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EFFECT OF CO-CONTAMINATION OF CADMIUM AND BUTACHLOR ON SEED GERMINATION AND EARLY SEEDLING GROWTH OF *C. ROSEUS* UNDER SIMULATED SOIL CONTAMINATION

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Abstract

Cadmium (Cd) and Butachlor (BC) alone or in combination revealed diverse effects on seed germination and early seedling growth of *C. roseus*. Root elongation was more sensitive compared to shoot both under single and/or joint toxicities. The effect of Cd alone was found to be more toxic followed by combined treatment of Cd and BC and BC alone as far as root growth is concerned. On the contrary, a 14.1% decrease in shoot length was observed at the lowest concentration under joint treatment, however, the individual effect of Cd toxicity was more pronounced followed by the combination treatment and application of BC alone. The study reveals the ability of the plant to germinate and grow in the admixture of Cd and BC and its prospective phytoremediation potential.

Keywords : Co-contaminants, Butachlor, Cadmium, Phytoremediation, Seedlings

Introduction

Cd, Arsenic (As) and (Pb) lead are the d-orbital elements of the contemporary periodic table, which have attained due importance owing to their patho-physiological impact attributed to their bioaccumulation in living organisms (Sharma *et al*., 2014). At the same time, large scale application of pesticides further leads to environmental pollution causing health hazards (Igbedioh, 1991). The living systems are more often exposed to admixtures of heavy metals and pesticides as compared to isolated exposure of either of them (Chen *et al*., 2004). A phytoremediation approach accounting individual exposure may not encounter the consequence witnessed under joint toxicity which is universally reflected under real life conditions. Therefore, a study encompassing the combined interactions between pesticides and pesticide and plant systems has parallel importance for effective mitigation of toxicity (Chen *et al*., 2004). An earlier study carried out by us (Ratnakar and Shikha, 2018) reflected extensive distribution of pesticides in agricultural land in and around Lucknow city, (U.P.) India, having co contamination of Cd, Cd and BC. The present study, examines the phytoremediation potential of co contaminated soil as above wherein the effect of Cd alone and jointly with BC on seed germination and early seedling growth of *C. roseus* was carried out under simulated soil contamination.

Catharanthus roseus, commonly known as Periwinkle, belongs to genus, *Catharanthus* and is a native of West Indies. It is perennial herb flowering all the year round in tropical regions. Although the lead uptake ability of this plant is widely documented (Imam, 2017), but no preview is available as yet on the effect of co contamination with Cd along with any xenobiotics or BC on phytoremediation potential of the herb. In this study *C. roseus* has been opted because it is an evergreen ornamental herb, available easily and is well known widely for its endurance under arid and deficient conditions.

Materials and Methods

The seeds of *C. roseus* were obtained from a certified dealer "Neelkanth Agroforestry, Kaiserbagh", in Lucknow city, U.P., India.

Seed Sterilization: The seeds of *C. roseus* were sterilized by rinsing with 10% (v/v) hydrogen peroxide for 20 min to prevent fungal growth for 5 min and then washed with distilled water for two to three times. All glass wares were autoclaved at 121°C for 15 min, prior to use.

Experimental procedure: A total of 15 seeds were placed maintaining appropriate distance in each petriplate lined with double layered filter paper, and initially soaked with 10 ml of the respective solution. Initially a dark condition was provided for germination followed by a photoperiod of 16/08 h, light/dark period. Each treatment was added with 3 ml of the respective solution after every 48 h. Germination percentage was recorded for 08 days at different intervals.

Determination of germination: Seed germination was observed at regular intervals for 08 days. A seed was considered as germinated if the radicle was emerged. The germination percentage was calculated from the total number of seeds and germinated seeds in a Petri plate.

- The germination percentage (G %) was calculated, as given by Tanveer *et al*. (2010):

$$\text{Germination Percentage (G \%)} = \frac{\text{Germinated seeds}}{\text{Total Seeds}} \times 100$$

- Seedling Vigour index (VI) was calculated by the following formula (Vashisth and Nagarajan, 2010).

  VI = Seedling length * Germination Percentage %

- Germination index (GI) was calculated according to the formula given by (USDA, 2001) (Tiquia *et al*.,1996)

  $$\text{Germination Index (GI)} = E \times \frac{G}{100}$$

Relative root elongation (E) = (Mean root length with treatment)/Mean root length with control)* 100

Seed germination (G) = (seeds germinated with treatment)/ Seeds germinated with control) * 100

- The Tolerance Index (T.I.) was calculated using the formula given by Iqbal and Rahmati (1992).

$$\text{Tolerance Index= T. I} = \frac{\text{Mean root length in treatment}}{\text{Mean root length in control}} \times 100$$

- The percent phytotoxicity (P.P.) was calculated according to Chou and Lin (1976) and Ray and Banerjee (1981).

Percent Phytotoxicity (%) =

$$\frac{\text{Radicle length of Control} - \text{Radicle length of treatment}}{\text{Radicle length of Control}} \times 100$$

Scanning electron microscopic (SEM) study

Root and shoot specimens were prepared for SEM study using the protocol adapted from standard procedures (O'Brien and McCully, 1981). In order to determine the cellular differentiation of plant tissues treated with Hg and BC alone and in combination, along with control, fresh root and shoot samples (5 mm square from similar middle portion) were dissected and immediately fixed in a solution of 2.5 % gluteraldehyde at 4ºC temperature. The specimens were washed three times in 0.1M sodium phosphate buffer (pH 6.8) and kept overnight at 4 °C and then dehydrated in absolute acetone using 30 minutes series with 30, 50,70, 95 and 100% acetone and then stored at 4°C until examination. The specimens were rinsed, post-fixed in 2 % osmium tetraoxide, critical point dried and sputter coated with aluminum stubs with double-sided carbon tape. The specimens were viewed and photographed using Scanning electron microscope (SEM, Model: JSM-6490LV, Make: JEOL, Japan).

Statistical Analysis

All data were analyzed by one way ANOVA to test the individual and combined treatment of Hg and BC. Significantly different means were calculated using the 'Duncan test'. The entire statistical tests were performed using SPSS software (SPSS Inc., version 25.00).

Results

Effect on seed germination: Seeds of *C. roseus* were treated with diverse concentrations of heavy metal ($CdNO_3$) and herbicide (BC) individually, and in combination, to study the effect on germination. The germination was scored after 8th day of imbibitions.

Effect of Cadmium on seed germination

The final germination percentage reduced from 100 to 23.12% relative to control treatments as the concentration of Cd in solution increased from 5 to 120 ppm (Figure 2). The final germination percentage of seeds of *C. roseus* at different concentrations of Cd showed 5.1% inhibition for the lowest concentration (5 ppm) and 91.67% (Table 1) for the highest concentration (120 ppm). Germination was also inhibited by 29.63% at 50 ppm of Cd in solution. However, there was no total inhibition of seed germination in all concentrations of Cd.

One-way ANOVA showed that the inhibition of germination was significant ($p<0.05$) as the concentration of Cd in solution increased from 5-120 ppm.

Over an 8-day germination period GI significantly decreased from 88.5 to 16.81 with an increase in Cd concentration from 5 to 120 ppm in solution. Significant difference in GI was recorded as the concentration of Cd in the solution increased from 5-120 ppm.

Effect of BC on seed germination: The final germination percentage reduced from 93.33 to 24.21% relative to control as the concentration of BC increased from 1 to 10 ppm (Figure 4). The final germination percentage of seeds of *C. roseus* at different concentrations of BC showed 5.07% inhibition for the lowest concentration (1 ppm) and 90.32% inhibition (Table 2) for the highest concentration (10 ppm). However, there was no total inhibition of seed germination in all concentrations of BC studied.

One-way ANOVA showed that the inhibition of germination was significant ($p<0.05$) as the concentration of BC increased from 1-10 ppm.

Over an 8-day germination period GI significantly decreased from 88.5 to 3.45 with an increase in BC concentration from 1 to 10 ppm (Figure 4). Significant difference in germination index was recorded as the concentration of BC in the solution increased from 1-10 ppm.

Joint toxicity of Cd and BC on seed germination: Combined effect of Cd (5-120 ppm) and BC (4 ppm) on seed germination showed a significant difference in both germination percentage and index as compared to the individual effects of heavy metal and herbicide. The final germination percentage under combined treatment reduced from 100 to 21.18% relative to control as the concentration of Cd in solution increased from 5-120 ppm (Figure 6). The final germination percentage of seeds of *C. roseus* under combined treatment showed 94.94% inhibition for the highest concentration (120 ppm) (Table 3). However, there was no total inhibition of seed germination in all concentrations of combinations studied.

Results showed a significant changes in germination percentage under combined effect of Cd and BC as compared to individual treatments, respectively. An 80 % germination was observed under combined treatment of 20 ppm of Cd and 4 ppm of BC which was higher than the individual treatment of BC (66.66 %) at 4ppm and marginally less than the isolated treatment of Cd, being 82.21% at 20 ppm (Figure 2, 4 and 6)

One-way ANOVA showed that the inhibition of germination was significant ($p<0.05$) as the concentration of Cd increased from 5-120 ppm in the presence of 4 ppm BC.

Over an 8-day germination period, the GI significantly decreased from 46.63 to 2.82% (Figure 6) with an increase in Cd concentration from 5 to 120 ppm in the presence of BC (4 ppm). Significant difference in GI was recorded as the concentration of Cd in the presence of BC in the solution increased from 1-10 ppm.

Effect on seedling growth: The effect of different concentrations of heavy metal ($CdNO_3$) and herbicide (BC) alone, and in combination, was studied on the root and shoot length of *C. roseus.*

Effect of Cd on seedling growth: The effect of various Cd concentrations (5-120 ppm) on the root and shoot length of *C. roseus* is shown in Figure 1. The shoot length and root length as measured on the 8th day of incubation revealed a gradual decline in root length from 2.16 cm in control to 0.08 cm at 120 ppm of Cd. However, the length of the plumule was found to be greater than the root length at all concentrations studied. The shoot length was 2.62 cm in the control and showed a concentration (5-120 ppm) dependent decline reaching to 0.11 cm at 120 ppm. It was further revealed that relative to control treatments, the shoot length of *C. roseus* was adversely affected by Cd and results showed a 13.98% and 81.73% significant ($p<0.05$) inhibition for 5 and 120 ppm of the metal in solution. Similar results were observed for roots, but the root length inhibition was lesser than the shoot length inhibition. The present result showed a marginal decrease (5.1% inhibition) in root length at 5 ppm of cadmium, however, as the concentration of Cd in solution increased from 20-120 ppm the inhibition to the root length of *C. roseus* increased significantly ($p<0.05$) from 21.3% to 91.67% relative to control treatments.

Early seedling growth of *C. roseus* showed a differential trend for R: S, it showed decrease as the concentration of Cadmium increased from 5 to 120 ppm relative to control. A 12.19% decrease in R: S ratio was recorded at the highest concentration (120 ppm) of Cd may be attributed to limitations of nutrient and water under metal stress.

Effect of BC on seedling growth: The effect of BC concentrations (1-10 ppm) on the root and shoot length of *C. roseus* is shown in **Figure 3**. The shoot length and root length as measured on the 8th day of incubation revealed a gradual decline in root length from 2.17 cm in control to 0.21 cm at 10 ppm of BC. However, the length of the plumule was found to be greater than the root length at all concentrations studied. The shoot length was 3.39 cm in the control and showed a marginal decrease up to 2 ppm of BC, followed by a major decline in root length with increase in concentration. A 79.64% decrease in plumule length was recorded at the highest concentration of BC (10 ppm) relative to control. It was further revealed that relative to control treatments, the root length and shoot length of *C. roseus* was adversely affected by BC. The present result showed a marginal decrease (5% inhibition) in root length at 1 ppm of BC, however, as the concentration of BC increased from 2-10 ppm the inhibition to the root length of *C. roseus* increased significantly ($p<0.05$) from 14.75% to 90.32% relative to control treatments (Table 2).

Early seedling growth of *C. roseus* showed a fairly consistent trend for R: S to decrease as the concentration of BC increased from 1 to 10 ppm relative to control. A 53.12% decrease in R: S ratio recorded at the highest concentration (10 ppm) of BC may be attributed to limitations of nutrient and water under metal stress.

Joint toxicity of Cd and BC on seedling growth: Combined effect of Cd (5-120 ppm) and BC (4 ppm) on the root and shoot length of *C. roseus* is shown in Figure 5. The shoot length and root length as measured on the 8th day of incubation revealed a gradual decline in root length from 2.17 cm in control to 0.11 cm at 120 ppm of Cd in the presence of BC. However, the length of the plumule was found to be greater than the root length at all concentrations studied. The shoot length was 3.9 cm in control and showed a concentration (20-120 ppm) dependent decline reaching to 0.39 cm at 120 ppm. It was further revealed that relative to control treatments, the shoot length of *C. roseus* was adversely affected by Cd and results showed a 15.65% and 90% significant ($p<0.05$) inhibition for 20 and 120 ppm of the metal in solution. Similar results were observed for roots, but the root length inhibition was greater than the shoot length inhibition. The present result showed a significant decrease (47.9% inhibition) in root length at 5 ppm of Cd, however, as the concentration of Cd in solution increased from 20-120 ppm the inhibition to the root length of *C. roseus* increased significantly ($p<0.05$) from 50.24% to 94.94% relative to control treatments.

Early seedling growth of *C. roseus* showed R: S to decrease as the concentration of Cd increased from 20 to 120 ppm relative to control. An approximate 1.13 fold decrease in R: S ratio recorded at the highest concentration (120 ppm) of Cd and (4 ppm) BC may be attributed to limitations of nutrient and water under metal stress.

Effect on seed vigour: Seed vigour of *C. roseus* declined with increase in Cd concentration (5-120 ppm), BC (1-10 ppm), both alone and in combination (Cd-5-120 ppm and BC-4 ppm) as measured after 48 h of germination. The seedling vigour index was reduced from 96 to 2.19 as the concentration of Cd increased from 5-120 ppm (Table 1). However, the vigour index in the presence of BC was found to decrease from 72.79 to 5.81 with the increase in concentration from 1-10 ppm (Table 2). The vigour index in combination on the contrary decreased from 70.19 to 3.38 when the concentration of Cd increased from 5-120 ppm in the presence of BC (Table 3). Results thus revealed that treatment of Cd alone bears greater impact on seed vigour followed by combination and BC alone. Under stress conditions there may be a decrease in uptake of water both during imbibitions and seedling establishment (Vieira, 1976), which bear physiological and biochemical changes in the metabolism of both seed and seedling (Gomes and Sodek, 1988).

Effect on Tolerance indices

The seedlings of *C. roseus* were tested for tolerance to heavy metal and herbicide (BC), using different concentrations of Cd (5-120 ppm) and BC (1-10 ppm), both alone and in combination.

C. roseus showed high percentage of tolerance at 5 ppm of Cd. An increase in Cd concentration (20-120 ppm) gradually decreased the tolerance of *C. roseus*. The treatment of Cd at 120 ppm showed the lowest percentage of tolerance in *C. roseus* as measured on the eighth day of exposure as compared to control. The tolerance index was found to decrease from 91.3 to 5.21 as the concentration of Cd increased from 5-120 ppm (Table 1). However, as compared to Cd better tolerance index was reported in the presence of BC. The tolerance index decreased from 92.59 to 14.28 as the concentration of BC increased from 1-10 ppm. Under combination treatment, the tolerance index was lesser than individual treatments of Cd and BC decreasing from 60.66 to 4.58 as the concentration of Cd in the presence of BC (4 ppm) increased from 5-120ppm (Table 3). Hence, according to tolerance test it may be inferred *C. roseus* was more tolerant to BC compared to Cd and combination treatments.

Effect on Percent Phtotoxicity: The phytotoxicity of Cd (5-120 ppm) and butachlor (1-10 ppm), both alone and in combination on root length of *C. roseus* is given in Table 1, 2 and 3. The increase of Cd levels increased significantly the percent phytotoxicity on root length under all treatments ($p<0.05$). The lowest percent phytotoxicity (5.09%) was observed at the lowest concentration of Cd (5 ppm). However, in the presence of BC, it was 5.06% at the lowest concentration (1 ppm). The joint toxicity of Cd and BC revealed 46.19% phytoxicity at the lowest concentration (5ppm Cd in the presence of 4ppm BC). Results clearly revealed that the joint treatment of Cd and BC was more toxic to root growth followed by the individual treatment of Cd and BC on the radical length of *C. roseus.*

Scanning Electron Microscopy study (SEM) analysis: The result of Scanning Electron microscope studies indicated the structural deformation in the tissues of root and shoot in comparison to control (Figure 7 and 8). The shrinkage in the parenchymatous cells of plant tissues is clearly visible which may be attributed to limitation of nutrient supply and toxic effect of combined treatment.

Toxicity thresholds:

The Cd, BC and combination toxicity thresholds were determined for radical and plumule of C. *roseus*. EC50 is the contaminant concentration that produced 50% inhibition in seedling length (root and shoot) with respect to the control treatment. The EC50 values for Cd, BC alone and in combination is given in Table 4. The 50% inhibitory concentration of Cd for radicle and plumule of *C. roseus* was found to be 70.15 and 71.37 ppm, which is contrary to the the observation of Guilherme *et al*., (2015) who reported that Cd at 0.12 mM inhibited the growth of *T. aestivum* and thus inhibited the germination of 50% of seeds.

A 50% reduction in radicle and plumule length of *C. roseus* in the presence of herbicide BC was recorded at 6.15 and 8.35 ppm respectively. Ateeq *et al*. (2002) was reported Butachlor induced dose-dependent root growth inhibition in *Allium* root tip with EC50 value registered at 5.13 ppm which is contrary to our results. However, the EC50 value for BC + Cd was found to be 17.95 ppm for radicle and 69.32 ppm for plumule. Overall results clearly revealed that joint treatment of Cd and BC was more toxic compared to individual toxicities of Cd and BC for both root and shoot.

Discussion

Filter paper soaked with heavy metal and or pesticides for seed incubation may reduce the effects of any other metals or xenobiotics that might be present in soil under natural conditions, due to their synergistic and/or antagonistic effect (Munzuroglu and Geckil, 2002). Heavy metals and pesticides are known to affect the development of plants and can be studied by determining the characteristics of seed germination.

In the present study, the effect of increasing concentrations of Cd (5-120 ppm) and BC (1-10 ppm) alone and in combination [Cd (5-120 ppm) and BC (4 ppm)] on germination and early seedling growth of *C. roseus* was studied. Results revealed that the individual and combined effect of the heavy metal and pesticide affected both, the percentage of germination and early seedling growth of *C. roseus,* differently.

There was no complete inhibition of germination even for the highest Cd and/or BC concentration used either singly or jointly (Figure 2, 4, 6). The joint effect of Cd and BC was found to be more toxic as compared with the individual toxicities of Cd and BC. Seed germination under Cd stress could be decreased which may be due to accelerated breakdown of reserved food material in seed embryo (Ahmad *et al*., 2012). The negative impact of heavy metals on seed germination has earlier been reported by several researchers (Sethy and Ghosh, 2003). It is well documented that the effect of metals on seeds germination may lower the water uptake and transport, causing embryonic damage and/or death. Selection of a specific plant species and metal element or pesticide, either alone or in combination may vary the degree to which the above mentioned toxicities affect negative germination.

Similar results were obtained for root elongation as well. Lower concentration of Cd (5 ppm) and BC (1 ppm) did not seem to affect the root length of *C. roseus* and revealed a marginal decrease of 5.09 and 5.06%, at the respective concentrations compared to control. However, a significant decrease in root length (47.92%) was observed at the lowest concentration under joint toxicity of Cd and BC. The overall results indicated that the joint effect of Cd and BC was more toxic followed by individual treatments of Cd and BC as far as root growth is concerned.

On the contrary the results obtained for shoot length revealed that lower concentration of Cd (5 ppm) and BC (1 ppm) affected a marginal decrease of 4.19% and 0.58% in shoot length of *C. roseus* respectively, as compared to control. However, under joint treatment of Cd and BC although a 14% decrease was observed in the length of shoot of *C. roseus* at lowest concentration (5 ppm Cd + 4 ppm BC) studied which was similar to Cd treatment alone, rest of the concentration dependent decline was improved. Overall results indicated that combined effect of Cd and BC was more pronounced followed by the application of Cd and BC alone. Similar results were observed by Cailin *et al*. (2009), their results confirm the inhibitory effects of Cd and Tri Chloro Benzene on the growth of wheat seedlings.

Roots are the initial part of the plant which comes in touch with any soil and any contamination therein; therefore, they tend to be more sensitive to toxicity compared to shoots (Magna *et al*., 2013). In the present investigation, length of the shoot was found greater than the root length at all concentrations studied, and a greater sensitivity was revealed on the root length (Khatamipour *et al*., 2011; Subin and Steffy, 2013). An increase in root/shoot ratio observed in the presence of BC and Cd, and under joint toxicities as well, might be attributed to structural and morphological changes in root (less root hairs, thickening of roots) induced by metal and/or pesticide.

The tolerance index of *C. roseus* was significantly reduced under joint application of Cd and BC, compared to individual application of BC and Cd. Results of vigour index revealed that Cd alone is more pronounced followed by combination and BC alone. Although, with the increase in concentration phytotoxicity found to be continuously increasing in Cd and BC either alone or under combined treatment. These findings are in line with Raziuddin *et al.* (2011) who reported that Cd stress decreased germination index, vigour index and seed germination, of *Brassica.*

The SEM images showed a significant anatomical difference between root and shoot. This study showed the structural deformation in morphology more in shoot as compared to root which is contrary to Godbold and Huttermann (1986) who highlighted that the structural deformation in root is more prominent than shoot which may have serious consequences for nutrient and water supply to aboveground plant parts.

Conclusion

This study revealed that Cd and BC either alone or in combination have differential effects on seed germination and early seedling growth of *C. roseus*. In spite of fact that, morphologically root was more sensitive to shoot both under single and/or joint toxicities however the SEM micrographs revealed more structural deformation in shoot as compared to roots. Based on EC 50 values it was inferred that the joint effect of Cd and BC was more toxic followed by individual treatments of BC and Cd as far as root and shoot growth is concerned. A differential toxicity of heavy metal and pesticide on seed germination and root and shoot elongation of *C. roseus* under different germination parameters reveals the ability of the plant to germinate and grow in the admixture of Cd and BC and its prospective phytoremediation potential. Our future endeavors shall be to work out the phytoremediatian potential of *C roseus* under co contamination, and envisage the reason behind structural deformations in plant tissues.

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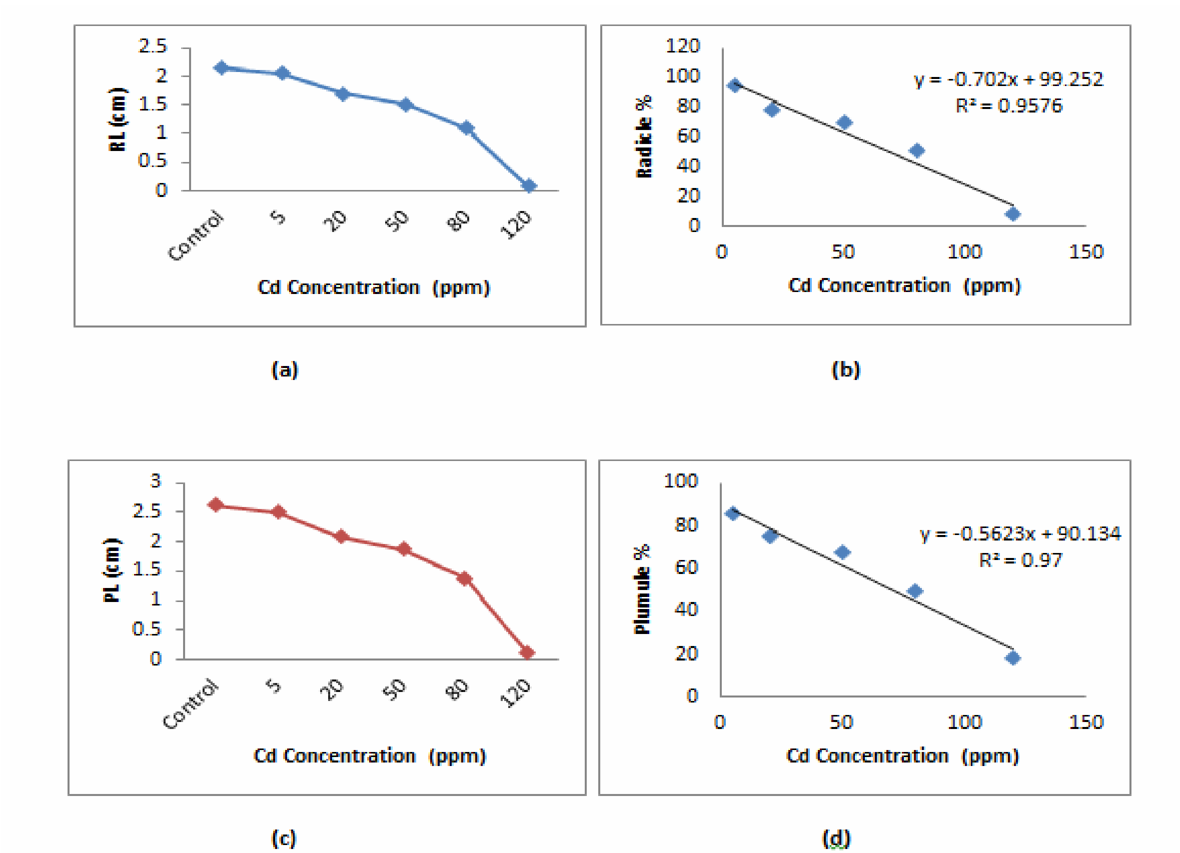

Fig. 1 : Effect of Cd on (a) (b) radicle and (c) and (d) plumule length of *C. roseus* seedlings

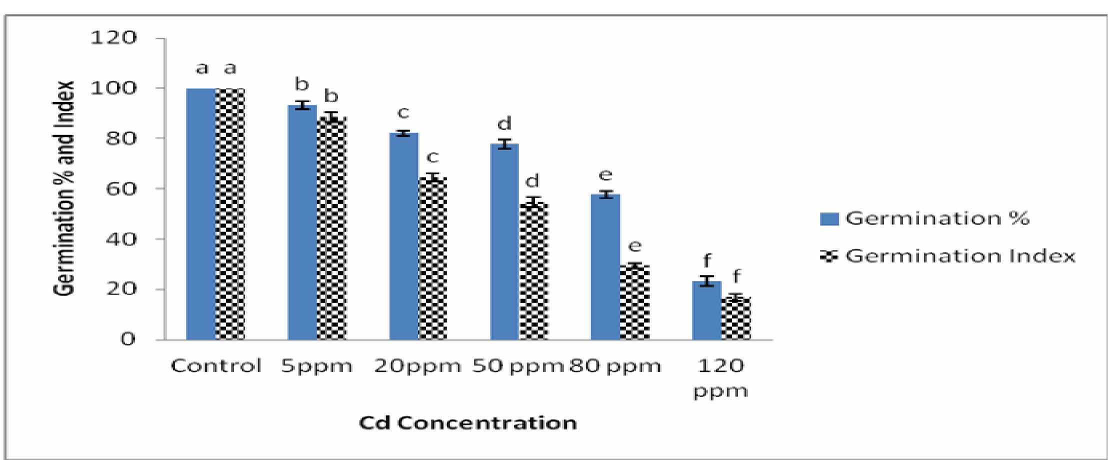

Fig. 2 : Effect of Different Concentration of Cd on Germination Percentage and Index of *C. roseus* (±S. D., n=3)
*Means with different letters are significantly different from each other [a,b,c,d,e] ($p < 0.05$) .

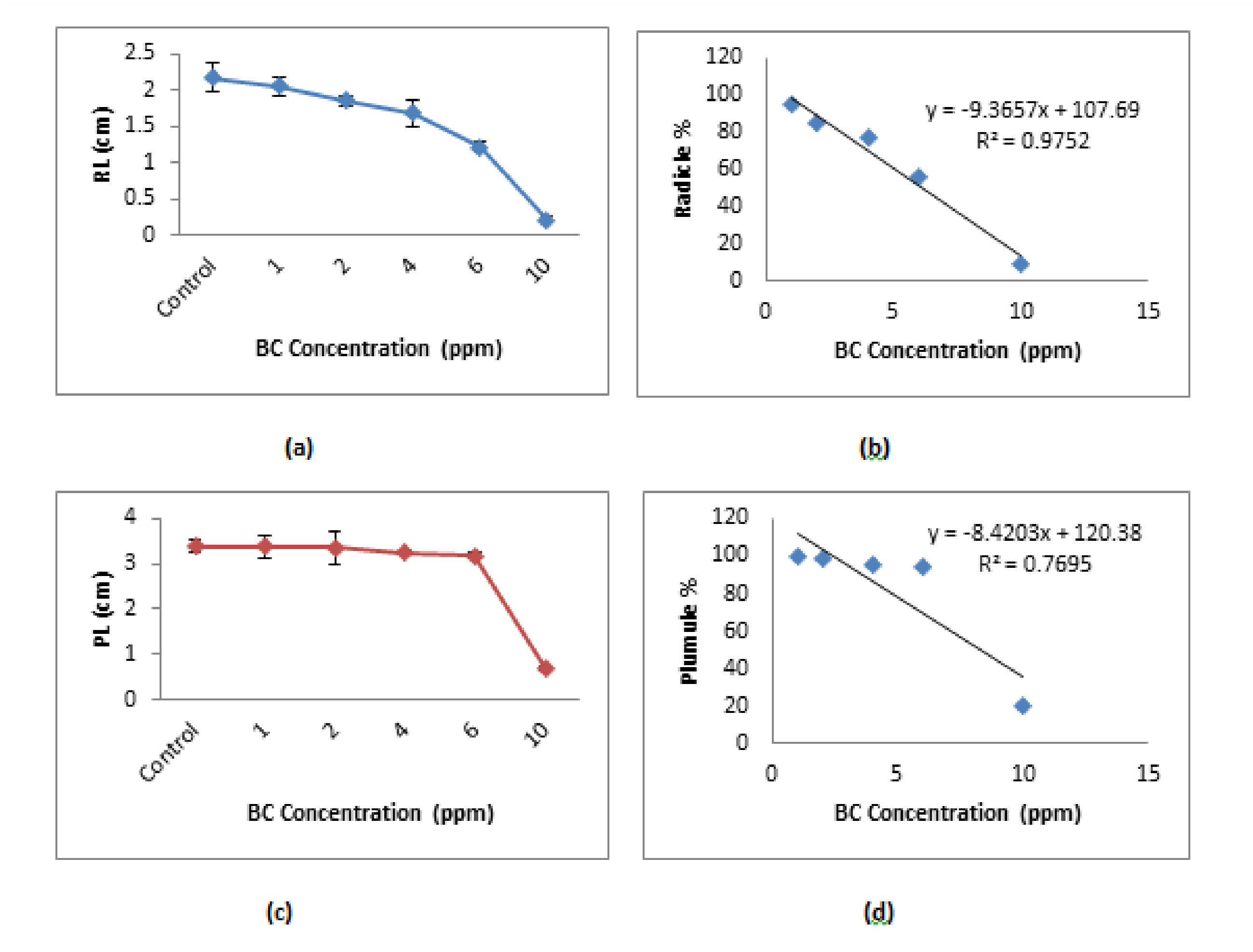

Fig. 3: Effect of BC Concentration on (a) (b) radicle and (c) and (d) plumule length of *C. roseus* seedlings (±S. D., n=3)

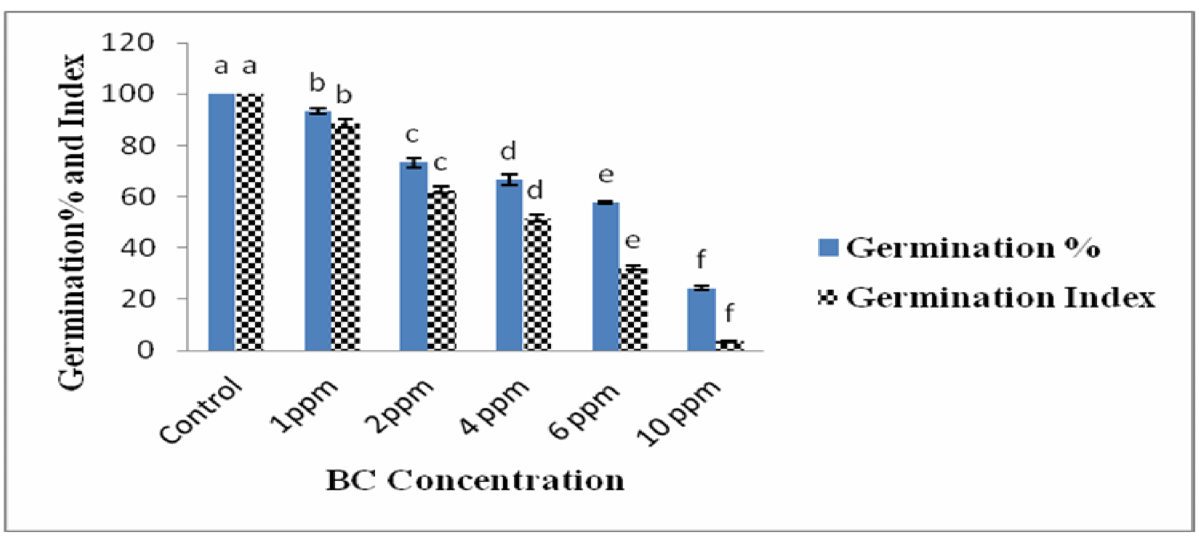

Fig. 4: Effect of Different BC Concentration on Germination Percentage and Index (±S.D., n=3)

**Means with different letters are significantly different from each other ^{a,b,c,d,e,f} ($p < 0.05$).

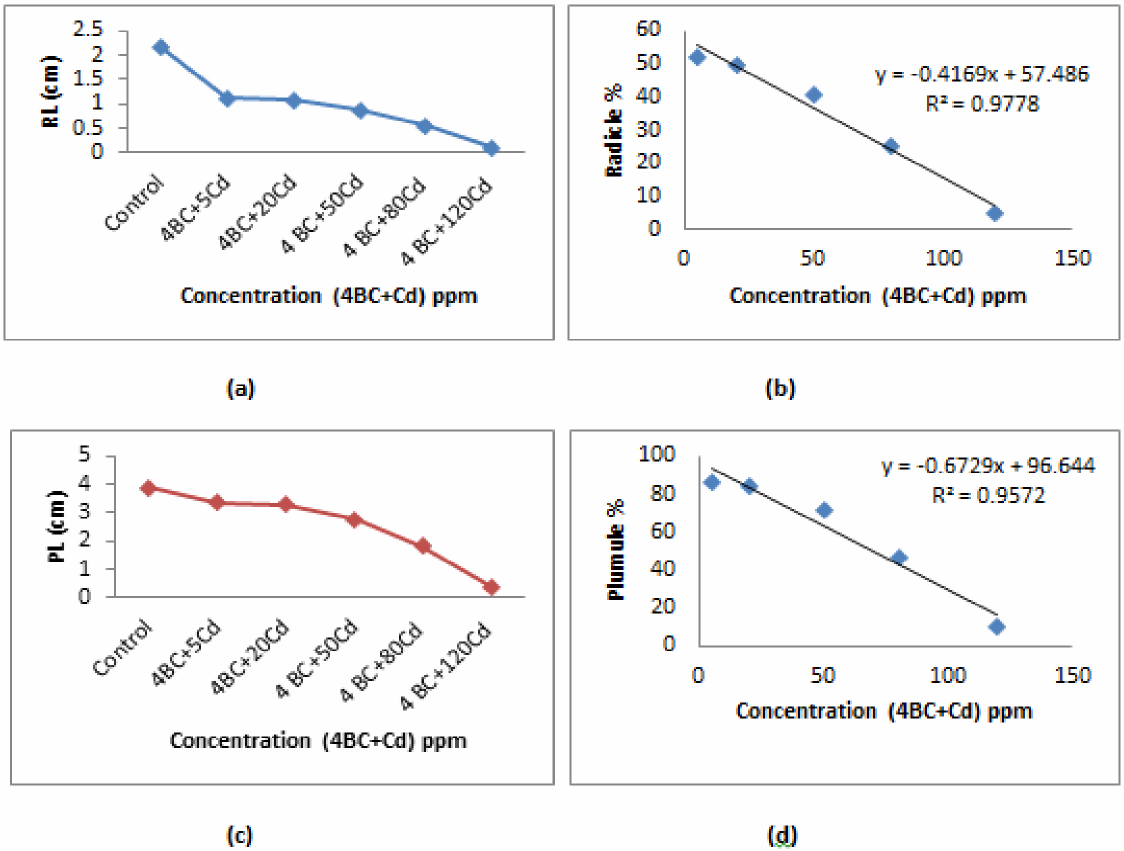

Fig. 5: Effect of Cd +BC (4 ppm) on (a) (b) radicle and (c) and (d) plumule length of *C. roseus* seedlings (±S. D., n=3)

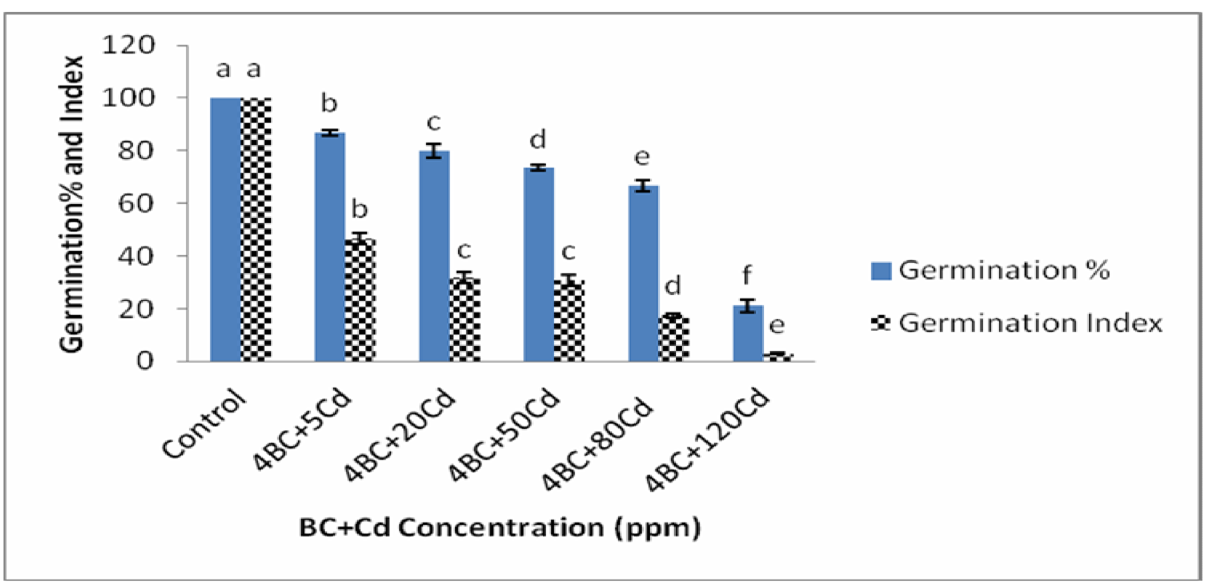

Fig. 6 : Effect of Different BC and Cd Concentration on Germination Percentage and Index (±S. D., n=3)
*Means with different letters are significantly different from each other [a,b,c,d,e,f] ($p < 0.05$).

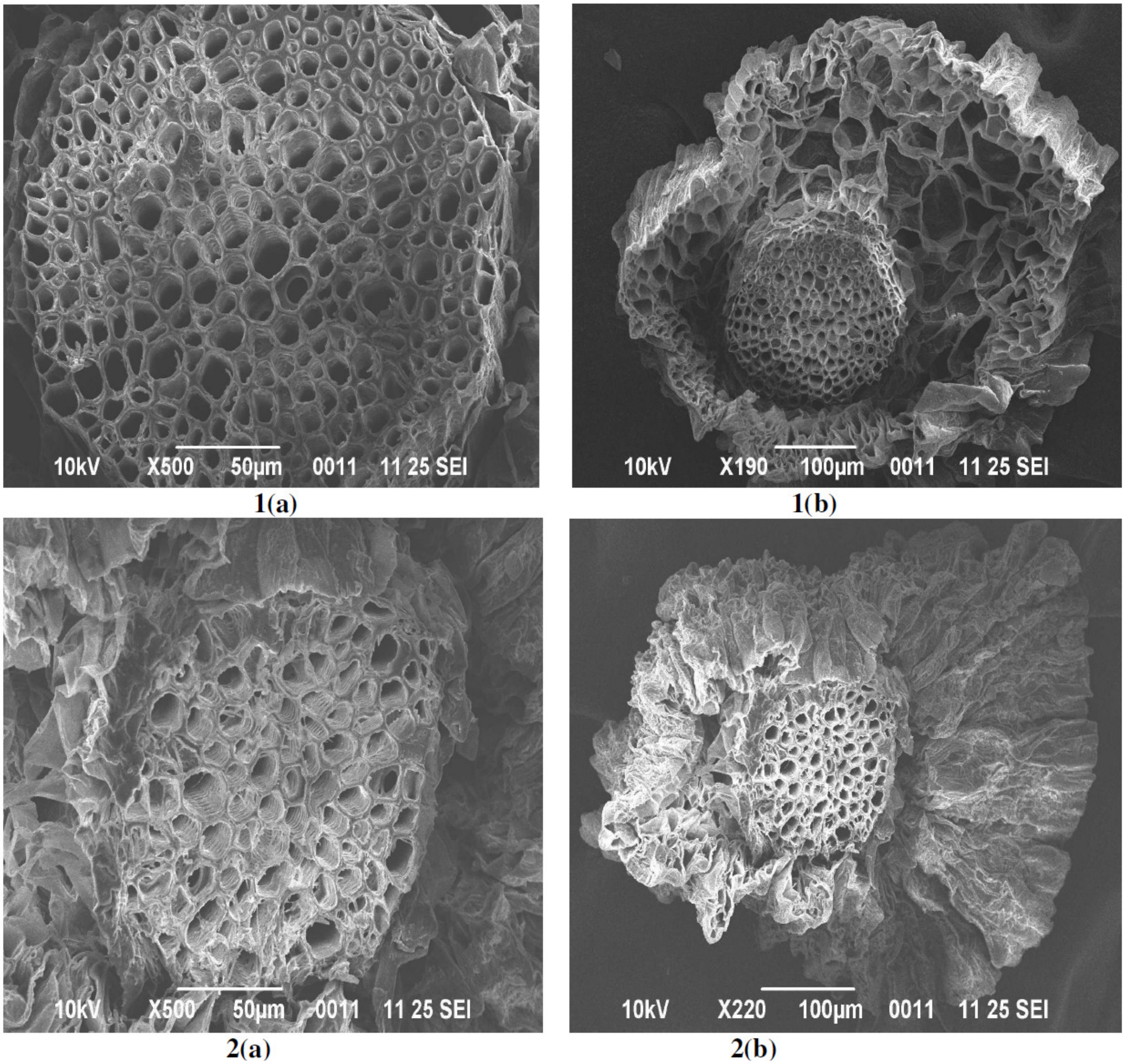

Fig. 7 : Scanning Electron Micrograph of Untreated and treated radicle of *C. roseus* under early seedling growth: 1(a,b) Control; 2(a,b) Cd+BC treated root

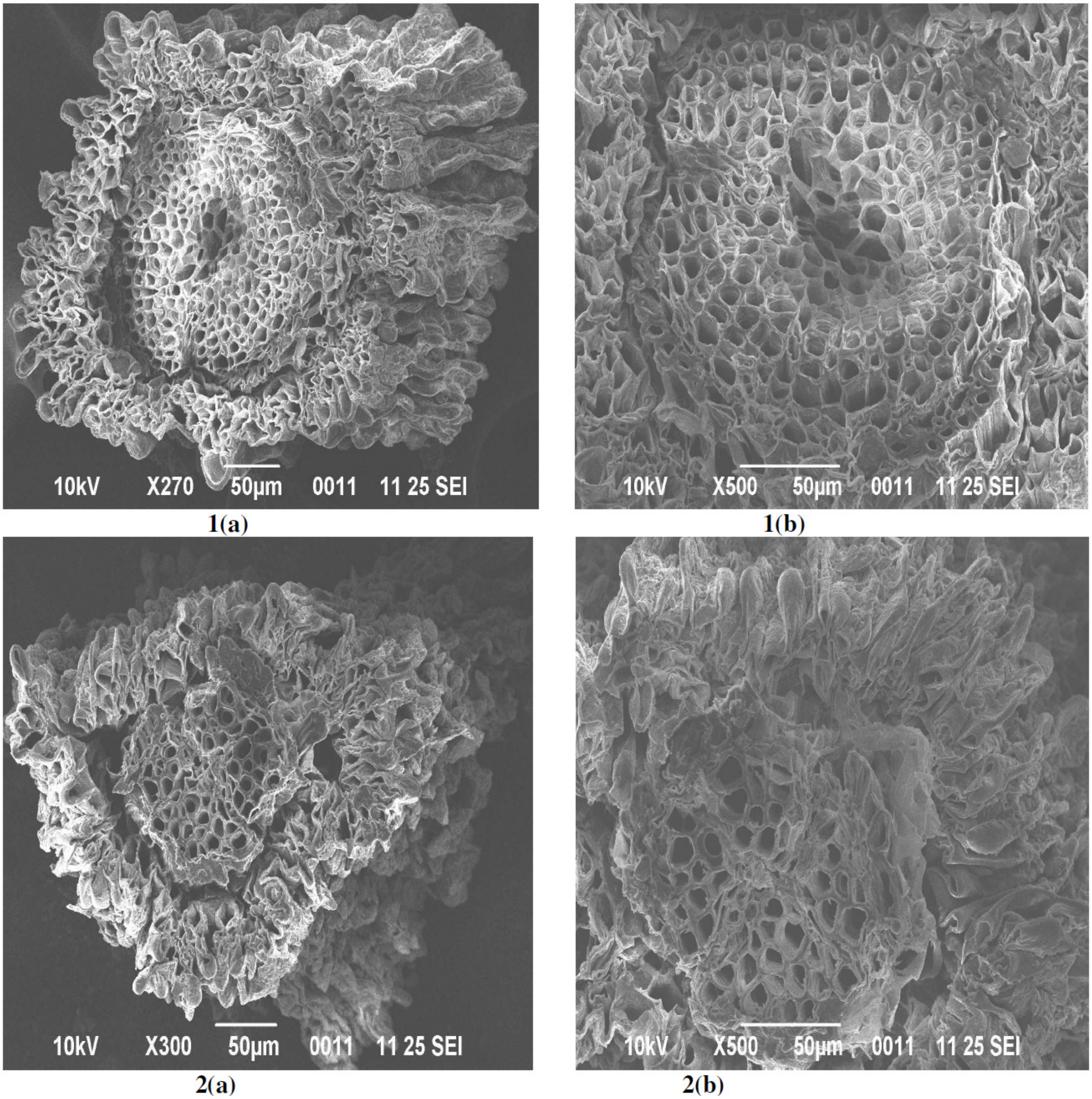

Fig. 8 : Scanning Electron Micrograph: 1(a,b) Control Shoot;2(a,b)Cd+BC Treated Shoot

Table 1: Effect of Different Cd Concentration on seedling growth parameters (±S. D., n=3)

	Inhibitory %	Vigour Index	Tolerance Index	Phytotoxicity %	R/S Ratio
Control	0±0.00[f]	96±4.58[a]	100±0.00[a]	0±0.0[e]	0.82±.15[a]
5ppm	5.1±.26[e]	76.5306±6.13[b]	91.3±2.60[b]	5.09±.08[d]	0.81±.81[a]
20ppm	21.3±.99[d]	50.1481±3.56[c]	78.69±3.09[c]	24.07±1.04[c]	0.81±.14[a]
50 ppm	29.63±.55[c]	42.7735±2.02[d]	66.08±1.78[d]	37.96±1.05[b]	0.80±.02[a]
80 ppm	49.08±1.04[b]	32.9289±2.22[e]	63.91±1.56[d]	39.35±1.53[b]	0.79±.06[a]
120 ppm	91.67±1.68[a]	2.1964±.253[f]	5.21±.23[e]	94.9±1.03[a]	0.72±.18[a]

**Means with different letters are significantly different from each other [a,b,c,d,e ,f] ($p < 0.05$).

Table 2: Effect of Different BC Concentration on seedling growth parameters (±S. D., n=3)

	Inhibitory %	Vigour Index	Tolerance Index	Phytotoxicity %	R/S Ratio
Control	0.00±0.00[f]	111±8.88[a]	100±0.00[a]	0.031±0.002[f]	0.64±0.07[a]
1ppm	5.07±0.56[e]	72.79±3.27[b]	92.59±1.13[b]	5.06±0.24[e]	0.61±0.01[a,b]
2ppm	14.75±0.37[d]	52.79±3.47[c]	77.77±1.52[c]	14.74±0.63[d]	0.55±0.07[a,b]
4 ppm	22.59±0.36[c]	43.32±3.86[d]	70.37±1.12[d]	22.58±0.94[c]	0.51±0.05[b]
6 ppm	44.24±0.80[b]	35.23±3.68[d]	68.23±0.97[e]	44.23±1.03[b]	0.38±0.01[c]
10 ppm	90.323±1.70[a]	5.81±0.42[e]	14.28±0.94[f]	85.71±1.57[a]	0.30±0.05[c]

**Means with different letters are significantly different from each other [a,b,c,d,e ,f] ($p < 0.05$).

Table 3 : Effect of different Cd and BC concentration on seedling growth parameters of *C. roseus* (±S. D., n=3)

	Inhibitory %	Vigour Index	Tolerance Index	Phytotoxicity %	R/S Ratio
Control	0±0.0[e]	111±4.35[a]	100±0.00[a]	0±0.00[e]	0.55±0.05[a]
4BC+5Cd	47.93±2.26[d]	70.19±2.90[b]	60.66±2.09[b]	46.19±0.97[d]	0.33±0.03[b]
4BC +20Cd	50.24±2.62[d]	53.60±5.17[c]	46.66±2.01[c]	58.09±1.03[c]	0.32±0.04[b]
4BC +50Cd	59.45±1.52[c]	47.66±4.65[c]	36.25±2.00[d]	60.47±3.07[c]	0.31±0.01b
4BC +80Cd	74.66±1.22[b]	40.66±3.68[d]	23.75±2.21[e]	73.8±1.35[b]	0.30±0.01[b]
4BC +120Cd	94.94±1.60[a]	3.38±0.46[e]	4.58±0.46[f]	94.93±2.59[a]	0.28±0.08[b]

**Means with different letters are significantly different from each other [a,b,c,d,e ,f] ($p < 0.05$).

Table 4: Effective Concentration 50 (EC 50) values for Cd, BC alone and in combination:

Treatment	**EC-50**	
	Radicle	**Plumule**
Cd (ppm)	70.15	71.37
BC (ppm)	6.15	8.35
BC+Cd (ppm)	17.95	69.32

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