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RESEARCH PAPER

Screening for Appropriate Dose of Chitosan to Remediate Cadmium Toxicity in Pea Genotypes through Seed Priming

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ABSTRACT

Six different concentrations of chitosan and five different concentrations of cadmium were selected and studied to determine the optimum concentration of chitosan for remediating cadmium toxicity through seed application. Results revealed that the morphological parameters (germination%, root length, shoot length, seedling vigour index, fresh weight and dry weight of seedlings) differed significantly among treatments and genotypes. Lower concentrations of chitosan (0.1% and 0.2%) were more effective in mitigating cadmium toxicity than higher concentrations in pea genotypes in terms of parameters studied as above. Chitosan mitigated adverse effects of cadmium in both the selected pea genotypes. The Cd 200 μM concentration was most detrimental for both the genotypes. Based on our results, we conclude that chitosan with lower concentrations has significant positive effects on all the parameters in response to cadmium toxicity and proves to be an important aspect for remediation of cadmium toxicity.

HIGHLIGHTS

- Cadmium toxicity is extremely detrimental to pea genotypes.
- Chitosan is an important biopolymer that mitigated toxic effects of cadmium in pea genotypes.
- Lower concentrations of chitosan (0.1% and 0.2%) were more effective in mitigating cadmium toxicity than higher concentrations in pea genotypes.

Keywords: Chitosan, Remediation, cadmium toxicity, Biopolymer

Pisum sativum, the common pea (also known as the garden or field pea), is an herbaceous annual from family Fabaceae, originated from the Mediterranean basin and Near East. It is a cool season crop now grown in many parts of the world. Pea is the third most important pulse crop at global level, after dry bean and chickpea and third most popular *rabi* pulse of India after chick pea and lentil. It is cultivated in more than 90 countries with estimated global annual production of 13.5 million metric tonnes (FAOSTAT, 2018). *Pisum sativum* is quite nutritious and contains fair amount of fibres, antioxidants and proteins. Pea proteins are composed of 7S/11S globulin (salt-soluble, 65% to 80% of total) and albumin 2S (water-soluble, 10% to 20%) protein classes and high levels of lysine (Ge *et al.* 2020). Regular dietary intake of pea proteins reduces the risk of chronic diseases and enhances human health. In addition to improving human health, proteins present in pea also play potential role in food processing (Burger & Zhang 2019; Ladjal-Ettoumi, Boudries, Chibane, & Romero, 2016; Lam, Karaca, Tyler, & Nickerson 2018; Lam, Warkentin, Tyler & Nickerson 2017). Like many legumes, it consists of symbiotic bacteria that have ability to fix atmospheric nitrogen.

Recent advancement of agriculture has led to increase in significant amount of heavy metals in the soil. Among heavy metals, cadmium pollution is

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a global environmental issue, and the development of modern industry and agriculture has led to more and more water and soil being polluted by cadmium every year. Cadmium can accumulate for long periods of time inside animals and plants, affecting growth and development and posing a great danger to human health. Cadmium toxicity induces changes in stress related enzyme activities and ultrastructure of pea root cells (Głowacka *et al.* 2019). Interaction of cadmium with plants leads to disruption in nutritional imbalance, impaired photosynthesis and disruption in membrane structural integrity. Application of biostimulators and elicitors could be a promising way to improve crop growth and yield without affecting soil health and environment and to cope up with the changes in environmental stresses (Hidangmayum and Richa 2017). Chitosan is a natural molecule which induces numerous biological responses in plants and plays important role in bioremediation. Chitosan is produced from chitin, an important component of crustacean shells, such as crab, shrimp and crawfish, and is mainly made up of (1–4)-2-amino-2-deoxy-β-D-glucan. Chitosan and its oligosaccharides have received much interest for potential application in agriculture, biomedicine and biotechnology due to their biocompatibility, biodegradability and bioactivity (Hidangmayum *et al.* 2019). Recent findings suggested that chitosan has evolved as promising elicitors in the field of agriculture. Chitosan was first characterized as an elicitor in plant (Limpanavech *et al*. 2008). It was reported to improve soil fertility and enhance nutrient uptake by plant (Dzung 2007), increase yield and quality of crops including cowpea (*Vigna unguiculata*), potato (*Solanum tuberosum*), common bean (*Phaseolus vulgaris*) and wheat (*Triticum aestivum*) under normal or stress conditions (Muriefah 2013). A hydroponic pot experiment was conducted to study the roles of chitosan solution with different molecular weight (MW) (10 kDa, 5 kDa and 1 kDa) in alleviating Cd toxicity in edible rape (*Brassica rapa* L .) which showed that chitosan treatment significantly mitigated cadmium induced stress (Zong *et al.* 2017). Chitoligosaccharides, a water soluble derivative of chitin (0.01%, 0.05%, 0.1%) has positive effect on mineral accumulation on hydroponically grown *Phaseolus* plants (Chatelain *et al.* 2014). Chitosan has evolved as a natural molecule which induces numerous biological responses in plants, depending on its structure and concentration and on species and developmental stage of the plant. Interesting results were obtained with chitosan treated plants against abiotic stresses. In *Pisum sativum*, chitosan at low concentration mitigated Cd toxicity by promoting antioxidant defense system, improving growth attributes and photosynthetic parameters (Rasheed *et al*. 2020). Chitosan application alleviates adverse effects of cadmium and promotes growth of wheat seedlings by enhancing antioxidant system and osmotic adjustment in cadmium stressed plants (Liu *et al*. 2021). Similarly, Qu *et al.* (2019) studied the role of chitosan in alleviating cadmium toxicity in maize seedlings. Furthermore, the changing climate conditions have adverse impact on crop growth and productivity, especially legumes like pea. Thus, the use of chitosan in seed technology through seed treatment methods provide new avenues and formulate coating methods for management of crops along with enhancement of physiological and functional responses during crop growth. Thus, the present study has been performed to identify the optimum concentrations of chitosan in different pea genotypes in response to cadmium toxicity by observing germination and seedling growth attributes.

MATERIALS AND METHODS

The experiment was performed in the lab of Stress Physiology, Department of Plant Physiology, Banaras Hindu University located in the south-eastern of the Varanasi, between 25.18°N latitude, 83.03°E longitude and 123.93 m above mean sea level. Chitosan of low molecular weight and deacetylation ≥ 75% was obtained from Sigma-Aldrich Co (St Louis, MO, USA). Cadmium chloride was obtained from Lobachemie. Genotypes of pea (*Pisum sativum* L.) (HUP-2/ HUDP-15) were obtained from the Department of Genetics and Plant Breeding, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, Uttar Pradesh. All the chemicals used were of analytical grade. The chitosan was dissolved in 0.5% acetic acid solution under constant stirring overnight and was adjusted to pH 5.6-6.0 using 1N sodium hydroxide. Cadmium concentration 25, 50, 75, 100, 200 μM were also prepared. Seeds were primed in different concentrations of chitosan viz. 0.1% 0.2% 0.3% 0.4% 0.5% 1.0% and control with distilled water for 12 h at room temperature in dark. After that seeds were thoroughly washed with distilled water and dried to their original weight. 20 seeds of each genotype were placed in two layers of Whatman no. 1 filtered paper placed in petri dishes moistened with distilled water. Cd stress was given at concentration 0, 25, 50, 75, 100, 200 μM in the petriplates. Chitosan along with Cd concentration was kept in order to determine suitable concentration of chitosan which could remediate Cd stress. Finally petriplates were placed in controlled germination incubator at 22°C±1°C. Taking emergence of 2 mm radicle from seed coat as criteria for germination and germination counts, were recorded. All the treatments were replicated three times. After 7 days, germination and seedlings parameters were measured. Treatments details are as follow:

Genotype: Two

Chitosan concentration: Six

Cadmium concentration: Five

T_0	**Control**
T_1	0.1 % chitosan
T_2	0.2 % chitosan
T_3	0.3 % chitosan
T_4	0.4 % chitosan
T_5	0.5 % chitosan
T_6	1.0 % chitosan
T_7	25 μM Cadmium
T_8	50 μM Cadmium
T_9	75 μM Cadmium
T_{10}	100 μM Cadmium
T_{11}	200 μM Cadmium
T_{12}	0.1 % chitosan + 25 μM Cadmium
T_{13}	0.2 % chitosan + 25 μM Cadmium
T_{14}	0.3 % chitosan + 25 μM Cadmium
T_{15}	0.4 % chitosan + 25 μM Cadmium
T_{16}	0.5 % chitosan + 25 μM Cadmium
T_{17}	1.0 % chitosan + 25 μM Cadmium
T_{18}	0.1 % chitosan + 50 μM Cadmium
T_{19}	0.2 % chitosan + 50 μM Cadmium
T_{20}	0.3 % chitosan + 50 μM Cadmium
T_{21}	0.4 % chitosan + 50 μM Cadmium
T_{22}	0.5 % chitosan + 50 μM Cadmium
T_{23}	1.0 % chitosan + 50 μM Cadmium
T_{24}	0.1 % chitosan + 75 μM Cadmium
T_{25}	0.2 % chitosan + 75 μM Cadmium
T_{26}	0.3 % chitosan + 75 μM Cadmium
T_{27}	0.4 % chitosan + 75 μM Cadmium
T_{28}	0.5 % chitosan + 75 μM Cadmium
T_{29}	1.0 % chitosan + 75 μM Cadmium
T_{30}	0.1 % chitosan + 100 μM Cadmium
T_{31}	0.2 % chitosan + 100 μM Cadmium
T_{32}	0.3 % chitosan + 100 μM Cadmium
T_{33}	0.4 % chitosan + 100 μM Cadmium
T_{34}	0.5 % chitosan + 100 μM Cadmium
T_{35}	1.0 % chitosan + 100 μM Cadmium
T_{36}	0.1 % chitosan + 200 μM Cadmium
T_{37}	0.2 % chitosan + 200 μM Cadmium
T_{38}	0.3 % chitosan + 200 μM Cadmium
T_{39}	0.4 % chitosan + 200 μM Cadmium
T_{40}	0.5 % chitosan + 200 μM Cadmium
T_{41}	1.0 % chitosan + 200 μM Cadmium

1. Germination percentage

The germination percentage of both pea genotypes was observed by following given formula:

Germination % = (Total number of seed germinated/ Total number of seed sown) × 100

2. Seedling Vigor Index (SVI)

Seedling vigor index of 7-day old seedling was measured and calculated by the formula given as under:

SVI= germination % × Seedling lengths (Root length + shoot length).

3. Measurement of Radicle Length and Plumule Length (cm)

Root length and shoot length of pea seedling was measured by the help of thread and centimeter scale.

4. Measurement of fresh weight and dry weight of pea seedlings (g plant^{-1})

The fresh and dry weight of seedlings were measured and expressed in g plant^{-1}. The fresh weight of seedlings was measured using Sartorious BT-224S Electronic Balance. Freshly weighed seedlings were placed in the envelope and kept in a hot air oven for one hour at 100°C. The temperature was then decreased to 65°C until the constant weight of the samples was achieved.

5. Statistical analysis

The data presented was subjected to one way ANOVA and the treatment means were compared using design CRD (Completely randomized design) at significant level of 5% ($p < 0.05$).

RESULTS

1. Germination %

Germination % of both genotypes is represented in the (Table 1). There was a considerable level of variation in magnitude of responses among pea genotypes to different concentrations of chitosan. The chitosan concentration 0.1% and 0.2% (T_1 &T_2) recorded highest germination % in both genotypes as compared to rest of the other treatments. HUDP-15 produced higher germination % than HUP-2. Meanwhile, chitosan concentrations 0.5% and 1% (T_5 & T_6) recorded lowest level of germination %. Moreover, the germination % of both pea genotypes decreased as concentration of cadmium toxicity increased, recording lowest germination for cadmium concentration 200 μM (T_{11}). When different concentrations of chitosan were combined with varying levels of cadmium, chitosan concentration 0.1% and 0.2% in both genotypes performed better with all the concentrations of cadmium as compared to other concentrations of chitosan.

Table 1: Germination % in pea genotypes with respect to treatments

	Germination %	
Treatments	**HUDP-15**	**HUP-2**
T_0	96.667 ± 3.333	96.000 ±3.055
T_1	98.333 ± 1.202	97.667±1.453
T_2	97.333±1.202	97.000±1.000
T_3	96.667±0.882	95.667±2.848
T_4	90.000±0.000	89.000±0.577
T_5	88.667±0.333	87.333±0.667
T_6	81.333±5.696	67.000±3.512
T_7	99.000±0.577	95.667±2.963
T_8	98.333±0.667	93.333±1.667
T_9	95.33±0.333	92.667±1.764
T_{10}	90.000±5.000	85.667±2.333
T_{11}	87.667±0.333	83.333±1.667
T_{12}	96.667±1.667	95.000±2.887
T_{13}	96.333±1.333	94.000±3.055
T_{14}	95.333±0.333	93.333±1.667
T_{15}	89.667±3.180	84.000±1.000
T_{16}	88.000±1.155	82.333±2.333
T_{17}	78.667±1.333	78.333±1.667
T_{18}	94.000±1.000	85.667±0.667
T_{19}	91.667±1.667	82.667±2.667
T_{20}	88.000±1.000	76.667±4.410
T_{21}	83.667±0.882	71.667±1.667
T_{22}	85.667±0.333	70.000±0.000
T_{23}	77.667±3.844	66.667±1.667
T_{24}	88.667±1.333	75.000±0.000
T_{25}	86.000±0.000	76.333±1.333
T_{26}	79.000±0.577	71.667±1.667
T_{27}	76.333±1.333	71.667±1.667
T_{28}	71.667±0.882	70.000±0.000
T_{29}	61.333±0.667	60.000±0.000
T_{30}	76.000±6.000	75.000±0.000
T_{31}	70.000±0.000	63.333±1.667
T_{32}	69.000±0.577	63.333±1.667
T_{33}	65.000±0.000	61.667±1.667
T_{34}	55.000±2.887	52.333±2.333
T_{35}	55.000±2.887	51.667±1.667
T_{36}	48.333±1.667	43.333±3.333
T_{37}	47.333±1.453	43.333±3.333
T_{38}	46.000±0.577	41.667±1.667
T_{39}	45.000±0.000	40.000±0.000
T_{40}	45.000±5.000	38.333±4.410
T_{41}	33.333±3.333	33.333±3.333
CD (0.05)	6.305	6.110
CV	4.938	5.110
S/NS	S	S

2. Seedling Vigour index (SVI)

SVI of both genotypes was found highest in treatment T_1 (0.1%) and T_2 (0.2%) among different concentrations of chitosan in (Table 2). The chitosan 1% showed least SVI among respective chitosan treatments in both the genotypes (T_6). It decreased significantly with increase in cadmium toxicity and found lowest in treatment T11 (200μM). The genotype HUDP-15 showed higher SVI than genotype HUP-2 in the respective treatments. Under combined effect of chitosan with cadmium, the concentration 0.1% and 0.2% chitosan showed better SVI than other concentrations in both the genotypes inresponse to different cadmium concentrations.

Table 2: Seedling vigour index in pea genotypes

	Seedling vigour index (SVI)	
Treatments	**HUDP-15**	**HUP-2**
T_0	911.667±28.333	868.067±42.774
T_1	943.967±11.572	908.533±22.212
T_2	918.200±12.601	902.000±9.406

T_3	860.467±17.252	845.167±27.126
T_4	777.000±12.000	759.400±11.904
T_5	750.700±2.902	698.533±15.602
T_6	636.200±38.735	518.267±28.456
T_7	824.833±9.597	793.033±9.630
T_8	793.200±4.715	678.167±10.933
T_9	724.600±16.332	660.733±12.095
T_{10}	595.833±23.278	519.667±14.333
T_{11}	464.733±19.554	386.000±5.000
T_{12}	808.833±17.622	756.667±20.276
T_{13}	802.733±9.872	782.933±19.157
T_{14}	784.900±8.307	743.500±14.344
T_{15}	732.400±27.826	669.067±10.112
T_{16}	698.200±11.816	642.433±22.552
T_{17}	584.667±8.667	566.667±13.532
T_{18}	770.700±4.486	648.167±2.167
T_{19}	718.667±35.225	622.667±18.809
T_{20}	668.600±5.188	566.667±27.187
T_{21}	638.567±4.477	523.167±12.167
T_{22}	611.100±4.539	471.333±2.333
T_{23}	424.433±19.732	359.833±5.890
T_{24}	659.000±7.000	535.000±2.500
T_{25}	642.133±2.867	544.467±8.497
T_{26}	571.633±18.685	484.833±9.714
T_{27}	549.600±9.600	465.833±10.833
T_{28}	494.400±2.203	434.000±4.041
T_{29}	308.667±1.333	284.000±7.211
T_{30}	522.600±29.725	467.500±2.500
T_{31}	476.000±10.693	386.167±7.328
T_{32}	448.500±3.753	346.000±5.000
T_{33}	400.833±4.333	328.667±5.812
T_{34}	322.333±13.956	270.467±13.671
T_{35}	275.167±16.412	244.333±5.207
T_{36}	246.167±6.930	196.000±12.220
T_{37}	236.400±3.451	197.667±13.667
T_{38}	207.033±4.583	165.500±9.777
T_{39}	199.500±10.817	140.000±4.619
T_{40}	163.833±19.833	122.667±19.238
T_{41}	92.667±11.795	79.000±8.544
CD (0.05)	43.9673	42.4785
CV	4.688	5.091
S/NS	S	S

3. Radicle length

Significant variations in radicle length were observed in both genotypes. In HUP-2, the radicle length was higher in T_2, T_3, T_4, T_9 and T_{13} treatments as compared to genotype HUDP-15. Radicle length of both genotypes decreased with increased cadmium toxicity and recorded least in T_{11} and under combined effect of chitosan with cadmium, the concentration 0.1% and 0.2% chitosan showed increased radicle length than other concentrations in both the genotypes in response to different cadmium concentrations which is shown in (Table 3).

Table 3: Radicle length for both pea genotypes

	Radicle length (cm)	
Treatments	**HUDP-15**	**HUP-2**
T_0	4.900 ±0.058	4.800±0.115
T_1	4.967±0.033	4.867±0.067
T_2	4.833±0.033	4.933±0.033
T_3	4.767±0.033	4.800±0.000
T_4	4.567±0.033	4.600±0.100
T_5	4.500±0.000	4.467±0.067
T_6	4.300±0.100	4.233±0.033
T_7	4.733±0.067	4.733±0.120
T_8	4.500±0.000	4.367±0.033
T_9	4.267±0.033	4.300±0.058
T_{10}	3.800±0.115	3.433±0.033
T_{11}	3.300±0.252	3.067±0.033
T_{12}	4.367±0.088	4.067±0.033
T_{13}	4.367±0.033	4.467±0.033
T_{14}	4.300±0.058	4.200±0.058
T_{15}	4.233±0.033	4.200±0.000
T_{16}	4.200±0.000	4.133±0.067
T_{17}	3.933±0.067	3.800±0.000
T_{18}	4.267±0.067	3.933±0.033
T_{19}	4.233±0.033	3.900±0.058
T_{20}	4.033±0.088	3.867±0.088
T_{21}	4.100±0.000	3.833±0.033
T_{22}	4.000±0.000	3.633±0.033
T_{23}	2.500±0.000	2.500±0.000
T_{24}	4.033±0.033	3.800±0.000
T_{25}	4.100±0.058	3.800±0.000
T_{26}	3.933±0.133	3.600±0.000
T_{27}	4.000±0.000	3.500±0.000
T_{28}	4.000±0.000	3.500±0.000
T_{29}	2.500±0.000	2.300±0.100
T_{30}	3.800±0.115	3.433±0.033
T_{31}	3.700±0.100	3.267±0.033
T_{32}	3.600±0.000	2.900±0.058
T_{33}	3.500±0.000	2.900±0.058
T_{34}	3.467±0.033	2.933±0.067
T_{35}	3.000±0.000	2.933±0.067
T_{36}	2.933±0.067	2.600±0.058

T_{37}	2.900±0.058	2.600±0.058
T_{38}	2.633±0.033	2.400±0.058
T_{39}	2.633±0.186	2.133±0.033
T_{40}	2.033±0.033	1.867±0.088
T_{41}	1.567±0.067	1.433±0.033
CD (0.05)	0.2066	0.155
CV	3.334	2.657
S/NS	S	S

4. Plumule length

In HUDP-15, chitosan concentration 0.1% (T_1) and 0.2% (T_2) showed higher plumule length as compared to other chitosan treatments (Table 4). Similar results were obtained in genotype HUP-2, and it showed least growth in T_6. Moreover, cadmium toxicity reduced plumule length and recorded least in T_{11} in the both genotypes. Under combined effects of chitosan with cadmium concentrations, treatments containing 0.1% and 0.2% chitosan performed better by increasing plumule length among different chitosan concentrations in varying cadmium concentrations in both the genotypes.

Table 4: Plumule length of pea genotypes

	Plumule length (cm)	
Treatments	**HUDP-15**	**HUP-2**
T_0	4.533 ± 0.088	4.233±0.120
T_1	4.633±0.067	4.433±0.033
T_2	4.600±0.058	4.367±0.067
T_3	4.133±0.088	4.033±0.033
T_4	4.067±0.120	3.933±0.067
T_5	3.967±0.033	3.533±0.267
T_6	3.533±0.033	3.500±0.000
T_7	3.600±0.100	3.567±0.067
T_8	3.567±0.033	2.900±0.058
T_9	3.333±0.167	2.833±0.088
T_{10}	2.833±0.033	2.633±0.033
T_{11}	2.000±0.058	1.567±0.067
T_{12}	4.000±0.000	3.900±0.000
T_{13}	3.967±0.033	3.867±0.033
T_{14}	3.933±0.088	3.767±0.033
T_{15}	3.933±0.033	3.767±0.133
T_{16}	3.733±0.033	3.667±0.033
T_{17}	3.500±0.000	3.433±0.033
T_{18}	3.933±0.033	3.633±0.033
T_{19}	3.600±0.306	3.633±0.033
T_{20}	3.567±0.033	3.533±0.033
T_{21}	3.533±0.067	3.467±0.033
T_{22}	3.133±0.033	3.100±0.058
T_{23}	2.967±0.033	2.900±0.058
T_{24}	3.400±0.058	3.333±0.033
T_{25}	3.367±0.067	3.333±0.033
T_{26}	3.300±0.058	3.167±0.067
T_{27}	3.200±0.000	3.000±0.000
T_{28}	2.900±0.058	2.700±0.058
T_{29}	2.533±0.033	2.433±0.033
T_{30}	3.100±0.058	2.800±0.000
T_{31}	3.100±0.058	2.833±0.033
T_{32}	2.900±0.000	2.567±0.033
T_{33}	2.667±0.067	2.433±0.033
T_{34}	2.400±0.058	2.233±0.033
T_{35}	2.000±0.058	1.800±0.058
T_{36}	2.167±0.088	1.933±0.033
T_{37}	2.100±0.058	1.967±0.033
T_{38}	1.867±0.033	1.567±0.067
T_{39}	1.800±0.058	1.367±0.088
T_{40}	1.600±0.058	1.300±0.058
T_{41}	1.200±0.058	0.933±0.033
CD (0.05)	**0.218**	**0.189**
CV	4.201	3.875
S/NS	S	S

5. Fresh weight

In HUP-2 the freshweight increased in the treatments T_1,T_4, T_5 and T_6 as compared to HUDP-15. In HUDP-15 it was recorded higher in T_1. The fresh weight of both genotypes decreased with increased cadmium concentration and recorded least in T_{11}. Among the treatments combined effect of chitosan with cadmium (T_{41}) showed the least fresh weight whereas treatments containing 0.1% and 0.2% chitosan performed better by increasing fresh weight among different chitosan concentrations in varying cadmium concentrations in both the genotypes (Table 5).

Table 5: Fresh weight for pea genotype seedlings

	Fresh weight (g plant^{-1})	
Treatments	**HUDP-15**	**HUP-2**
T_0	0.109±0.038	0.083±0.004
T_1	0.172±0.054	0.184±0.065
T_2	0.171±0.054	0.113±0.014
T_3	0.095±0.008	0.073±0.014
T_4	0.094±0.005	0.095±0.008
T_5	0.078±0.018	0.093±0.003

T_6	0.065±0.028	0.077±0.013
T_7	0.085±0.012	0.083±0.004
T_8	0.084±0.004	0.073±0.002
T_9	0.057±0.001	0.052±0.001
T_{10}	0.054±0.001	0.052±0.001
T_{11}	0.038±0.001	0.035±0.004
T_{12}	0.065±0.003	0.059±0.000
T_{13}	0.063±0.002	0.056±0.002
T_{14}	0.052±0.000	0.051±0.000
T_{15}	0.052±0.002	0.051±0.002
T_{16}	0.052±0.002	0.049±0.000
T_{17}	0.050±0.000	0.048±0.000
T_{18}	0.064±0.002	0.051±0.001
T_{19}	0.066±0.003	0.050±0.001
T_{20}	0.063±0.002	0.059±0.000
T_{21}	0.049±0.000	0.049±0.000
T_{22}	0.048±0.000	0.048±0.000
T_{23}	0.039±0.000	0.039±0.000
T_{24}	0.045±0.003	0.044±0.003
T_{25}	0.049±0.000	0.049±0.000
T_{26}	0.044±0.001	0.043±0.001
T_{27}	0.044±0.002	0.041±0.002
T_{28}	0.037±0.001	0.037±0.001
T_{29}	0.037±0.001	0.036±0.001
T_{30}	0.030±0.000	0.030±0.000
T_{31}	0.029±0.001	0.029±0.001
T_{32}	0.029±0.000	0.027±0.001
T_{33}	0.027±0.001	0.027±0.000
T_{34}	0.025±0.000	0.024±0.001
T_{35}	0.020±0.005	0.020±0.006
T_{36}	0.019±0.000	0.019±0.001
T_{37}	0.020±0.000	0.018±0.000
T_{38}	0.020±0.001	0.017±0.000
T_{39}	0.016±0.001	0.014±0.001
T_{40}	0.012±0.000	0.011±0.000
T_{41}	0.009±0.000	0.008±0.001
CD (0.05)	0.04053	0.03069
CV	46.0186	37.5142
S/NS	S	S

6. Dry weight

There was no significant difference between dry weight in T_0 in both the genotypes. In HUDP-15, T_1 and T_2 showed similar results. HUP-2 performed better in various treatments T_3, T_4, T_6, T_8, T_{26}, T_{28} than HUDP-15. With increased Cd toxicity, dry weight showed significant decrease and showed the least in T_{11} in both the genotypes. Under combined effects of different chitosan concentrations with varying cadmium concentrations, T_{41} showed least dry weight among all the treatments whereas treatments containing 0.1% and 0.2% chitosan performed better by increasing dry weight among different chitosan concentrations in varying cadmium concentrations in both the genotypes (Table 6).

Table 6: Dry weight of pea seedlings of both genotypes

	Dry weight (g plant^{-1})	
Treatments	**HUDP-15**	**HUP-2**
T_0	0.061±0.004	0.061±0.004
T_1	0.068±0.004	0.061±0.001
T_2	0.068±0.011	0.056±0.001
T_3	0.051±0.012	0.056±0.001
T_4	0.049±0.010	0.057±0.005
T_5	0.038±0.001	0.038±0.001
T_6	0.029±0.001	0.030±0.001
T_7	0.045±0.005	0.044±0.004
T_8	0.033±0.001	0.034±0.002
T_9	0.033±0.000	0.032±0.000
T_{10}	0.028±0.000	0.027±0.000
T_{11}	0.019±0.000	0.019±0.001
T_{12}	0.062±0.002	0.060±0.000
T_{13}	0.061±0.002	0.060±0.000
T_{14}	0.056±0.002	0.054±0.000
T_{15}	0.054±0.000	0.051±0.001
T_{16}	0.049±0.000	0.048±0.000
T_{17}	0.038±0.000	0.035±0.002
T_{18}	0.047±0.006	0.040±0.000
T_{19}	0.044±0.007	0.040±0.000
T_{20}	0.039±0.000	0.039±0.000
T_{21}	0.038±0.001	0.039±0.000
T_{22}	0.036±0.000	0.036±0.000
T_{23}	0.023±0.006	0.022±0.006
T_{24}	0.032±0.000	0.031±0.001
T_{25}	0.031±0.001	0.030±0.001
T_{26}	0.031±0.001	0.036±0.001
T_{27}	0.031±0.001	0.029±0.000
T_{28}	0.026±0.001	0.027±0.001
T_{29}	0.027±0.001	0.026±0.001
T_{30}	0.027±0.000	0.024±0.001
T_{31}	0.025±0.000	0.023±0.001
T_{32}	0.021±0.000	0.020±0.000
T_{33}	0.020±0.001	0.019±0.001

T_{34}	0.019±0.001	0.018±0.001
T_{35}	0.017±0.000	0.017±0.000
T_{36}	0.020±0.001	0.019±0.000
T_{37}	0.021±0.002	0.018±0.000
T_{38}	0.019±0.002	0.017±0.001
T_{39}	0.018±0.000	0.017±0.001
T_{40}	0.015±0.000	0.014±0.000
T_{41}	0.011±0.000	0.010±0.000
CD (0.05)	0.01026	0.00474
CV	17.9507	8.55351
S/NS	S	S

DISCUSSION

Chitosan is the naturally occurring polysaccharide which plays important role in plant growth and development (Pichyangkura and Chadchawan, 2015; Katiyar *et al.* 2015; Malerba and Cerana, 2016). It is a potent plant growth promoter and has significant role in plant metabolism. Chitosan application enhance growth parameters such as root length and shoot length, fresh weight and dry weight of and shoots roots in bean, Mentha and lemon grass (Sheikha & Al-Malki, 2011, Ahmad *et al.* 2017; Jaleel *et al.* 2017). Seed priming with chitosan enhanced seedling establishment, uniform seed germination and stress resistance in many crops like wheat, maize, pepper, soybean and spinach (Hindangmayum *et al*. 2022). The present experiment was conducted to identify the optimum concentration of chitosan having beneficial effects on germination and seedling growth parameters in addition to comprehend the effects of different concentrations of chitosan on mitigation of cadmium toxicity. According to results, germination percentage and seedling vigour index were highest in optimum concentration of chitosan (0.1% and 0.2%) while highest concentration (1%) showed negative effect, although this varied with genotype specific responses. Jogaiah *et al*. (2020) reported that seed priming with chitosan increased germination % and vigor index in cucumber seeds. Soybean seeds coated with chitosan showed increased yield, seed germination, and plant growth (Zeng, Luo, & Tu 2012). Chitosan treatment also resulted in increased radicle and plumule length. Higher concentration of chitosan decreased radicle and plumule length in both the pea genotypes (HUDP-15 and HUP-2). Cadmium is a toxic element that causes inhibition of plant growth by altering with plant metabolism. Cadmium toxicity causes decreased root growth and inhibits root elongation (Grat˜ao *et al.* 2009; Shanying *et al.* 2017), inhibits seed germination by interfering with imbibitions in species like *Dorycnium pentaphyllum* (Lef`ever *et al*. 2009). Findings from present experiment indicated that germination % and seedling growth parameters decreased with increased cadmium toxicity in HUDP-15 and HUP-2. Cd induced growth reduction has been documented in many species like pea, wheat, maize, rice and tomato (Jan *et al.* 2018; Tajti *et al.* 2018; Qu *et al.* 2018; Fu *et al.* 2019; Ahmad *et al.* 2018). Chitosan is an excellent biopolymer and plant elicitor which circumvented the toxic effects of cadmium in the plants. Lower doses of chitosan mitigated the adverse effect of cadmium in pea plant by enhancing growth parameters like fresh weight and dry weight of radicle and plumule (Rasheed *et al.* 2020). Similar results have been obtained in the present study implying that lower concentrations of chitosan (0.1% and 0.2%) were more effective than higher concentrations in mitigating cadmium stress by minimizing the negative effect of cadmium on germination % and seedling growth parameters in HUDP-15 and HUP-2 genotypes. This result is consistent with the findings that chitosan mitigated adverse effects of cadmium in flamingo anthurium (Gu *et al.* 2010), rapeseed (*Brassica napus*) (Shaheen and Rinklebe 2015; Zong *et al.* 2017), radish (Farouk *et al.* 2011). Therefore, the present evidences are strongly supported by results on germination and seedling growth parameters from previous studies.

CONCLUSION

Chitosan is a natural biopolymer that enhances germination and growth of pea plants. Treatments consisting 0.1% and 0.2% chitosan had significant effect on growth in both genotypes. Although higher concentration of chitosan had negative impact on genotypes which decreased germination % and other growth parameters. Seed treatments with low levels of chitosan had beneficial effect on seedling growth concluding that 0.1% and 0.2% were optimum concentrations of chitosan beneficial for pea genotypes. Results showed that cadmium stress caused drastic decrease in germination %, SVI, radicle and plumule length, dry weight and fresh weight of seedlings. There was a significant decrease in these parameters with increased concentration of Cd showing 200 μM Cd conentration as most detrimental for both the genotypes. Chitosan mitigated adverse effect of Cd toxicity in both the selected pea genotypes. Observations depicted that 0.1% and 0.2% chitosan concentration had better results in both pea genotypes in germination % and other growth parameters as compared to other chitosan concentration when combined with varying levels of cadmium. Thus, the present study indicates that chitosan mitigates cadmium toxicity to a significant level. Further studies should be implicated which focus on physiological and molecular aspects related to combined effects of interaction of chitosan with cadmium responses.

Disclaimer

The chemicals and other products used for this research usually and predominantly occur and utilized in our area of research and nation. Here, no conflict of interest between authors and producers of the products exist because we are not intend to use these products as an avenue for any litigation but for the progression of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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