



Phytotoxic Effects of Cadmium on Seed Germination and Seedling Growth of (*Brassica juncea* L.Czern Coss) cv.

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Abstract

Cadmium (Cd^{2+}) is extremely toxic heavy metal which adversely affects the growth and yield of plants. Therefore, study was conducted to evaluate the effects of various Cd^{2+} concentrations (0.5mM, 1 mM, 1.5 mM and 2mM) on seed germination and seedling growth of twenty *Brassica juncea* Cultivars. Various parameters tested in present study includes root and shoot length, seedling size, fresh and dry matter, seed germination percentage, tolerance indices and percent phytotoxicity. Results indicate that the significant inhibitory effect was observed at all the four concentrations of Cd^{2+} as compared to control. As the Cd^{2+} concentration increases, all the tested parameters except Tolerance Indices decrease. Present study also reveals that Cultivar RB-50 may show better performance under Cd^{2+} stresses while Cultivar RH-0406 might have poor performance. From these results it can concluded that Cultivar RB-50 might germinate well on Cd^{2+} contaminated soils, however more studies are needed to signify its effects on growth and yield of *Brassica juncea*.

Keywords: Cadmium, *Brassica juncea*, seed germination, tolerance indices, percent phytotoxicity.

Introduction

Indian mustard (*Brassica juncea*) is an important oil seed crop being cultivated in 53 countries all over the world. *Brassica juncea* (*B. juncea*) is a fast growing plant which produces a high biomass even in heavy metal contaminated soils. Yield of *B. juncea* is negatively affected by presence of heavy metals in the soil. The unrestricted development activities e.g. Urbanization and Industrialization, carried out haphazardly during past few years have caused serious problems of environmental contamination. Agricultural soils are continuously polluted with harmful heavy metals that hamper the growth of plants and cause significant yield reduction and loss in their productivity¹. Deshveer and Singh² reported that the yield of *Brassica juncea* is of low magnitude, despite the use of N and P fertilizers. Foy *et al*³ and Sheoran *et al*⁴ observed that indiscriminate usage of phosphatic fertilizers in agricultural fields builds up cadmium level in the soil which negatively affects plant growth and economic yield. Among various heavy metals Cd^{+2} is a non essential and extremely deleterious, it is discharged into the agricultural soils worldwide from various sources such as industrial, mining and farming practices⁵ and has been ranked Number 7 among the top 20 toxins which has harmful effects on the human health by entering into the food chains^{6,7}. It is quickly absorbed by roots of plants growing on Cd^{+2} polluted soils and transported to leaves in the same way as the essential micronutrient metal ions^{8,9}. Plants growing on Cd^{+2} polluted soil accumulate it, in all plant parts, resulting in growth retardation, affects nutrient uptake, cause alteration in the chloroplast structure, inactivates enzymes, reduce photosynthesis and activates enzymatic and non enzymatic antioxidant machinery^{10,11}. Phytotoxicity of Cd varies with its concentration

in the soil as well as with the plant species and cultivars. Some plant species or cultivars have developed tolerance to Cd. Plant varieties show difference in their Cd detoxification capacity in between and within the plant species, which has a beneficial role in the expression of high tolerance in crop plants to Cd toxicity. Therefore screening of plants varieties with the ability to detoxify Cd in the agricultural soils could be the best strategy to overcome the harmful and deleterious consequences of Cd in crop plants. The aims of present study were: *In vitro* analysis for the impact of Cd toxicity in twenty *Brassica juncea* Cultivars. Screening of *Brassica juncea* Cultivars for Cd tolerance using germination markers.

Material and Methods

Approximately 20 Cultivars of *Brassica juncea* were procured from SKN University Jobner and CCSHAU Hisar. Uniformly selected seeds were surface sterilized with 5% NaOCl for 5 minutes and then washed repeatedly for two to three times with distilled water to prevent fungal/bacterial contamination. Filter papers were also sterilized in autoclave to reduce any chances of microbial growth. Heavy metal test solutions were prepared using four different concentrations of Cadmium (Cd^{+2}) viz. 0.5mM, 1mM, 1.5mM and 2 mM. The chemicals used were of analytical reagent grade. Distilled water was used as control. Seeds were germinated in glass Petri dishes of 15 cm diameter lined with filter paper circles moistened with distilled water as control and various concentrations of Cadmium to impose four levels of stress. Nearly 10 seeds were sown in each Petri dish and incubated in growth chamber set at $25 \pm 2^\circ C$ for 7 days and each treatment was replicated thrice. Occurrence of Germination was considered when roots were 2mm long; Germination

percentage was recorded in every 24h, till the end of experiment. The following parameters were analyzed for the study.

Germination percentage: A mean of 10 seeds were taken and expressed as percentage.

$$G.P (\%) = \frac{\text{Total number of seeds germinated}}{\text{Total number of seeds}} \times 100$$

Root length and Shoot length: The length of root and shoot of seedlings were recorded at 7th day of germination. Mean values were calculated for both root and shoot length and values were expressed in cm.

Seedling Height: Heights of seedling were recorded at 7th day of germination. Mean values were calculated and expressed in cm.

Fresh Matter: The Fresh Weight was recorded from the 7th day old seedling. The whole seedling was surface dried with the blotting paper and their fresh weight were recorded

Dry Matter: The same seedlings were used and dried in oven for 24 h at 80°C and weighed again. This represented the dry matter.

Tolerance Indices (T.I): Tolerance indices were determined with the formula given by Iqbal and Rahmati¹²

$$T.I = \frac{\text{Mean root length in metal solution}}{\text{Mean root length in control}} \times 100$$

Percent Phytotoxicity: Percent Phytotoxicity was determined using the formula of Chou *et al*¹³.

$$PP = \frac{\text{Radicle length in control} - \text{Radicle length of test}}{\text{Radicle length of control}} \times 100$$

Results and Discussion

Root length, Shoot length and Seedling size: The statistical analysis (ANOVA) for root length, shoot length and seedling size revealed that cultivars and Cd concentration along with their interactions were highly significant. The data presented in table-1 and table-2 indicates the decrease in trend with respect to root length (RL), shoot length (SL) and seedling size (SS) of Cd⁺² treated seedlings over control. As the concentration of Cd⁺² increases root length, shoot length and seedling size decreases figure-1. Cultivar RB-50 showed minimum decrease in RL, SL and SS as compared to other 19 Cultivars at all four concentrations of Cd⁺² (0.5 mM, 1 mM, 1.5 mM, 2mM). Whereas, cultivar RH-0406 showed maximum decrease in RL, SL and SS at all four concentrations of Cd⁺². The metal imposed

high toxicity at 1.5 mM and 2 mM to all the 20 cultivars. In all the cultivars, the roots were found to be more sensitive to Cd⁺² than shoots, which correspond to higher content of Cd⁺² in roots than that in the shoots. The greater reduction of root length than shoots could be a result of more absorption of Cd⁺² in roots¹⁴.

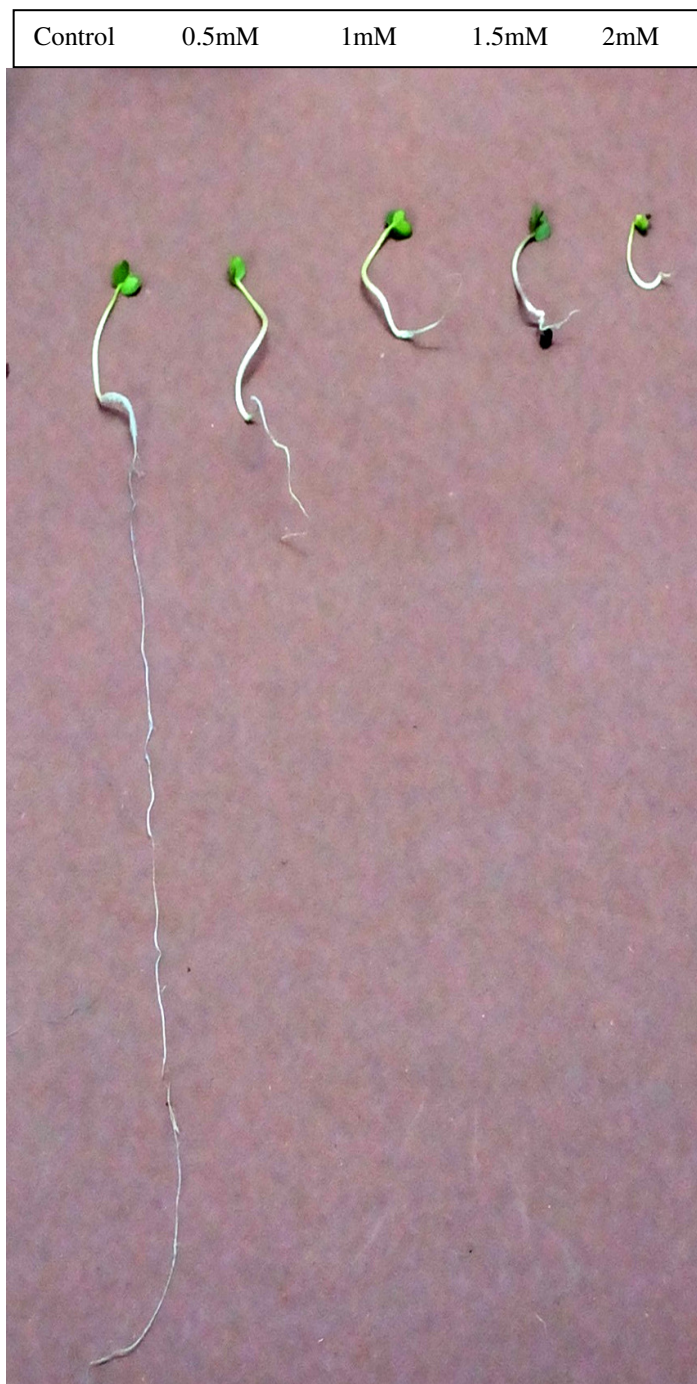


Figure-1
 Effect of different concentration of Cd⁺² on the growth of germinated seedlings of *Brassica juncea* grown for 7 days *in vitro*

Table-1
Effect of different concentrations of Cd⁺² on Root and Shoot length of Brassica juncea cv

Varieties	Root Length						Shoot length					
	Control	Cd 0.5mM	Cd 1mM	Cd 1.5mM	Cd 2mM	Variety Mean	Control	Cd 0.5mM	Cd 1mM	Cd 1.5mM	Cd 2mM	Variety Mean
RH-0749	11.07	5.78	1.48	1.34	1	4.13	2.47	2.06	1.71	1.45	1.32	1.8
Pusa Bold	8.24	3.14	1.91	1.76	1.45	3.3	2.28	1.84	1.51	1.46	1.11	1.64
GM-3	7.29	5.11	1.01	0.82	0.77	3	2.56	1.97	1.29	1.17	1.23	1.64
RH-0119	10.04	2.41	1.08	0.74	0.5	2.95	1.97	1.76	1.36	1.28	1.16	1.5
RH-9304	7.55	2.11	1.67	1.36	0.65	2.67	2.14	1.95	1.79	1.77	1.68	1.86
RGN-145	9.46	1.54	1.47	0.49	0.48	2.69	2	1.61	1.51	1.5	1.49	1.62
RH-781	6.48	2.78	2.19	0.74	0.47	2.53	2.5	2.39	1.74	1.14	1.06	1.77
I-2	6.93	6.01	2.58	2.28	0.33	3.63	2.37	1.61	1.59	1.46	1.13	1.63
V-7	8.63	5.87	0.67	0.58	0.34	3.22	3.09	2.39	1.53	1.12	0.9	1.81
RB-50	7.7	7.49	2.27	1.42	1.02	3.98	2.96	2.5	2.26	1.64	1.26	2.12
RH-8113	11.63	2.15	1.43	0.84	0.87	3.38	2.23	1.62	1.55	1.54	1.45	1.68
RH-8812	7.61	5.87	2.83	1.74	1.03	3.82	2.01	1.81	1.66	1.39	1.24	1.62
RH-0406	8.51	1.89	1.83	0.81	0.69	2.75	1.73	1.65	1.51	1.11	0.99	1.4
PBR-357	7.64	6.6	1.97	1.14	1.04	3.68	2.27	2.06	1.97	1.67	1.64	1.92
ROHINI	8.77	5.93	1.53	1.16	0.67	3.61	2.55	2.46	1.73	1.71	1.2	1.93
VARUNA	8.57	3.64	1.28	0.49	0.49	2.89	2.85	2.83	2.17	1.66	1.31	2.16
PCR-7	9.7	1.85	0.89	0.58	0.49	2.7	2.92	2.11	1.71	1.32	1.19	1.85
RL-1359	5.99	5.93	0.69	0.63	0.48	2.74	2.42	2.33	1.44	1.44	1.28	1.78
RH-819	8.56	4.09	1.47	1.36	0.85	3.26	2.43	1.98	1.8	1.21	1.11	1.71
RH-30	8.33	5.23	2.16	1.09	0.57	3.48	2.36	2.21	1.69	1.45	1.41	1.82
Conc Mean	8.43	4.27	1.62	1.07	0.71	3.22	2.41	2.06	1.68	1.42	1.26	1.76
-	-	-	-	-	-	-	-	-	-	-	-	-
For means of	S.Em±	C.D.5%	-	C.V.%=	14.85	-	S.Em±	C.D.5%	-	C.V.%=	12.18	-
Variety(var)	0.12	0.34	-	-	-	-	0.06	0.15	-	-	-	-
Concentration (conc)	0.06	0.17	-	-	-	-	0.03	0.08	-	-	-	-
VarXConc	0.28	0.77	-	-	-	-	0.12	0.35	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-	-	-

Each data is an average of three replicates

Seed Germination Percentage: The statistical analysis (ANOVA) for seed germination percentage reveals that cultivars and Cd⁺² concentrations along with their interactions were highly significant. The effect of different concentrations of Cd⁺² on the germination percentage on 20 cultivars of *B. juncea* is shown in table-3. The germination was highest when seeds were grown in distilled water as control; however Cd⁺² treatments reduced seed germination of *B. juncea* at different concentrations. The inhibition of seed germination increased with increasing concentration of Cd⁺². It was observed that maximum germination percentage recorded is (91.33%) for Cultivar RH-0119 and minimum germination percentage recorded is (76%) for Cultivar PCR-7 at 2mM Cd⁺². Whereas Cultivar RB-50 showed better performance at all four concentrations of Cd⁺² treatments. The results of the present study clearly revealed that metal Cd⁺² negatively affect the seed germination in *Brassica juncea*. This observation is in similarity with the previous findings of Seyed *et al*¹⁵ who reported that germination rate was reduced in Canola (*Barassica napus*), Wheat (*Triticum aestivum*) and Safflower (*Carthamus*

tinctorios) with increase in concentration of Cd⁺². Reduction in seed germination is may be due to the interference and alterations in the cell membrane permeability properties by Cd⁺², which resulted in reduction of water absorption and transport¹⁶.

Seedling Fresh and Dry Matter: The statistical analysis (ANOVA) for seedling fresh and dry matter reveals that cultivars and Cd⁺² concentrations along with their interactions were highly significant. Effect of different concentrations of metal Cd⁺² on the fresh and dry weight of germinated seedlings of *B. juncea* is shown in table-4. Seedling fresh weight and dry weight were highest when seeds were treated and grown in distilled water as control; however Cd⁺² treatments reduced fresh weight and dry weight of *B. juncea* at different concentrations. The reduction was increased with increasing concentration of Cd⁺². Cultivar RB-50 showed minimum reduction in fresh and dry weight at all four concentrations of Cd⁺² treatments as compared to rest of the 19 Cultivars and Cultivar RH-819 showed maximum reduction in fresh weight

and Cultivar RH-781 showed maximum reduction in dry weight at all four concentrations of Cd⁺² treatments. The total dry matter accumulation was comparatively less affected by Cd⁺² in all 20 cultivars of *B.juncea*. It has been reported that with an increasing concentration of Cd⁺² biomass accumulations reduces and it may be the result of inhibition in chlorophyll synthesis and photosynthesis¹⁷

Tolerance Indices and Percent Phytotoxicity: The Tolerance indices and phytotoxicity, both are the parameters which depend on the root length of the seedlings. The phytotoxicity was found to be increased in all the 20 cultivars with increased in Cd⁺² supply figure-2. The present work showed that there exist a perfect negative correlation between Tolerance indices and Percent Phytotoxicity. The Cultivar RB-50 showed least phytotoxicity (86.8%) and high metal tolerance (13.2%) at 2mM concentration of Cd⁺² as compared to other 19 cultivars where as Cultivar RH-0119 showed highest phytotoxicity (95.02%) and least metal tolerance (4.98%) at 2mM concentration of Cd⁺² as compared to other 19 cultivars. The decrease in metal tolerance index and increase in percent phytotoxicity with increase in Cd⁺² concentrations in the root have been observed earlier in *Cicer arietinum* and *Phaseolus mungo*¹⁸.

Conclusion

The results of present investigation reveals that Cd⁺² treatments played an inhibitory role for the seed germination and seedling growth of *Brassica juncea* Cultivars. Cd⁺² treatments was found to be responsible for decrease in root- shoot length, seedling size, germination percentage, fresh and dry matter, and percentage of tolerance indices in all the *Brassica juncea* cultivars. The intensity of inhibition was found to be directly proportional to the concentration of Cd⁺² solutions employed. Results presented shows the existence of genotypical variations among *Brassica juncea* Cultivars with respect to tolerance in Cd⁺² toxicity. Results of present study also indicates that Cultivar RB-50 showed least reduction in Root Length, Shoot Length, Seedling Size, Fresh matter and Dry matter; It also showed high metal tolerance and least phytotoxicity as compared to other cultivars. Whereas, Cultivar RH-0406 showed maximum reduction in Root Length, Shoot Length and Seedling Size. These findings reveals that Cultivar RB-50 might be the most tolerant cultivar and may be successfully grown on Cd⁺² contaminated soils.

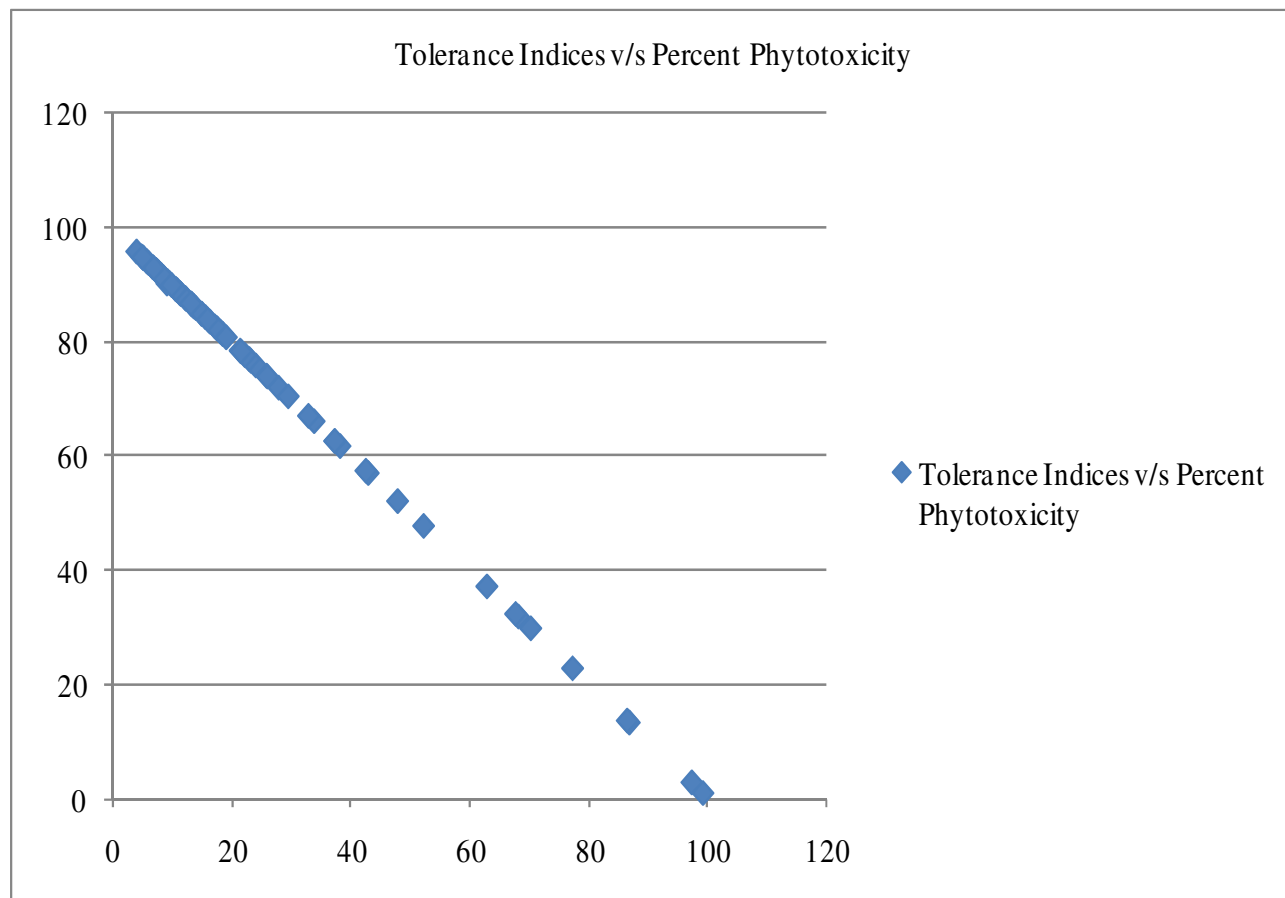


Figure-2
 Correlation graph of Tolerance Indices and Percent Phytotoxicity on *Brassica juncea* at different Cd⁺² concentrations

Table-2
Effect of different concentrations of Cd²⁺ on Seedling size of *Brassica juncea* cv

Varieties	Control	Cd 0.5mM	Cd 1mM	Cd 1.5mM	Cd 2mM	Variety Mean
RH-0749	13.54	7.84	3.17	2.79	2.33	5.93
Pusa Bold	10.52	4.98	3.42	3.22	2.89	5.01
GM-3	9.85	7.05	2.3	2.02	1.98	4.64
RH-0119	12.01	4.17	2.45	2.02	1.66	4.46
RH-9304	9.7	4.05	3.46	3.14	2.33	4.54
RGN-145	11.33	3.15	2.98	1.99	1.98	4.29
RH-781	8.99	5.18	3.93	1.88	1.53	4.3
I-2	9.31	7.62	4.17	3.73	1.46	5.26
V-7	11.75	8.27	2.2	1.7	1.25	5.03
RB-50	10.67	9.99	4.37	3.06	2.27	6.07
RH-8113	13.87	3.77	2.75	2.38	2.32	5.02
RH-8812	9.61	7.68	4.49	3.13	2.27	5.44
RH-0406	10.24	3.54	3.34	1.93	1.68	4.14
PBR-357	9.91	8.66	3.94	2.8	2.69	5.6
ROHINI	11.32	8.39	3.25	2.87	1.87	5.54
VARUNA	11.42	6.47	3.45	2.15	1.8	5.06
PCR-7	12.62	3.96	2.6	1.9	1.67	4.55
RL-1359	8.41	8.26	2.13	2.07	1.76	4.53
RH-819	10.99	6.07	3.27	2.58	1.96	4.97
RH-30	10.69	7.44	3.86	2.49	1.98	5.29
Conc Mean	10.84	6.33	3.28	2.49	1.98	4.98
For means of	S.Em±	C.D.5%	-	C.V.%=	10.26	-
Variety(var)	0.13	0.37	-	-	-	-
Concentration (conc)	0.07	0.18	-	-	-	-
VarXConc	0.3	0.82	-	-	-	-

Each data is an average of three replicates.

Table-3
Effect of different concentrations of Cd²⁺ on Germination percentage (φ-value) of *Brassica juncea* cv.

Varieties	Control	Cd 0.5mM	Cd 1mM	Cd 1.5mM	Cd 2mM	Variety Mean
RH-0749	77.71	71.57	68.86	66.14	66.14	70.09
Pusa Bold	75.00	68.86	68.86	66.14	63.43	68.46
GM-3	77.71	68.86	66.14	63.43	61.22	67.47
RH-0119	90.00	77.71	71.57	68.86	68.86	75.40
RH-9304	77.71	68.86	66.14	61.22	61.22	67.03
RGN-145	68.86	68.86	66.14	63.43	63.43	66.14
RH-781	90.00	77.71	68.86	66.14	63.43	73.23
I-2	90.00	68.86	66.14	66.14	63.43	70.91
V-7	77.71	68.86	63.43	63.43	63.43	67.37
RB-50	68.86	66.14	63.43	61.22	59.00	63.73
RH-8113	90.00	77.71	68.86	66.14	63.43	73.23
RH-8812	68.86	68.86	66.14	63.43	63.43	66.14
RH-0406	68.86	68.86	63.43	63.43	61.22	65.16
PBR-357	83.86	77.71	66.14	63.43	61.22	70.47
ROHINI	71.57	68.86	68.86	59.00	56.79	65.02
VARUNA	68.86	66.14	63.43	63.43	61.22	64.62
PCR-7	66.14	63.43	61.22	59.00	54.78	60.92
RL-1359	68.86	68.86	68.86	63.43	61.22	66.24
RH-819	71.57	66.14	63.43	61.22	61.22	64.72
RH-30	68.86	66.14	66.14	66.14	56.79	64.82
Conc Mean	76.05	69.95	66.30	63.74	61.74	67.56
For means of	S.Em±	C.D.5%	-	C.V.%=	7.24	-
Variety (var)	1.26	3.52	-	-	-	-
Concentration(conc)	0.63	1.76	-	-	-	-
VarXConc	2.82	7.88	-	-	-	-

Each data is an average of three replicates.

Table-4
Effect of different concentrations of Cd⁺² on Fresh and Dry matter on *Brassica juncea* cv

Fresh matter Varieties	Dry matter Control	Cd 0.5mM	Cd 1mM	Cd 1.5mM	Cd 2mM	Variety Mean	Control	Cd 0.5mM	Cd 1mM	Cd 1.5mM	Cd 2mM	Variety Mean
RH-0749	0.313	0.292	0.281	0.202	0.196	0.257	0.063	0.062	0.059	0.055	0.049	0.058
Pusa Bold	0.27	0.198	0.161	0.159	0.13	0.184	0.053	0.05	0.045	0.043	0.039	0.046
GM-3	0.327	0.303	0.224	0.192	0.189	0.247	0.062	0.062	0.061	0.058	0.053	0.059
RH-0119	0.59	0.117	0.115	0.086	0.082	0.198	0.046	0.046	0.045	0.045	0.044	0.045
RH-9304	0.176	0.213	0.206	0.204	0.194	0.199	0.056	0.05	0.049	0.048	0.048	0.05
RGN-145	0.279	0.267	0.213	0.239	0.202	0.24	0.043	0.038	0.037	0.036	0.034	0.037
RH-781	0.373	0.261	0.219	0.191	0.171	0.243	0.046	0.041	0.034	0.021	0.02	0.032
I-2	0.265	0.229	0.213	0.196	0.189	0.218	0.056	0.049	0.045	0.044	0.043	0.047
V-7	0.255	0.233	0.215	0.159	0.144	0.201	0.051	0.046	0.045	0.045	0.043	0.046
RB-50	0.182	0.211	0.199	0.184	0.182	0.191	0.049	0.045	0.046	0.044	0.042	0.045
RH-8113	0.244	0.218	0.193	0.184	0.165	0.201	0.064	0.063	0.063	0.063	0.058	0.062
RH-8812	0.263	0.22	0.197	0.184	0.165	0.206	0.052	0.05	0.045	0.043	0.042	0.046
RH-0406	0.286	0.23	0.213	0.207	0.178	0.223	0.061	0.055	0.053	0.051	0.05	0.054
PBR-357	0.319	0.317	0.302	0.232	0.225	0.279	0.054	0.053	0.049	0.046	0.045	0.049
ROHINI	0.279	0.26	0.219	0.212	0.209	0.236	0.058	0.054	0.046	0.044	0.039	0.048
VARUNA	0.333	0.307	0.241	0.23	0.226	0.268	0.045	0.04	0.036	0.038	0.038	0.039
PCR-7	0.492	0.364	0.271	0.27	0.191	0.318	0.062	0.058	0.048	0.047	0.042	0.051
RL-1359	0.274	0.272	0.199	0.194	0.173	0.222	0.055	0.05	0.049	0.046	0.044	0.049
RH-819	0.177	0.176	0.165	0.101	0.055	0.135	0.051	0.048	0.044	0.044	0.042	0.046
RH-30	0.315	0.253	0.251	0.228	0.222	0.254	0.055	0.054	0.054	0.051	0.049	0.053
Conc Mean	0.301	0.247	0.215	0.193	0.174	0.226	0.054	0.051	0.048	0.046	0.043	0.048
-	-	-	-	-	-	-	-	-	-	-	-	-
For means of Variety(var)	S.Em±	C.D.5%	-	C.V.%=	15.609	-	S.Em±	C.D.5%	-	C.V.%=	8.712	-
Concentrations (conc)	0.005	0.013	-	-	-	-	0.001	0.002	-	-	-	-
VarXConc	0.02	0.057	-	-	-	-	0.002	0.007	-	-	-	-

Each data is an average of three replicates.

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