Effects of Abiotic Stress Conditions on Seed Germination and Seedling Growth of Medical Plant, Hyssop (*Hyssopus officinalis* L.)

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**ABSTRACT:** Plants in nature are continuously exposed to several biotic and abiotic stresses such as drought, salinity and exposure to heavy metals. The aim of the study was to determine the influence of heavy metals (Cd and Ni), osmotic stress caused by treatment with polyethylene glycol (PEG) and salinity (NaCl) on germination and seedling growth of *Hyssopus officinalis*, an important medicinal plant in the Lamiaceae. Germination rate decreased with increasing concentrations of the tested factors. Maximum germination (97%) was recorded in PEG (5%) treatment and the minimum percentage of germination was observed in NaCl (150 mM) with 58%. The maximum of root and shoot length were observed in Ni (100μM) with 80 and 113 mm respectively. In this study, osmotic stress has been improved seed germination of *Hyssopus officinalis*. In general it can be expressed that root length was more affected by the studied treatments in compared to shoot length.

**Key words:** abiotic stress, Germination, *Hyssopus officinalis*, Medical plant.

**INTRODUCTION**

*Hyssopus officinalis* is an important medical plant in the Lamiaceae. This species is widely used in the pharmaceutical industry (Jankovasky and Landa, 2002), and it is cultivated in some countries such as Russia, Spain, France, Italy and Iran. Despite its bitter taste, it used as a flavor enhancer in foods and sauces (Kazazi et al., 2007). The essential oil of this plant has anti-bacterial and anti-fungal properties (Jankovasky and Landa, 2002; Kazazi et al., 2007; Mazzanti, 1998). It used in traditional and modern medicine as an appetizer. *H. officinalis* also is used as a cure for digestive disorders, laryngitis, asthma, bronchitis, herpes, and to help heal wounds (Jankovasky and Landa, 2002).

During their lifecycle, plants experience a variety of abiotic stresses such as drought and low temperatures, heavy metals, UV and highlight, which can have a profound effect on viability, growth, morphology, and reproduction (Deborah vicuna et al., 2011). These stresses lead to significant reduction of agricultural products around the world. Soil contamination by heavy metals one of the main reasons of ecological and environmental degradation in large area (Raskin and Ensley, 2000). Various operations such as using of pesticides in agriculture, mining operations, transportation of untreated industrial waste, and metallurgical industry lead to the release of various heavy metals like Cu, Fe, Cd, Cr and Hg into the environment (Chandra et al., 2009). Accumulations of chemical environmental pollutants cause interruptions in ecological processes and energy chain such as chemical weathering and reduction in plant growth and energy production. It also causes physiological damage such as genotoxicity, impaired photosynthesis, reduced uptake and transport of mineral nutrients and growth retardation in plants (Babich and Stotzky, 1978; Nagajyoti et al., 2010).

Nickel is a heavy metal that is essential in small amounts for plant growth and development because it is involved in the structure of some important enzymes such as urease. Urea accumulates in leaves when nickel is deficient, and urease activity within the cell completely disappears resulting in the leaf tip necrosis (Taiz and Zeiger, 2007). Toxic and even fatality properties of nickel for humans, animals and plants in high concentrations have been found. In the culture medium, high concentrations cause a change in absorption of necessary elements, chlorosis,
reduced absorption of CO₂, irregularities in the exchange of gases, changes in water absorption and free radical production and production of any reactive oxygen that cause oxidative stress (Ali et al., 2009). Nickel is a metallic element that is easily absorbed by plant roots. It is moving in plant and its excessive accumulation in plants will reduce the value of plant products. Due to the toxic effects, high concentrations of nickel can inhibit plant growth (Zdeneck, 1999).

Cadmium is a very strong pollutant due to extreme toxicity at low concentrations and its high solubility in water. The availability of cadmium in soil depends on organic matter in soil, root exudates, presence of mycorrhiza, pH, and soil cation exchange capacity of the soil, temperature and concentration of other elements in the soil. Soils with Cd concentrations from 0.32 to 1 mM in the soil solution are moderately contaminated to highly toxic (Wagner, 1993). Cadmium alters nutrient absorption by plants by competing with potassium (K), magnesium (Mg), calcium (Ca), manganese (Mn), copper (Cu), zinc (Zn) and nickel (Ni) (Moreno et al., 1999). Cadmium has a negative effect on plant metabolism, such as decreased nutrient absorption, inhibition of photosynthesis through effect on chlorophyll metabolism and chloroplast structure, photosynthetic carbon metabolism enzyme, photosystem II activity and changes in metabolism of nitrogen. Cadmium causes the closing of stomata and reduces the amount of plant water in the long term. Cadmium leads to lower growth and lower biomass in plants. Damage to DNA and changes in RNA synthesis is the effect of Cd toxicity (Liang, 2005; Karantev, 2006; Popova, 2009).

Drought stress is one of the most restrictive of seed germination. Seed germination is negatively affected by unfavorable conditions of soil moisture due to lack of rainfall and irrigation (Mwale et al., 2003). At planting, inadequate moisture leads to irregular germination and seedling emergence, which influence establishment and performance of the species as a crop (Mwale et al., 2003; Okcu et al., 2005). In arid and semi-arid regions, water is usually one of the most important factors limiting crop production. Seed germination and emergence are critical stages for plant establishment and crop growth in arid and semi-arid regions and help determine standing crop density and yields (Hadas, 1976).

High levels of salt in the soil can often create limitations for agricultural production and development. The main factors that lead to this problem are the semi-arid climate and addition of salt by water that is used for irrigation. Soil salinity may lead to deleterious effects of on plant growth and development, physiological and biochemical in plants (Munns, 2002). These effects could be due to low osmotic potential of soil solution, specific ion effects and nutritional imbalances, or a combination of these factors (Marchner, 1995; Zalba and Peinemann, 1998). High levels of salt in the germination media can lead to a high osmotic pressure that prevents water absorption necessary for germination. High concentrations of salts have toxic effects on the embryo and can reduce or delay germination (Ramden, 1986). Salinity reduces germination, root length, callus size, coleoptile length and seedling growth (Lallu and Dixit, 2005; Ghannadha et al., 2005; Bera et al., 2006; Agnihotri et al., 2006).

Different stages of the plant life cycle, such as seed germination, seedling growth, vegetative growth, flowering and fruiting are adversely affected by non-biological stresses. In general, seeds and seedlings may have less tolerance than mature plants (Bhattacharya et al., 2012). The aim of the study was to determine the influence of heavy metals (Cd and Ni), osmotic stress caused by treatment with polyethylene glycol (PEG) and salinity (NaCl) on germination and seedling growth of Hyssopusofficinalis.

MATERIAL AND METHODS

Seeds of Hyssopusofficinalis were obtained from the National Seed Gene Bank, Karaj, Iran. To destroy seed-borne microorganisms, all seeds were sterilized by 10% sodium hypochlorite solution for 5 min and washed three times with sterilized distilled water. The seeds were placed in Petri dishes on two layers of filter paper. Each dish contained 50 seeds. The experiment used a randomized complete block design with four replicates per treatment. Treatments included i) control ii) PEG (5%, 10%, 15%), iii) NaCl (50, 100, 150 mM) and, iv) Cd⁺² (50, 100, 150μM) and v) Ni⁺² (50, 100, 150μM). The experiments were carried out in a programmed incubator at 25±2°C. Radicle emergence to a length of 2-5 mm was the criterion for seed germination (ISTA, 1966). Germination was recorded every day for 20 days, at which time the germination percentage was calculated. The seedlings were harvested after 20 days of incubation and root and shoot lengths were recorded.

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 test at 5% level was carried out to determine the statistical significance of the differences between means of treatments.
RESULTS

Seed germination was different at various levels of treatments. First germination was observed in PEG (5%) treatment and control on the third day. Seed germination began in the NaCl (50mM) treatment on the fifth day and Ni (50μM) and Cd (50μM) treatments on the sixth day, after which germination rates increased. The maximum germination (97%) was recorded in PEG (5%) and minimum germination (58%) was observed in NaCl (150 mM) (Fig. 1. A, B, C, D). Maximum length of root and shoot (80 and 113 mm, respectively) was observed in Ni (100μM). Root length increased with increasing PEG concentration, but it was reduced with increasing concentrations of Cd and NaCl. Also shoot length was reduced with increasing concentrations of PEG, Cd and NaCl(Fig. 2. A, B).

![Figure 1](image1.png)

Figure 1. A, B, C and D: Time course of seed germination of *Hyssopus officinalis* as affected by PEG, NaCl, Ni and Cd. Data are the mean ± S.E. n=4

![Figure 2](image2.png)

Figure 2. Effect of PEG, NaCl, Ni and Cd on plant growth (A. Root length, B. Shoot Length) of *Hyssopus officinalis*. Growth measured after 20 days of incubation. Data are the mean ± S.E. n=15.
DISCUSSION

Effects of salt and osmotic stress (PEG) on plants have been investigated in many studies (Hampson and Simpson, 1990; Falleri, 1994; Huang and Redmann, 1995; Katembe et al., 1998; Raza et al., 2006; Arves et al., 2013). In this study, osmotic stress has been improved seed germination rates of Hyssopusofficinalis. Other researchers have expressed that osmotic conditions improves seed germination in Soybean (Giudice et al., 1998), neem (Vanangamudi et al., 2000) and Asparagus (Bittencourt et al., 2004). Also the results showed that root length increased with increasing PEG concentration. As a rule of thumb, species with greater root length are better able to tolerate drought conditions. And plant roots play an important role in plant survival in drought conditions. Also drought-resistant species to drought stress depends on root system development and reduced shoot growth. The results showed that shoot length decreased with increasing PEG concentration, it can help to endure drought stress conditions by species. In this study, with increasing of NaCl concentration seed germination, root length and shoot were reduced. In dry climate may be due to high evaporation rates and insufficient leaching due to low precipitation, salt accumulates in the soil and affect the plants. High concentrations of salts are toxic effect on the embryo. It leads to delayed and reduced germination (Ramden, 1986). Salinity reduces seed germination percentage, root and shoot length (Bera et al., 2006; Agnihotri et al., 2006). The results showed that with increasing of Cd concentration, seed germination, root and shoot length were decreased. In general, Cd is known as disturbing element for several elements such as Ca, Mg, P and K (Das, 1997). Cadmium makes limit root growth more than shoot length. Although the various mobility of metal ions in plants but in general the content of these ions in the root is higher than in the above-ground tissues. In the most environmental conditions, cadmium enters the root first and so it can damage to the root first (Benavides et al., 2005). The results showed that the germination percentage decreased with increasing Ni concentration. The results showed that root and shoot length were increased in the 100 μM concentration of this element. Nickel is an essential component of the urease enzyme. However, when the Ni concentration in plant tissues increases, it can have toxic effects (Aydinalp and Marinova, 2009). In general it can be expressed that root length was more affected by the studied treatments in compared to shoot length. Environmental factors such as high temperature, drought, salinity and heavy metal have decisive effects on growth, development, production and plant seed quality. Thus, understanding the response of plants to environmental conditions is necessary.

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REFERENCE


