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Effect of Cadmium on Growth, Nitrogen Metabolism, Photosynthesis and Yield of Soybean

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ABSTRACT: *A field study was conducted to study the effects of soil applied cadmium chloride concentrations (25, 50, 75 and 100 mg kg-1 of soil) on growth of above/belowground parts, biomass accumulation, nitrogen metabolism, photosynthetic performance and yield of soybean var. JS 335. All the parameters studied were decreased progressively with increasing Cd concentrations in soil. Higher cadmium concentrations especially at 75 and 100 mg Cd kg-1 soil, severely inhibited the leaf area, root length, plant and root dry mass, number and fresh weight of root nodules and significant decline in photosynthetic pigments, net photosynthetic rate (*Pn*), stomatal conductance (*gs*), rate of transpiration (*Tr*), nitrate reductase activity and leghemoglobin and hemechrome content drastically as compared to the controls. Thus high Cd concentration perturbs the soybean growth drastically by interfering with the photosynthetic performance and disrupting the coordination between carbon and nitrogen metabolism. Thus Cadmium caused remarkable decrease in carbon and nitrogen fixation which caused the reduction in biomass and rate of photosynthesis it ultimately results in reduction of yield of soybean.*

Key words: *Cadmium, hemechrome, leghemoglobin, nitrate reductase, photosynthesis, yield.*

INTRODUCTION

The problem of heavy metal pollution is increasing throughout the world. In many parts of the world, heavy metal contamination had influence on the biosphere [1]. One of the major abiotic stresses that cause environmental pollution in recent decades is heavy metal stress. Cadmium (Cd) is a toxic pollutant primarily released into agricultural environments via human activities [2] and negatively affects the plant growth. It exists at low concentrations in most soils under natural conditions. It enters the soil with phosphorus fertilizers, sewage sludge and air pollutants.

Cadmium is added to the environment by different sources and in the environment it persistent for a long time; through plants, it comes into the food chain and threatened the ecosystems [3]. Cadmium is taken up by the plant roots and loaded into the leaves through the phloem and can be accumulated in all parts of the plants [4]. Thus, instead of just reducing the crop productivity and quality [5], it causes a severe health risk to mammals and humans [6].

In plants the increasing amount of cadmium in the environment affects various physiological and biochemical processes [7]. John *et al.* [8] found that the presence of excessive amounts of Cd in soil usually bring out many stress symptoms in plants, such as reduction of growth, especially root growth, disturbances in mineral nutrition and carbohydrate metabolism and may thus strongly reduce biomass production. Reductions in both biomass production and nutritional quality have been observed in crops grown on soils contaminated with moderate levels of heavy metal [9]. Even at low concentration Cd inhibits plant growth and disturbs photosynthesis, sugar metabolism, sulphate assimilation and several enzyme activities [7,10].

Early studies of the effects of heavy metals on nitrogen fixation by legumes found little evidence that symbiotic N_2-fixation was sensitive to heavy metal toxicity [11,12]. Soybean (*Glycine max*) is an important economic crop that has been reported to be sensitive to the effects of Cd [13]. There are many reports on the effect of heavy metals on the morphological and biochemical characteristics of soybean [14]. The effect of heavy metal on the root nodule formation, leg-hemoglobin and hemechrome content in root nodules and nitrate reductase activity in leaves of soybean

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crop plants attracted little attention. The objective of present investigation was to study the effects of heavy metal Cadmium stress on nodulation, N_2-fixation capabilities of root nodule through the changes in leghemoglobin and hemechrome content of the root nodule and nitrate reductase activity in leaves of soybean along with growth, photosynthesis and yield using Cadmium (Cd) as a model for toxic substances.

MATERIAL AND METHODS

Seeds of soybean variety JS-335 were obtained from the Directorate of Soybean Research, Indore. A completely randomized block design experiment was arranged on the terrace of Department of Life Sciences, Devi Ahilya University, Indore, India during November'2013 to Febuary'2014 under the ambient environmental conditions. The seeds of soybean var. JS 335 were treated with recommended fungicides viz Bevistin and Diathane M at 2gm/kg seeds and then inoculated with slurry of *Rhizobium Japonicum* at 3g/kg seeds before sowing. The seeds of soybean were sown in plastic bags (34 cm H x 34 cm B) filled with 5 kg of soil containing sandy loam soil and farmyard manure (6:1 v/v). Soil in the selected plastic bags was added and mixed thoroughly with Cadmium ($CdCl_2$ -0, 25, 50, 75 or 100 mg $CdCl_2$ kg^{-1} of soil) and watered on alternate days.

GROWTH ANALYSIS

Aboveground growth parameters

At crop maturity the 15 plants (five plants from each replicate) were randomly selected to measure the plant and total dry weights. Plant height was measured from the soil level to shoot tip and total biomass accumulation was mesearured after oven drying of whole plant parts at 60°C for 72 h. The area of third trifoliate leaf was measured manually using a graph sheet, where the squares covered by the leaf were counted.

Below ground growth parameters

The below ground growth parameters like- root length, root biomass, number of root nodules and nodule fresh weight per plant were taken in soybean plants at 45 days after emergence of the seedlings. Nodules on each root were counted carefully and recorded per plant and nodule weight was recorded as gm/plant for all treatments. Roots were taken out carefully, washed and the root length was measured against a cm scale. For root biomass, plant roots with nodules were dried at 60°C for 72 hours and weighed.

NITROGEN METABOLISM

Extraction and estimation of Leghemoglobin (Lb) content

Leghemoglobin (Lb) was extracted from the root nodules of 45 day old soybean plants and measured by the method of Jun *et al.* [15] in all the treatments.

Heme concentration in leghemoglobin was measured by pyridine hemechromogen assay as described by Appleby and Bergerson [16].

Determination of nitrate reductase (NR; E.C. 1.6.6.1) activity

Nitrate reductase (E.C. 1.6.6.1) activity in the third trifoliate leaf of soybean was determined by the intact tissue assay method of Jaworski [17].

PHOTOSYNTHETIC PERFORMANCE

Determination of chlorophyll

Photosynthetic pigments were extracted from the leaves (fully opened matured third trifoliate leaves at 45 DAE) of each treatment with 80% acetone. The extract was centrifuged at 10,000 rpm for 10 min. Absorbance at 663, 646 and 470 nm was recorded. The amount of total chlorophyll was quantified using the formulae of Wellburn and Lichtenthaler [18].

Gas exchange parameters

Gas exchange parameters-CO_2 assimilation (*Pn*), intracellular CO_2 concentration (*Ci*), stomatal conductance (*gs*) and rate of transpiration (*Tr*) were recorded in each treatment by using a portable Infra Red Gas Analyser (LI-6200, LICOR Inc., Lin coln, USA) in intact plants grown in under field conditions at midday between 11.00 and 12:00 at 45 DAE in each treatment.

Crop Yield

All yield parameters were measured at harvest maturity (120 DAE) in all the treatment. Yield parameters including number of pods/plant, number of seeds/plant and seed weight/plant have been taken at the crop maturity in three replicates of five plants each.

RESULTS

Effect of Cadmium on growth

Effect of different concentrations of Cadmium on plant growth showed reductions of plant growth, leaf area and biomass accumulation as shown in Figure 1 and 2. Retarded development in Cd-treated plants compared to the controls was observed. It showed a concentration response, the plant growth and development was decreased as the concentration of Cd was increased (Fig. 1, 2). In the presence of 100 mg Cd kg^{-1}soil, significant reduction was found in plant height (P<0.0001), and a marked decrease of 78% in leaf area was observed also at 100mg Cd kg^{-1} soil. (Fig. 1A,B). Total biomass accumulation was drastically reduced by 78% and 81% at 75 and 100 mg Cd kg^{-1}soil (Fig.1C).

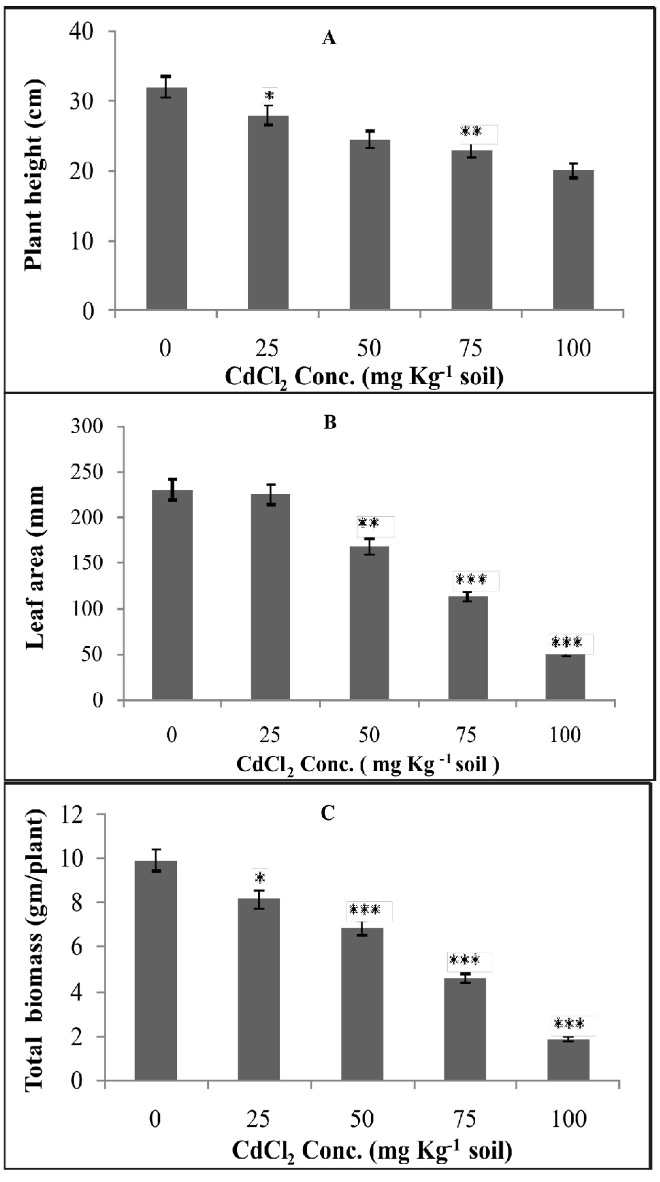

Figure 1: Effect of different concentrations of Cd (0, 25, 50 or 100 mg Cd kg^{-1} soil) on (a) plant height, (B) leaf area and (C) total biomass accumulation of soybean plant. The vertical bar indicates ±SE for mean. The values are significantly different from (*P <0.05, ***P <0.001) from control (Newman-Keulis Multiple Comparison Test).

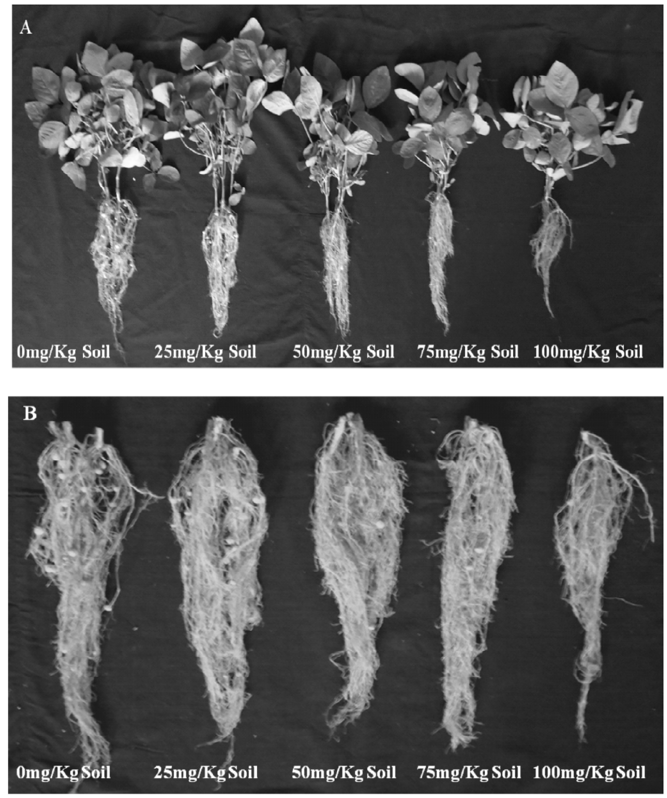

Figure 2: Photographs showing effect of different concentrations of Cd (0, 25, 50 or 100 mg Cd kg″1 soil) on (A) plant growth and plant biomass and (B) root growth and root biomass of soybean plant.

Compared with the non-treated soybean plants, root length and root biomass in soybean was significantly suppressed at 100 mg Cd kg^{-1}soil (Fig. 3 A,B), number of nodules and nodules fresh weight was noticeably decreased at all the concentration from 25 to 100mg Cd kg^{-1} soil (Fig. 3 C,D). The magnitude of the reduction was increased with the increase in the concentrations of Cd (Fig. 3 C,D).

Effect of Cadmium on Nitrogen Metabolism

The absorption of leghemoglobin (from 1.25 g of root nodules) isolated by ammonium sulphate fractionation (50 to 90% Pellet) is shown in Figure 4A. The higher amount of leghemoglobin was present in the plants grown under control conditions as compared to those plants grown under soil supplemented with different concentrations of Cadmium (25 to 100 mg Kg^{-1} soil). Lb content decreased with the increasing concentrations of Cd. There was 20% to 300% reduction was observed at different concentration of cadmium from 25 to 100 mg Cd Kg^{-1} soil (Fig. 4A). Heme concentration in leghemoglobin of the root nodules was measured by the pyridine hemochromogen assay. A remarkable decrease in the hemchrome content by increasing concentrations of Cd was observed in the root nodules of soybean (Fig. 4B).

The Nitrate reductase (NR), which catalyses the reduction of nitrate to nitrite, was also decreased with the increasing concentration of Cd. It caused 33% decrease in NR activity at the highest concentration of Cadmium i.e.100 mg Cd Kg^{-1} soil (Fig. 4C).

Effect of Cadmium on Photosynthetic Performance

The photosynthetic pigments Chl *a*, Chl *b*, total Chl and carotenoids were significantly decreased by the presence of Cd in the soil at all the concentration of Cd used in the present study but the magnitude of reduction in all the pigments were more at higher concentration of Cd used as compared to the lower concentrations of Cd (Fig.5). The decrease in amount of total Chl was due to the significant decreases in Chl *a* and Chl *b* both, but the extent of reduction was more in Chl *b* rather than Chl *a* in the presence of Cd in the soil (Fig. 5).

A dramatic decrease in the net photosynthesis rate (*Pn*) was observed in the leaves of soybean plants grown in the Cd supplemented soil (Fig. 6A). This was concomitant with a decrease in stomatal conductance (*gs*) and rate of transpiration (*Tr*) (Fig. 6 B, C) and the intercellular concentration of CO_2 (*Ci*) was increased slightly (12%) at the higher concentrations of Cd (Fig. 6D).

Effect of Cadmium on Crop Yield

Yield attributes like number of pods per plant, number of seeds per plant and seed weight per plant were remarkably decreased with the increasing concentrations of Cd supplementation in the soil. The maximum reduction of 84% in number of pods per

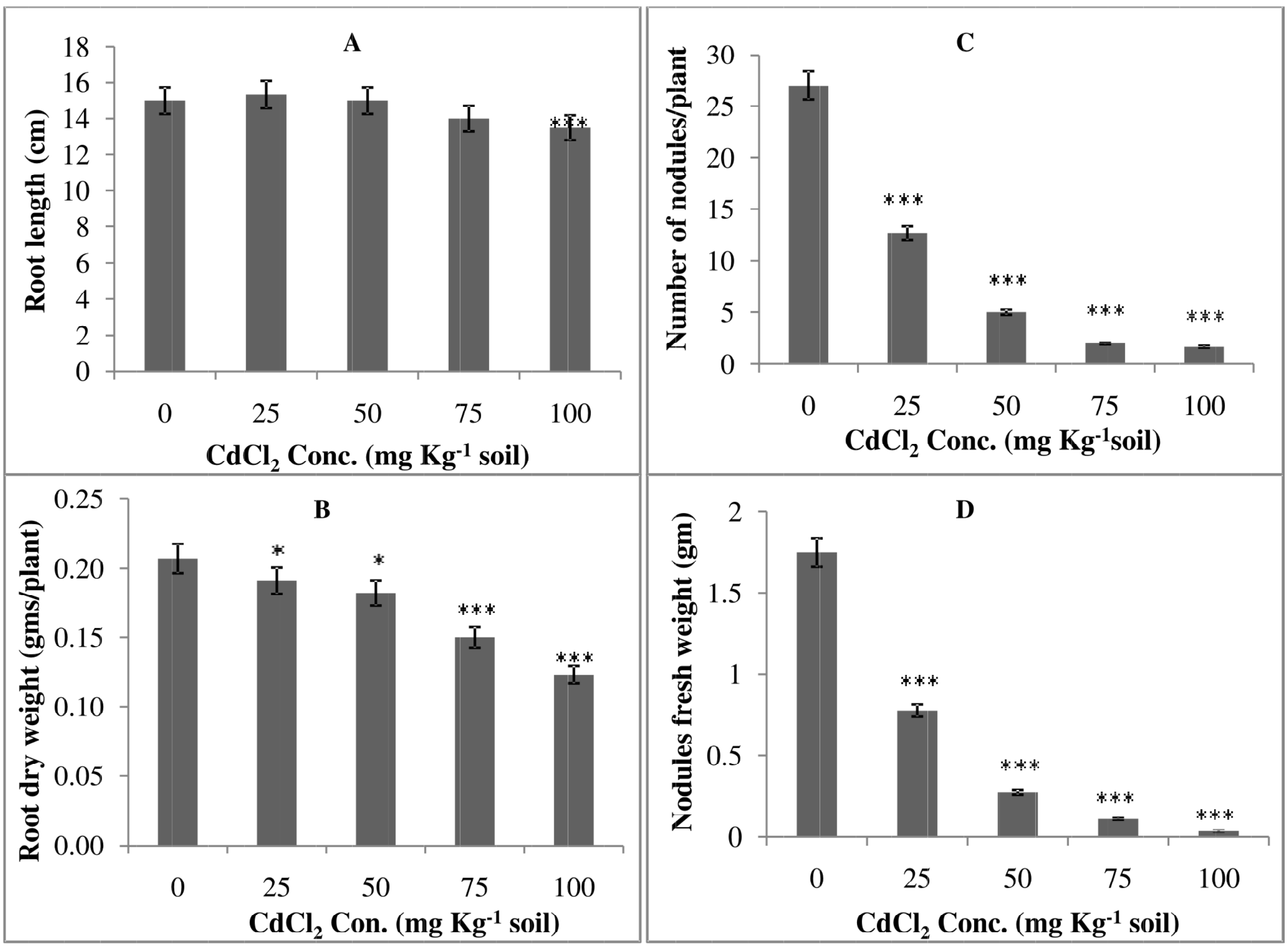

Figure3: Effect of different concentrations of Cd (0, 25, 50 or 100 mg Cd kg^{-1} soil) on (A) root length, (B) root biomass (C) number of root nodules and (D) nodules fresh weight of soybean var.JS-335. The vertical bar indicates ±SE for mean. The values are significantly different from ($^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$) from control (Newman-Keulis Multiple Comparison Test).

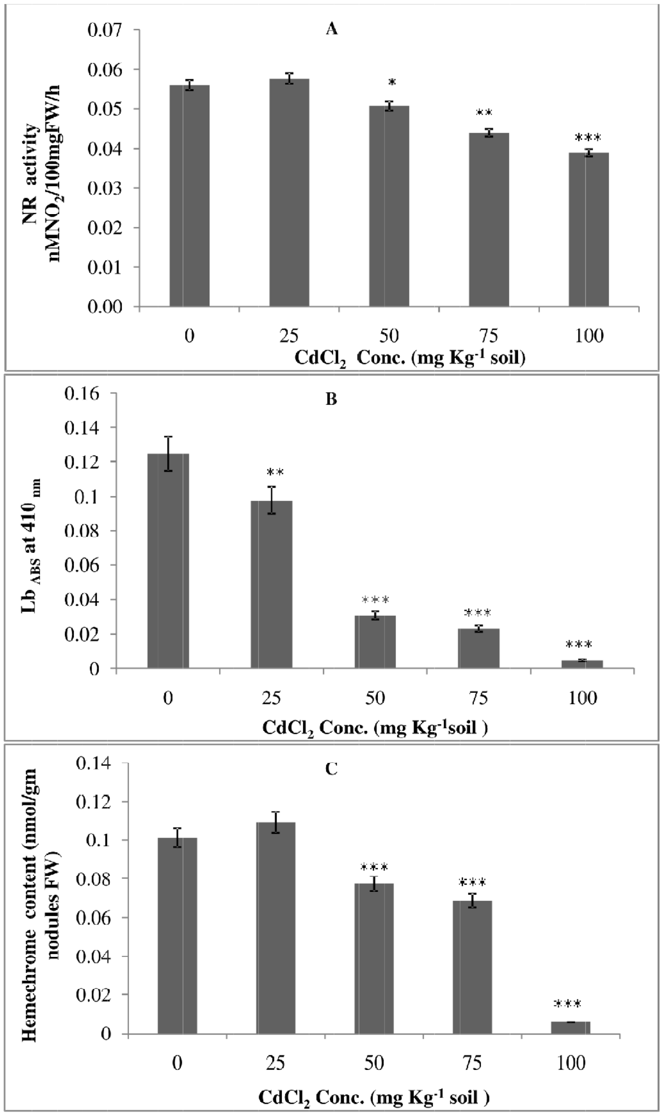

Figure 4: Effect of different concentrations of Cd (0, 25, 50 or 100 mg Cd kg^{-1} soil) on (A) nitrate reductase activity (B) leghemoglobin content in root nodules and (C) hemechrome content in root nodules of soybean var. JS-335. The vertical bar indicates ±SE for mean. The values are significantly different from ($^{*}P <0.05$, $^{***}P <0.001$) from control (Newman-Keulis Multiple Comparison Test).

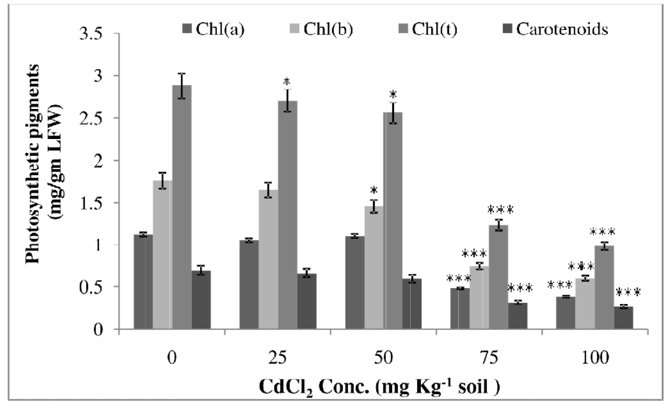

Figure 5: Effect of different concentrations of Cd (0, 25, 50 or 100 mg Cd kg^{-1} soil) on photosynthetic pigments *Chl a*, *Chl b*, total *Chl* and Carotenoids in the leaves of soybean var. JS-335. The vertical bar indicates ±SE for mean. The values are significantly different from ($^{*}P <0.05$, $^{**}P <0.01$, $^{***}P <0.001$) from control (Newman-Keulis Multiple Comparison Test).

plant; 87% in number of seeds per plant and 84% in seed weight per plant was recorded in plants grown under Cd supplemented soil at 100 mg Cd Kg^{-1} soil (Fig. 7 A,B,C).

DISCUSSION

Most commonly found metal in soil is Cadmium, which limits the crop productivity worldwide as this metal tend to accumulate within plant organs and negatively interfere with essential physiological processes [19]. Our research has clearly showed that Cadmium produced a significant growth inhibition, decline of physiological and biochemical activities as well as the yield of soybean plants. Both above and below ground growth parameters were decreased progressively with increasing Cd concentrations in soil and significantly higher reductions were observed at higher Cd concentration used in the present study. Thus toxic effects on plant growth were observed at higher doses of Cd treatment, although lower concentration, i.e. 25 mg Cd kg^{-1} of soil showed stimulatory effects in root length, nitrate reductase activity and hemechrome content of soybean plants. Furthermore the result of the present study indicates that reduction in all the measured parameters was directly proportional with the increase of metal concentration in the soil.

The dramatic decrease in the biomass production of soybean plants fed with Cd in the present study may be a consequence of a reduced uptake and transport of several essential nutrients within the plant [20]. In several plants, previously it was reported that Cadmium at higher doses reduced the growth [21,22,23]. These studies suggested that abnormal transport of some essential nutrients such as Zn, K and Fe are responsible for reduced growth of plants under Cd stress. Excess amount of Cd caused the deficiency of Zn, it might be a reason of reduced growth as Zn deficiency is reported to be involved in the reduction of auxin through its involvement in the synthesis of tryptophan, a precursor of auxin [24]. Cd treatment caused the reduction in root length in bean [25], *Solanum melongena* [26] and alfalfa [27], these results are similar to our results.

The nitrogen requirement of legumes can be met by both inorganic N assimilation and symbiotic N_2

fixation, and in practice, legumes obtain their N through both processes. Both nitrate uptake and assimilation seems to be affected by the presence of Cd in the present study as it is evident by decrease in Lb and hemechrome content in root nodules of soybean and decrease in NR activity in leaves of soybean in the presence of Cd in soil. The decrease of NR activity in Cd treated plants could also reflect a decrease in photosynthesis, since sugars are essential for NR expression [28,29] or during Cd stress treatment the toxic oxygen species generated which induced an increase in the enzyme breakdown. Bhandal and Kaur [30] reported that reduction in nitrate reductase activity due to stress might be a result of a reduced uptake of nitrogen. Cadmium also reduced the absorption of nitrate and its transport from roots to shoots by inhibiting nitrate reductase activity in the shoots [31]. The leghemoglobin contents of nodule of horse gram plants increased at 50 mg/kg soil level and decreased further with an increase in cobalt level in the soil at 250 mg/kg cobalt level [32]. To best of our knowledge this is the first report showing decrease in Leghemoglobin and hemechrome content in root nodules of soybean by cadmium in the soil. Gill et al. [23] found that the physiological disorder greatly depended on nitrogen metabolism perturbation by Cd.

In the present study, Cd caused reduction in the growth of soybean plants in terms of plant dry mass and leaf area which is consistent with the previous findings as shown in *Brassica juncea, Pisum sativum* and *Lepidium sativum* [21, 23,33]. Strong decrease in leaf area was correlated to accumulation of chlorophyll pigments as disturb integration of

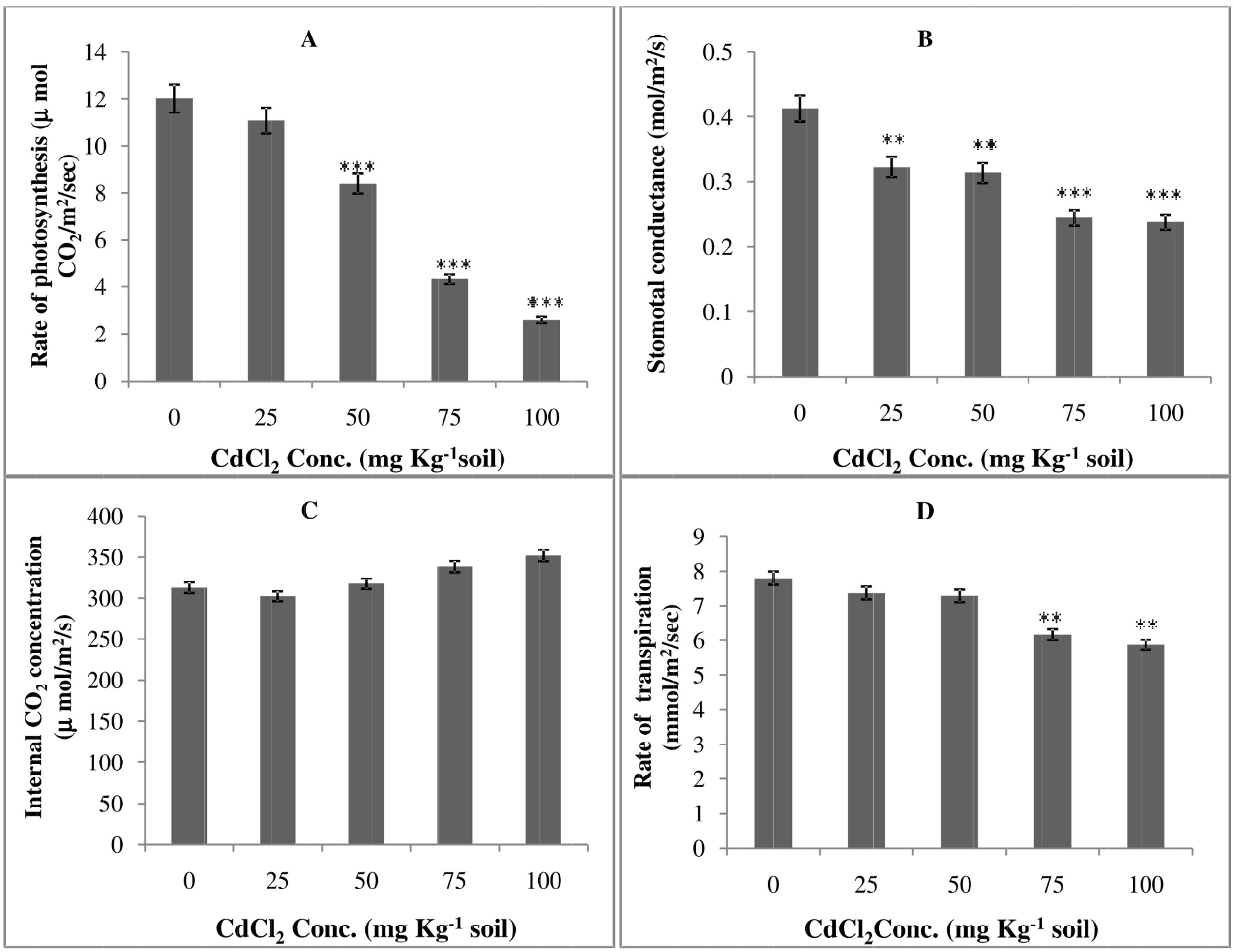

Figure 6: Effect of different concentrations of Cd (0, 25, 50 or 100 mg Cd kg^{-1} soil) on (A) rate of photosynthesis, (B) stomatal coductance (C) internal CO_2 concentration and (D) rate of transpiration in the leaves of soybean var. JS-335. The vertical bar indicates ±SE for mean. The values are significantly different from ($^{**}P < 0.01$, $^{***}P < 0.001$) from control (Newman-Keulis Multiple Comparison Test).

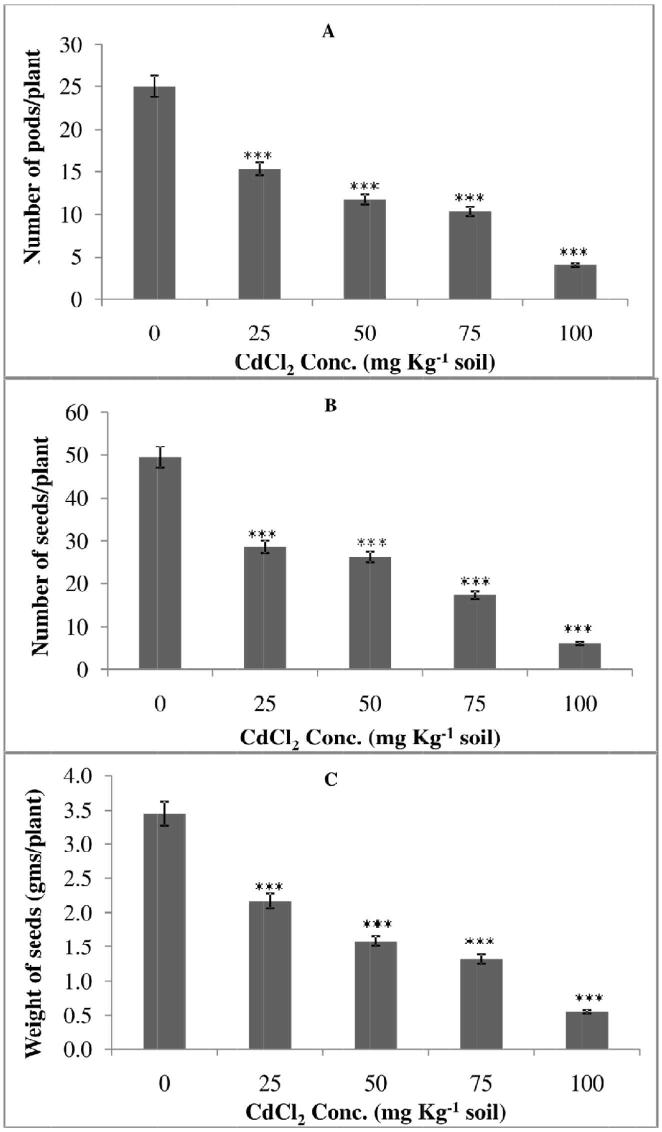

Figure 7: Effect of different concentrations of Cd (0, 25, 50 or 100 mg Cd kg^{-1} soil) on (A) number of pods/plant, (B) number of seeds/plant and (C) seed weight/plant of soybean var. JS-335. The vertical bar indicates ±SE for mean. The values are significantly different from (***P <0.001) from control (Newman-Keulis Multiple Comparison Test).

chlorophyll molecules into stable complex [34]. In the present study the photosynthetic pigments were significantly reduced and resulted in lower *Pn* which led to the inhibition of plant growth. The cadmium stress has been shown to enhance the stomatal closure and inhibit the photosynthesis via chlorophyll degradation in plants [35]. Similarly, higher level of cadmium has been shown to reduce the synthesis of chlorophyll *a*, *b* and total chlorophyll contents in gram, sorghum and garden cress [23,36,37]. The accumulation of Cd in plant tissues caused damage to the photosynthetic apparatus and decrease in the *Pn* in *L. sativum* plants [23].

Accompanied with the reduction of *Pn*, lower *gs* was simultaneously observed in soybean leaves in the present study, however, the reduction of *Pn* results from a low stomatal conductance (*gs*) and low CO_2 concentration in chloroplasts, because the intracellular CO_2 concentration (*Ci*) levels were not significantly affected in leaves treated with Cd compared to the control (Fig. 6). Similarly, inhibition in the net photosynthetic rate due to decreased stomatal conductance and photosynthetic pigment contents in *Arachis hypogaea* plants under Cd stress [38].

In the present study, yield attributes of soybean crop like number of pods per plant, number of seeds per pod and seed weight per plant decreased significantly with the increasing concentration of Cd. Deleterious effects of Cd on these parameters have also been reported in mungbean [39,40]. Results obtained by Manisha and Dhingra [41] indicated that the reduction in seed yield in pea has been found to be associated with decline in number of flowers, number of seeds and seed size. Biological yield and harvest index/plant was decreased at higher concentrations of Cd in terms of reduction in number of pod and leaf, root and stem growth while lower concentrations promoted the growth of these attributes [26, 42]. Thus Cadmium caused remarkable decrease in carbon and nitrogen fixation which caused the reduction in biomass and rate of photosynthesis it ultimately results in reduction of yield of soybean.

CONCLUSIONS

It can be concluded from present investigation that all the concentration of Cadmium used are toxic for soybean plants to all plant attributes. Present investigation revealed that higher concentrations 75 and 100mg Cd kg^{-1} soil severely hampered the plant height, phytomass, rate of photosynthesis, nitrogen fixation and assimilation which ultimately reduced the crop yield significantly. Phytotoxicity percentage was observed in the following order -25mg Cd kg^{-1} soil >50mg Cd kg^{-1} soil >75mg Cd kg^{-1} soil >100 mg Cd kg^{-1} soil concentration. High Cd concentration (100 mg Cd kg$''^{1}$ soil) perturbs the soybean growth by interfering with the photosynthetic performance and disrupting the coordination between carbon and nitrogen metabolism.

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