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STUDIES ON SEED GERMINATION AND SEEDLING GROWTH IN KALMEGH (*ANDROGRAPHIS PANICULATA* WALL. EX NEES) UNDER ABIOTIC STRESS CONDITIONS

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Abstract: The changing environmental conditions and increasingly heavy metal concentration in soil has been a major concern for growth of medicinal plants widely used in pharmaceutical and ayurvedic formulations. The need is to develop effective means to regenerate and grow medicinal plants under changing environmental conditions. The realization of this strategy requires an understanding of agronomic requirements of the medicinal plant species including the responses to variations in growth conditions on account of abiotic stresses. Present study is an attempt to determine the influence of abiotic stress viz., water-deficit (PEG), salinity (NaCl) and heavy metal (Cd), germination, seedling growth and seed vigour index (SVI) of Kalmegh (*Andrographis paniculata* Wall. ex Nees), an important indigenous medicinal plant of Acanthaceae family. Seed germination was enhanced by all the three factors tested i.e. PEG, NaCl and Cd; the degree of enhancement was found to be maximum in case of PEG. Seedling growth and SVI was suppressed by PEG, NaCl and Cd. Cadmium stress was most inhibitory.
Keywords, Kalmegh, Medicinal plant, Stress, Germination, SVI.

1. INTRODUCTION

Kalmegh (*Andrographis paniculata* Wall. Ex Nees) belonging to the family Acanthaceae has wide range of medicinal and pharmacological application. It is also known as ‘King of bitters’, Maha-tita or bhui-neem because of its similarity in appearance and bitter taste as that of neem (*Azadirachta indica* A. Juss) though it is much smaller in size (Niranjan et al. 2010). Kalmegh is one of the nineteen species of the genus *Andrographis,* which is indigenous to India. It is an active constituent in majority of Ayurvedic preparations and is official in the Ayurvedic Pharmacopoeia (Rammohan et al. 2011). It is distributed in tropical Asian countries having hot and humid climatic conditions but it can be cultivated in subtropical regions during the monsoon season (Niranjan et al. 2010; Kumar, 2011). The herb is having a preventive effect from many diseases, due to its powerful immune strengthening benefits

(Chauhan, 2009). The therapeutic activities of this plant are attributed to andrographolide and related diterpens i.e., deoxyandrographolide, 14- deoxy-11, 12- didehydro-andrographolide and neo-androrapholide (Bahn, 2006). The demand of Kalmegh is increasing day by day (Chauhan. 2009). The propagation of kalmegh generally occurs through seeds, inspite of several germination problems. The production of any crop heavily relies on quality of planting seeds and for producing good quality seeds it would be desirable to have information regarding germination and associated germination parameters like germination energy, germination period etc.(Kumar, 2011).

During their life-cycle, plants experience a variety of abiotic stresses. Among the major abiotic stresses that affect the plant growth and yield are water-logging, drought, high or low temperature, excess soil salinity, heavy metals, inadequate mineral nutrients in the soil and too little or too much light. They cause considerable (upto 80%) economic losses in agriculture. Water-deficit, salinity and heavy metal stress affect the water relations of a plant on the cellular as well as whole plant level causing damages and reduction in growth rate and development. Processes such as seed germination, seedling growth and vigour, vegetative growth, flowering and fruit set are adversely affected by abiotic stresses. In general, the seeds and seedlings may be less stress tolerant than adult plants. The plants respond in a species-specific manner to different stresses. Keeping this in view, the present study was an attempt to standardize germination parameters under varying abiotic conditions.

1. Materials and methods

2.1. Seed Source

The seeds of *Andrographis paniculata* Wall. ex Nees were obtained from Dr. Yashwant Singh Parmar University of Horticulture and Forestry, Nauni, Solan. The experiment was conducted during Aril-May in laboratory conditions of Shoolini University of Biotechnology and Management Sciences, Solan. The methodology adopted is described below.

2.2. Germination test

Seeds of *Andrographis paniculata* selected for uniformity (on the basis of colour and size), were surface sterilized with 0.1% $HgCl_2$ for 5 min. followed by thorough washing under tap water. Then, the seeds were soaked in distilled water (control) or in solutions of stated concentrations of the effector substances namely, i) PEG (5%, 10%), ii) NaCl (25, 50 mM) and, iii) $CdSO_4$ (50, 100μM) for 24 h at 25±2ºC. Thereafter, the seeds were transferred to petriplates lined with two layers of filter paper moistened with distilled water (control), PEG, NaCl and $CdSO_4$ solutions of same concentrations. The treatments were replicated six times and each petriplate contained 30 seeds. The seeds were allowed to germinate in an incubator at 25±2ºC under continuous illumination provided by fluorescent white light (PAR: 40 µmol m^{-2} s^{-1}). Emergence of 2-5 mm radicle was taken as seed germination (ISTA, 1966). Germination was recorded regularly until the final count.

2.3. Seedling growth & vigour: Seedling growth was measured after 30 d of incubation in terms of root and shoot length, seedling fresh weight and seed-vigour index (SVI). Seed-vigour Index (SVI): Germination percentage × Seedling length (cm). Experiments were performed in triplicate; each replicate comprised 15 seeds.

At the end of experiment, data was subjected to analysis of variance (ANOVA) and mean separation. The statistical analysis was done using GraphPad Prism® 5.2. The least significance difference (LSD) at 5% level was used to compare the means of different test parameters under different stress conditions.

1. Results and discussion

The seeds of *Andrographis paniculata* were subjected to the described germination conditions in order to determine the status of germination and the effects of different effectors namely, PEG, NaCl and Cd there on. The seeds of *A. paniculata* were non-dormant and started germinating within 4 d of incubation. Seeds responded differently to water-deficit (PEG), salinity (NaCl) and cadmium (Cd) treatments (Fig. 1). The percent germination and days to germination varied with stress conditions. The first emergence of seedling was observed on 4^{th} day in control, 5% PEG & 25mM NaCl, 6^{th} day in 10% PEG & 50mM NaCl, 8^{th} day in 50µM Cd and 14^{th} day in 100µM Cd. During the initial period (until 12 d in case of 50 and 14 d in case of 100µM) Cd treatment led to the suppression of seed germination but after that it was increased. The degree of increase was maximum in case of 50µM $CdSO_4$. Seedling growth was measured after 30 d of seed incubation for germination in terms of root, shoot length and seedling fresh weight. All stresses applied (PEG, NaCl, Cd) adversely affected the seedling growth (Fig. 2). In general, the root length was affected more than the shoot length.

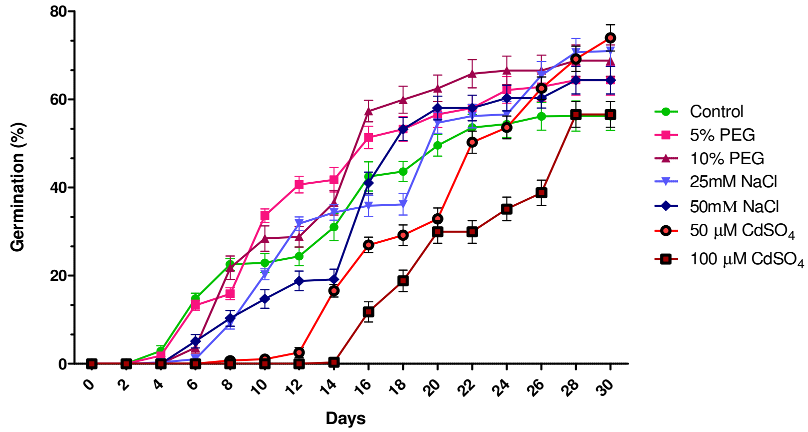
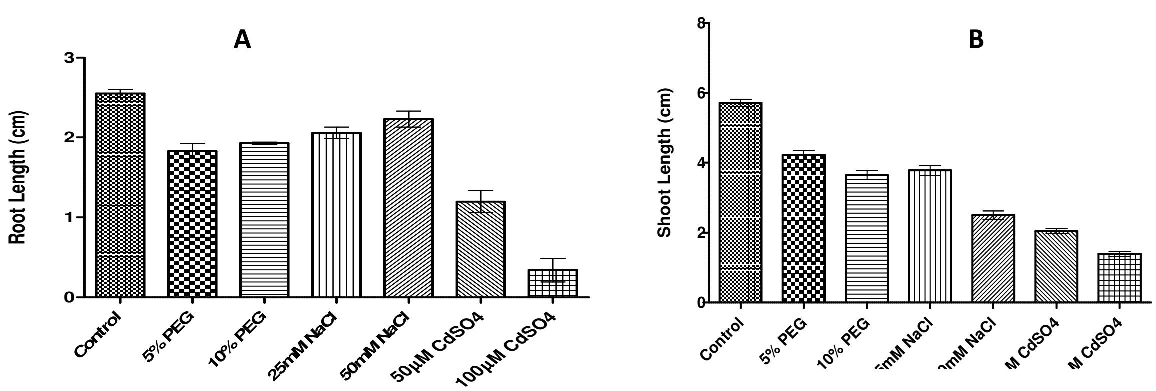


Fig. 1: Time course of seed germination of *Andrographis paniculata* as affected by PEG, NACl and Cd. Data are the mean ±S.E. n=6

The salt and osmotic stress (PEG) effects have been extensively studied in a wide range of plant species (Hampson and Simpson, 1990; Falleri, 1994; Huang and Redmann, 1995; Katembe et al., 1998; Raza et al., 2006; 2007). The osmotic conditions improved seed germination of soybean (Guidice et al., 1998), neem (Vanangamudi et al., 2000) and asparagus (Bittencourt et al., 2004). Under the dry climatic conditions, salt might accumulate in soil because of high evaporation demand and insufficient leaching of ions due to low precipitation. Cadmium is a non-essential element that affects plant growth and development (Sanita di Toppi and Gabrjelli, 1999). The seeds treated with Cd showed decreased seed germination at higher concentration. The root length of *A. paniculata* in PEG treatment increased with an increase in concentration.



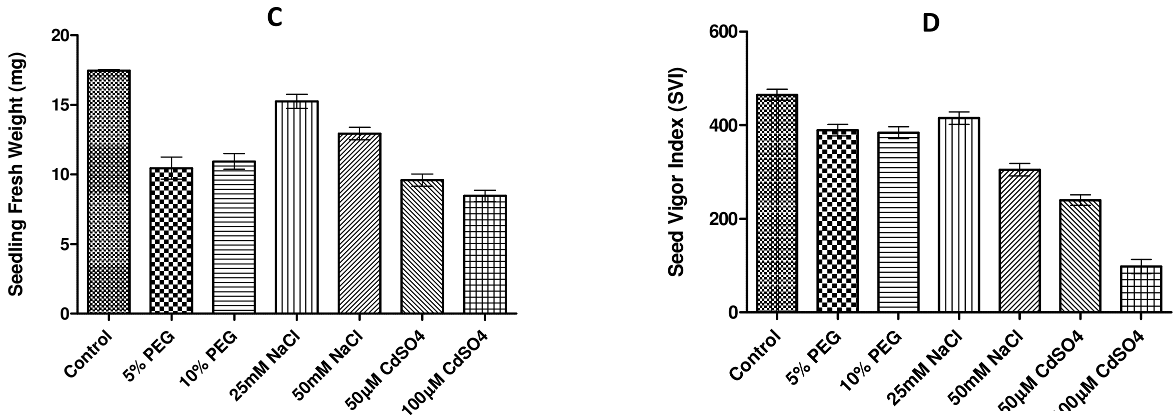


Fig. 2: Effect of PEG, NaCl and Cd on plant growth (A. Root length, B. Shoot Length, C. Seedling fresh weight & D. Seed vigour index of *Andrographis paniculata.* Growth measured after 30 d of incubation. Data are the mean ± S.E. n=15

This suggests that *A. paniculata* tolerate drought even at high concentration. It is known that species with longer root length are drought resistant (Leishman and Westoby, 1994). Roots play an important role in plant survival during periods of drought (Hoogenboom et al., 1987) and also drought resistance is characterized by an extensive root growth and small reduction in shoot growth in drought stressed conditions (Guoxiong et al., 2002). A detailed analysis of root-to-shoot ratio under stressful conditions would be useful. At 25 and 50mM NaCl concentrations, the reduction in shoot length was greater than in the root length. This effect has been shown earlier (Khan, 2004; Alam and Sheikh, 2007). In response to the cadmium stress, both root and shoot length and the seedling fresh weight & SVI of *A. paniculata* seedlings were inhibited in a concentration- dependent manner. In general, Cd has been shown to interfere with the uptake, transport and use of several elements (Ca, Mg, P and K) and water by plants (Das et al., 1997). Cd treatment induced a greater inhibition of root length than that of shoot length and seedling fresh weight. Despite the different mobility of metal ions in plants, the metal content is generally greater in roots than in the above-ground tissues (Ramos et al., 2002). In most environmental conditions, Cd enters the roots first, and consequently they are likely to experience Cd damage first (Sanita di Toppi and Gabrielli, 1999). In conclusion, the findings from present study reveal the differential sensitivity of seed germination and seedling growth of *A. paniculata* to the tested abiotic stresses namely, water-deficit, salinity and heavy metal stress. All the three stress factors tested increased the seed germination of *A. paniculata*. Unlike seed germination, seedling growth, measured in terms of root and shoot length, seedling fresh weight and seed vigor, was inhibited by the stress factors tested.

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