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Effect of Cadmium (Cd) stress on the germination and early seedling growth of Mustard seeds (*Brassica campestris* L.): deducing a dose – response relationship

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Abstract

Due to severe heavy metals contamination in agricultural soil worldwide, several studies specifically focused on the development of metal accumulating crop plants, which can maintain their optimum growth and production in contaminated soil. Mustard plants (*Brassica campestris* L.) are in generally considered as the hyper accumulator, can be grown advantageously for phytoremediation of the lands contaminated by industrial wastes. Therefore, the effect of varied cadmium (Cd) concentrations on the germination and initial growth of mustard seeds were investigated under controlled laboratory conditions. Results showed a differential response of mustard seeds under varied Cd concentrations. The lower levels of Cd affected the mustard seeds positively or neutrally; but the higher Cd levels showed significant adverse effect. We conclude that as mustard being a hyper-accumulator of several metals can only be grown at lower Cd levels for their optimum performance.

Key words: Cadmium stress; *Brassica campestris* L.; Germination; Early vegetative growth

Introduction

Over the last couple of decades, global agricultural production is seriously suffering from emerging load of pollution, especially the soil pollution. Mining, manufacturing, and the use of synthetic products (e.g. pesticides, paints, batteries, industrial waste, and land application of industrial or domestic sludge) can result in heavy metal contamination of urban and agricultural soils. Heavy metals also occur naturally, but rarely at toxic levels. Cadmium (Cd), a common heavy metal, is a wide spread pollutant with a long biological half life (Wagner, 1993; Chien *et al.*, 2002). Its addition to the agricultural soil is generally due to continuous application of Cd-rich phosphate fertilizers (Mc.Laughlin *et al.*, 1999). Cadmium is easily taken up by plants and enters food chain resulting in a serious health issue for human being (Hall, 2002). Cadmium has been shown to disturb photosynthetic activity and normal growth and development of plants (Wu and Zhang, 2002; Zhang *et al.*, 2002). Cadmium generates oxidative stress through overproduction of reactive oxygen species (ROS) in plants (Qadir *et al.*, 2004; Khan *et al.*, 2007). The Cd induced formation of ROS, superoxide anion (O_2^{-}), hydroxyl radical (OH^{-}) and H_2O_2 result in the damage of chloroplast (Mobin and Khan, 2007). One of the strategies is to develop or select the species or genotypes with high Cd tolerance, which was reported in some crops like sunflower (Li *et al.*, 1995), durum wheat (Penner *et al.*, 1995), rice (Hassan et al. 2005; Cheng et al. 2008) and barley (Wu and Zhang 2004). Some plant growth regulators (PGRs), such as jasmonic acid (JA), abscisic acid (ABA), gibberellins (GA) and salicylic acid (SA) also play important roles in activating the defense system of the plants in the adverse environment. Crosstalk between transduction pathways of external and/or internal signals in plant cells gives rise to the possibilities for techniques aiming to increase plant resistance to abiotic or biotic stress factors (Fujita et al. 2006).

Rapeseed-mustard (*Brassica sp.*) group of crops is the second most important oilseed crop after groundnut, contributing nearly 25 - 30% of the total oilseed production globaly (FAO, 2007). The crop is mainly grown in subtropical and tropical parts regions. Its total production in the world is 36.15 million tonnes from 22.94 million hectare land area. India with 5.39 million hectares land under cultivation and 6.20 million tonnes of production, ranks second and third, respectively in rapeseed-

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mustard scenerio in the world. Rajesthan is the largest producer of the crop followed by Uttar Pradesh, Haryana, Madhya Pradesh, West bengal, Gujrat and Assam (FAO, 2007). It can also be used for biodiesel production to replace a part of the fossil fuel. For biodiesel, it is acceptable that it contains a small amount of toxic metals to some extent. In contrast, a trace of toxic elements in food chain could result into serious health consequences. Oilseed rape can be used as a crop for phytoremediation because of its relatively higher biomass, which could extract more heavy metals from the soil as compared to other crop species, including mustard (*Brassica sp.*) (Ishikawa et al. 2006). Most of the Cd uptake occurs during the early vegetative stage in mustard (Ishikawa et al. 2006). However, little is known about the toxic effect of Cd on the early establishment of oilseed rape seedlings, and further, or how the growing plants cope with the toxicity through physiological and biochemical adaptations. In some rapeseed producing areas like Zhejiang province of China, rape seed-mustard is grown in rotation with rice.

Keeping these in mind, the present experiment has been designed to understand the effect of induced Cd-stress on the germination and early seedling growth of mustard seeds. We hope that the obtained results will definitely help farmers and fellow researchers to prepare a future road map for mustard cultivation in Cd - rich / polluted soil.

MATERIALS AND METHODS

Plant Material

One high yielding genotype of rapeseed-mustard seeds (*Brassica campestris* L. cv. Aashirwad) with medium life span were selected for the present experiment. This variety was developed by mating involving cvs. Krishna and Vardan. The life span of this genotype is 125 days and is suitable in late sown conditions. This has a yield potential of 1, 800 – 2, 500 kg ha^{-1} and is moderately resistant at leaf and pod stages for *Alternaria* blight (*Alternaria brassica; Alternaria brassicicola*) and resistant for white rust. The seeds contain approximately 40% w/w oil ratio. The seeds were collected from the HOD, Department of Genetics and Plant Breeding, Institute of Agricultural Sciences, Banaras Hindu University, India.

Experimental Design

Obtained mustard seeds were surface sterilized with 0.5% (v/v) sodium hypochlorite solution and bedded on the double layered sterilized Whatman paper soaked in the $Cd(NO_3)_2$ solutions of different Cd_2^+ concentrations (0, 5, 10, 20, 40, 80, 100, and 150 ppm) in petri-dishes and then incubated at 25 ± 2°C. All the experimental sets were supplemented with minimal nutritional support in the form of Hoagland solution (contains macro elements – KH_2PO_4, KNO_3, $Ca(NO_3)_2$, $MgSO_4$, $Fe(NO_3)_2$, and micro elements – H_3BO_3, $MnCl_2$, H_2MoO_4. pH was adjusted at 6.8 - 7). The experiment was continued for a period of 144 h.

Plant Response Analysis

Germination assay

For germination assay, we mainly considered a seed as a 'germinated seed', where a prominent radical emergence occurred after proper incubation. All the experimental sets were continuously evaluated till 144 h, with an interval of 24 h for the germination test. The germinated seeds were indexed and documented properly till the completion of the experiment.

The germination percentage was calculated as follows –

Germination percentage = (Total no. of germinated seeds / Total no. of seeds) × 100

Growth parameters

For early vegetative growth of germinated mustard seeds, we mainly assessed two major growth parameters – i. shoot length, and ii. root length. As till the end of experiment, we could only obeserved three leaves including two cotyledonary leaves (evident in Figure 2) as the highest number of leaves in any experimental set. So, we decided not to add the number of leaves as a growth parameter. At 144th h after sowing of all the seeds, the number of established seedlings was counted and documented. All the seedlings were drawn from each treatment for the measurement of root lengths and shoot heights.

Statistical Analysis

All the obtained results were subjected to one-way and two-way ANOVA for assessing the significance of quantitative changes in germination and growth parameters due to different Cd-treatments on mustard seeds. Duncan's multiple range test was performed as post hoc on parameters subjected to ANOVA (only if the ANOVA was significant). All the statistical tests were performed using SPSS software (SPSS Inc., version 16.0).

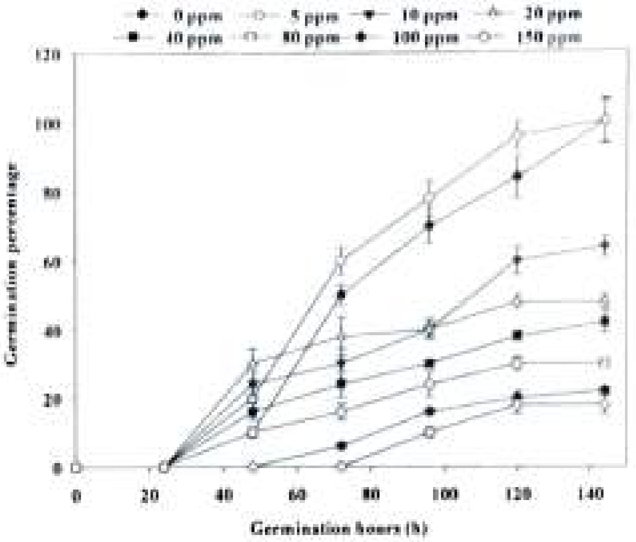

Figure 1. Effect of different Cd levels (ppm) on the germination percentage of mustard seeds (*Brassica campestris* L.) at different germination hours (h). Values represent mean ± SE.

RESULTS AND DISCUSSION

Over the last couple of decades, global agriculture is severely suffering from several crises, amongst which heavy metal contamination of soil is of serious concern. From the viewpoint of successful crop production in contaminated environments, the knowledge of plant growth sensitivity stages has immense significance. Different plant species / genotypes exhibit variable responses to different metals at various growth stages. Like, the initial growth of *Arabidopsis thaliana* seedling was more sensitive to heavy metals like - Hg_2^+, Pb_2^+, Cu_2^+ and Zn_2^+, in comparison to seed germination. However, Cd inhibited both of these processes at similar concentrations (Li et al. 2005). Till date, literature provides little information for the toxicity effect of Cd on mustard (*Brassica campestris* L.) at the germination and / or early vegetative growth stage. Meng *et al.* (2009) reported a varied response of mustard seed germination under graded Cd concentrations. The results of the present study also pointed towards similar direction. We found that lower concentrations of Cd, like - 5, 10 and 20 ppm, significantly promoted the germination percentage of mustard seeds at early germination hours, but not all of them carried the similar effect till the later stages (Figure 1). Some of the previous workers suggested that as during initiation of germination, generally roots transported Cd rapidly to the shoot parts; so, the lower Cd stress might activate the initial defense mechanism of root / seed system and induce the germination process at lower Cd levels (Meng et al. 2009). Obtained results also showed that the effect of 5 ppm Cd was better or almost similar, i.e. – with non significant differences, to control set (0 ppm Cd) (Figure 1 and 3). Even the concentration of 10 and 20 ppm showed significant positive effect on seed germination at initial germination hours, but not in later phases (Figure 1 and 2). Two way ANOVA also showed significant effects of Cd concentrations and germination hours on the germination of mustard seeds (Table 2). Some of the previous researchers have reported that several crops appeared to be capable of tolerating more severe Cd stress, as sorghum could tolerate Cd stress up to 500 ppm in the medium at seed germination. However, at concentrations above 1000 ppm Cd, the seed germination was affected adversely with a complete cessation of seedling growth, caused by the inhibition of hydrolysis of carbohydrate reserves and translocation of hydrolyzed sugars (Kuriakose and Prasad 2008). Cheng et al. (2008) observed that rice was able to tolerate Cd stress up to 250 ppm during seed germination. Seed germination was even significantly enhanced for most of rice genotypes given Cd concentration under 500 ppm in the medium. But higher Cd levels inhibited germination drastically.

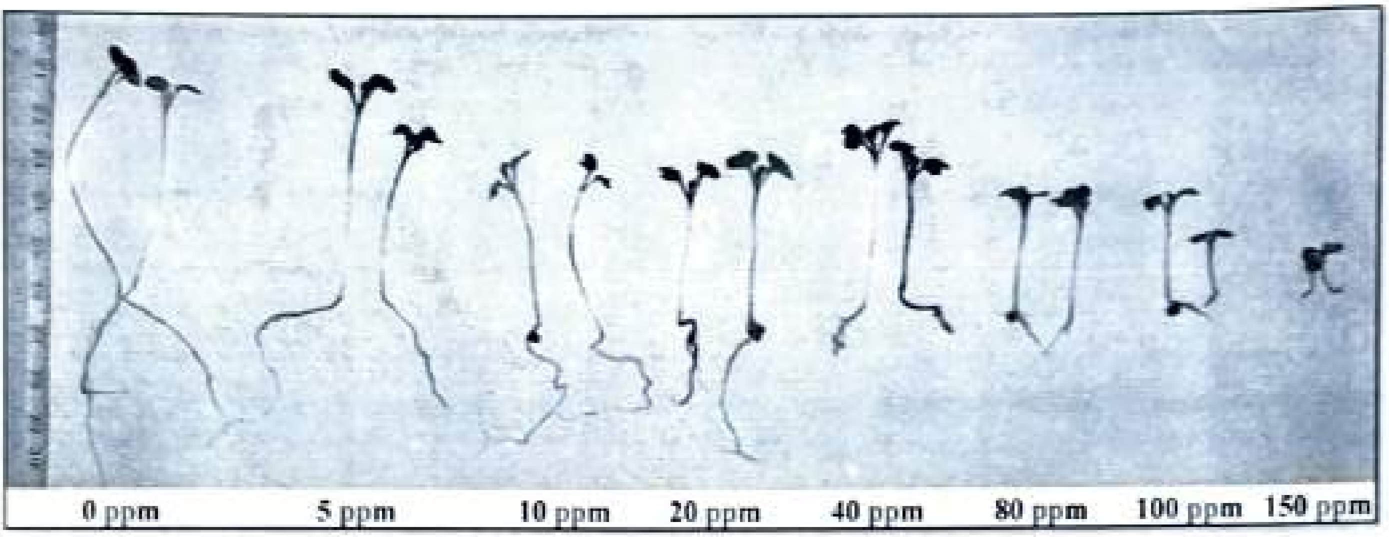

Figure 2. Effect of different Cd levels (ppm) on the seedling development of germinated mustard seeds (*Brassica campestris* L.) at 144 h of germination.

The next stage of germination, i.e. – seedling growth of germinated seeds of mustard showed a prominent gradient response to varied Cd concentrations (Table 1 and Figure 2). Though both the shoot and root lengths were observed best at control set (0 ppm Cd); but the concentrations up to 20 ppm also showed considerable positive impact on seedling growth (Figure 2). Meng *at al.* (2009) also reported similar trends in seedling growth of mustard seeds at different Cd concentrations. However, in that study they found that some growth regulators like – jasmonic acid, abscisic acid and gibberellins significantly help mustard seedlings to alleviate Cd stress. Though, it is obvious that plants absorb Cd from soil / medium through roots, and transport it to shoots. But in present study we found that higher amount of Cd in medium can suppress the root growth, in comparison to shoot growth (Figure 2). Two way ANOVA also depicted significant negative effect of Cd concentrations on both shoot and root growth of mustard seedlings. Anjum *et al.* (2008) reported that Cd stress can significantly inhibit both the photosynthetic rate and net photosynthesis, which should be resulted in

Table 1. Shoot and root length of germinated mustard (*Brassica campestris* L.) seedlings at different levels of Cd at 144 h of germination

Cd concentrations (ppm)	Shoot length (cm)	Root length (cm)
0	6.2 ± 0.08 ^{a}	5.6 ± 0.04 ^{a}
5	5.8 ± 0.06 ^{ab}	3.4 ± 0.08 ^{b}
10	4.2 ± 0.02 ^{c}	3.2 ± 0.07 ^{bc}
20	3.9 ± 0.04 ^{d}	2.9 ± 0.02 ^{bc}
40	3.6 ± 0.06 ^{de}	1.2 ± 0.04 ^{d}
80	2.2 ± 0.04 ^{f}	0.8 ± 0.01 ^{de}
100	1.6 ± 0.01 ^{fg}	0.6 ± 0.01 ^{de}
150	0.9 ± 0.01 ^{h}	0.1 ± 0.08 ^{f}

Table 2. Results of two-way ANOVA showing level of significance for germination percentage shoot length and root length of mustard seeds (*Brassica campestris* L.) at different levels of Cd.

Parameters	Cd concentrations	Germination hours	Cd concentrations Germination hours
Germination percentage	***	***	**
Shoot length	***	**	ns
Root length	***	***	ns

Level of significance: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; ns : not significant.

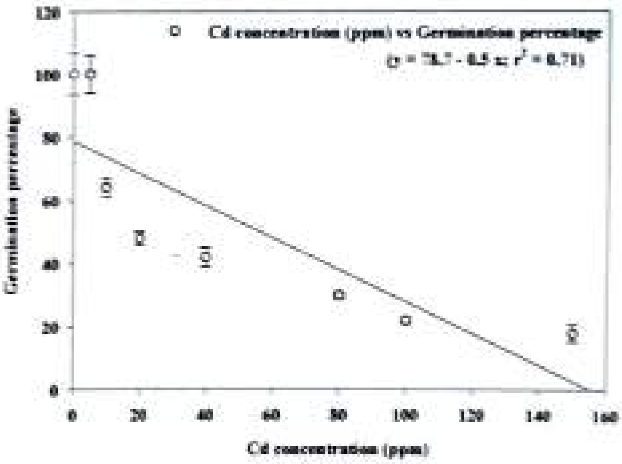

Figure 3. Correlation between Cd concentrations (ppm) and germination percentage of mustard seeds (*Brassica campestris* L.) at 114 h of germination.

decrease in shoot and root growth, and total biomass of mustard seedlings (Figure 2). While correlating the Cd concentrations with germination percentage of mustard seeds, we found an obvious negative correlation (Figure 3) between both of them. But the interesting observation was that the Cd levels – 5 and 10 ppm can able to maintain the germination percentage more than 50% till the end of experiment (Figure 3).

CONCLUSION

Cadmium being a major heavy metal is toxic to plant's growth and development. In present study also we found the similar effect of varied Cd concentrations on germination and initial vegetative growth of mustard seeds. However, obtained results showed that especially mustard seeds can tolerate a Cd levels, though lower than 20 ppm in medium, and growth positively. This can be useful for cultivating mustard plants at Cd rich soils.

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