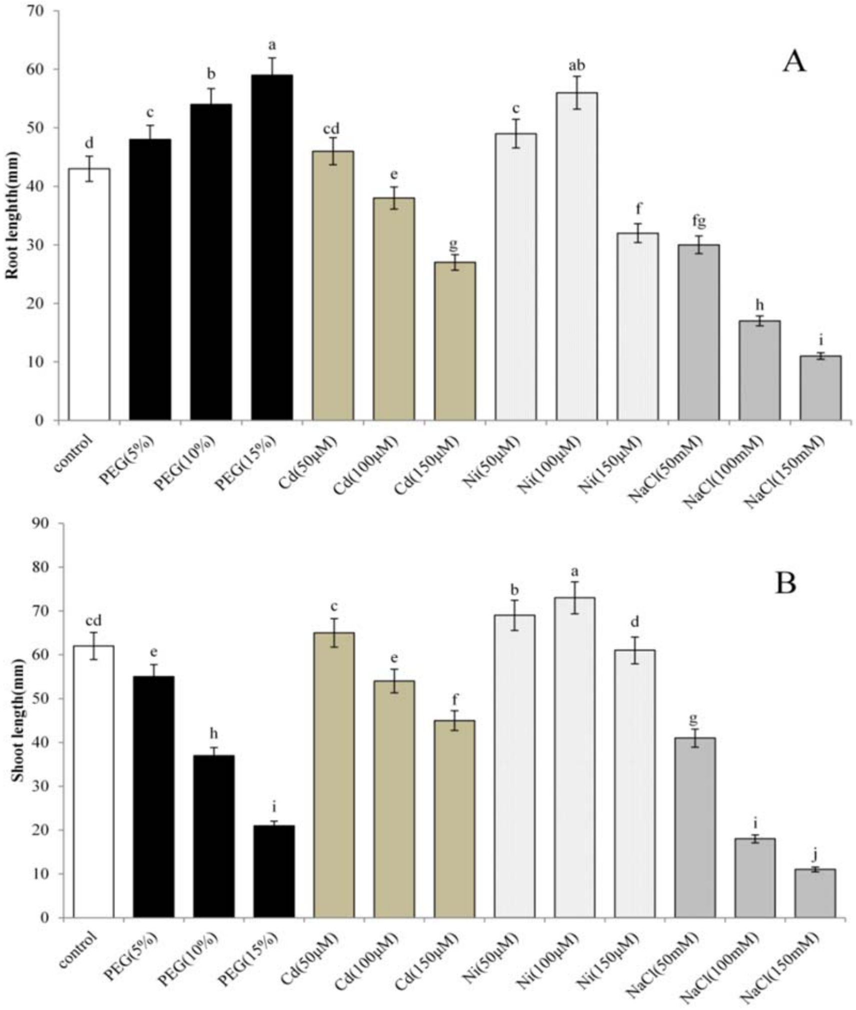

Diagram 1) Effect of different concentration of polyethylene glycol (PEG), heavy metals (Cd and Ni), and salinity (NaCl) on root length (A) and shoot length (B) of *S. arvensis*. (Different letters show significant differences between means at p≤0.05 [LSD test]).

Effect of PEG, Cd, Ni, and NaCl on Root and Shoot Fresh and Dry Weight: The effects of the concentrations of PEG, Cd, Ni, and NaCl on root and shoot fresh and dry weight of *S. arvensis* are presented in Table 1. The results showed that with increasing Cd and NaCl concentrations, root fresh and dry weight significantly decreased (Root Fresh Weight: F=1808.124; p=0.000; Root Dry Weight: F=194.623; p=0.000; Shoot Fresh Weight: F=6716.250; p=0.000; Shoot Dry Weight: F=765.940; p=0.000). The maximum values of root fresh and dry weight were recorded in Ni (100µM) with 2.42 and 0.81 g and the minimum of their values were observed in NaCl (150mM) with 0.68 and 0.22 g, respectively. Also, the results showed that PEG, Cd, and NaCl treatments significantly decreased the shoot fresh and dry weight ($p \leq 0.05$). The maximum of shoot fresh and dry weight was related to Ni (100µM) with 3.37 and 1.12 g, and the minimum shoot fresh and dry weight was recorded in NaCl (150mM) with 0.45 and 0.15 g, respectively.

Discussion

Research on plant responses under various abiotic stresses in the phases of seed germination and early seedling growth is necessary for completely understanding the traits in early life stages and, to a sure extent, in comprehending the internal deals for low recruitment of seedlings under natural conditions. Drought and salt stress conditions extremely affect germination of seeds, but the intensity of response and injurious effects of stress depends on the plant species.

In this study, osmotic stress conditions have enhanced seed germination rates of *S. arvensis*. Other studies have expressed that osmotic conditions enhance seed germination in Wheat [38], Neem [39], Asparagus [40], and Hyssop [8]. Also, the results showed that with increasing osmotic stress, the root length increased. As a rule of thumb, plants with greater root system are better able to endure drought conditions and roots of plants have an important role in plant survival in drought conditions.

Also, drought-resistant plant to drought stress condition depends on the development of root system and decreasing shoot growth. The results showed that the shoot length reduced with increasing osmotic stress, which can help plants in tolerating drought stress conditions.

In this research, with increasing NaCl concentrations, seed germination, root and shoot length, and root and shoot weights were decreased. In dry climate conditions, as a result of high evaporation rates and deficient leaching due to low precipitation, salt might accumulate in the soil and affect the plants. High levels of salts have toxicity effect on the embryo. In turn, this cause delayed and decreased seed germination [15]. Salinity decreases seed germination rate, root and shoot length, and root and shoot weights [18-19].

Also, our results showed that with increasing Cd concentration, seed germination, root and shoot length, root, and shoot weights were reduced. Generally, Cd is known as the upsetter component for several elements such as Ca, Mg, P, and K [41]. Cadmium restricts the root growth more than shoot growth. Although the mobility of metal ions in plants is various, generally the content of these ions in the root is higher than in the above-ground biomass. Cadmium enters the root at the first and, thus, it can damage to the plant root first [42]. The results showed that seed germination percentages decreased with increasing Ni concentration. The results showed that root and shoot length and root and shoot weights were increased in the 100µM concentration of this element. Nickel is an essential element for the urease enzyme. However, increasing Ni concentration in plant tissues has toxicity effects on plants [43]. In general, it can be expressed that root length and weights were more affected by the studied treatments in comparison shoot length and weights.

Conclusion

Germination conditions and the range of tolerance, under which seeds will germinate, differ with species, and depend on the environment, in which the plants usually grow. Environmental parameters such as high temperature, drought, salinity, and heavy metals have important effects on plant growth, development, production, and seed quality. Thus, understanding plant responses to environmental stresses can help select suitable plants in order to obtain sustainable products.

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