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COMPARATIVE STUDY OF MITIGATING EFFECT OF FOLIAR SPRAYED PACLOBUTRAZOL AND SALICYLIC ACID ON GROWTH CHARACTERISTICS IN MUNGBEAN UNDER CADMIUM STRESS

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Abstract: *The present investigations were carried out to assess the extent of cadmium stress-induced morpho-physiological responses in mungbean (Vigna radiata L.) genotype HUM-1 and its mitigation in the presence of plant growth regulators salicylic acid (SA) and paclobutrazol (PBZ), at different growth stages. All the treatments resulted in improvement of plant growth characteristics viz. germination percentage, root length, plant height, leaf numbers, leaf area, total dry weight. The germination percentage was found to be significantly enhanced with PBZ application @ 50µM. Highest root length was observed with PBZ application at 50µM with both the levels of Cd stress; 75 µM (C1T1) and 150 µM CdSO4(C2T1).The plant height was found to be significantly enhanced with PBZ treatment @ 100 µM, with 150 µM CdSO4 (C2T2). A reduction in both number of leaves and leaf area was observed in plants which were exposed to Cd stress. The dry weight seen in the light of the comparative better performance of the treatment PBZ application @ 50 µM, with 150 µM CdSO4 (C1T2) at both early as well as the later growth stages point out to a possibility of an effective means to combat Cd stress. The results regarding cell membrane injury well explain the effectiveness of PBZ in maintaining permeability and selectivity of cell membrane against cadmium stress. Electrolytic leakage was found to be considerably higher in plants under both, high and low levels of Cd stress and devoid of SA and PBZ application at all growth stages, as a result of membrane damage and deterioration of membrane integrity. SA application @ 0.5mM, helped to maintain membrane function thus reduced electrolytic leakage significantly.*

Key words: *Mungbean (Vigna radiata L.), Paclobutrazol (PBZ), Salicylic acid (SA), Cadmium (Cd) stress.*

Introduction: Pulses have been given the status of ‘wonder crop’ a unique gift bestowed by nature to mankind for a number of reasons but mainly for two special attributes. Firstly, pulses have relatively higher protein content in their seed, which makes the diet more balanced in its nutritive value. Pulse seeds are also sources of other nutritionally important materials, such as vitamins and minerals. Mungbean (*Vigna radiata* L.), which is one of the most important short summer season pulse crops which is grown primarily for its protein rich edible seeds. The mungbean has been grown in India since ancient times. It is still widely grown in South-east Asia, Africa, South America and Australia. Mungbean is a very rich source of easily digestible proteins; it contains about 24.5% protein, 59.9% carbohydrates and 1.2% fat [1].

Stress can also be defined as “Disadvantageous influences exerted on a plant by external abiotic or biotic factor(s), such as infection, or heat, water, and anoxia heavy metals. Germination and early seedling growth have been regarded as critical phases in plant growth and are greatly influenced by stressful conditions [2]. Among stresses, heavy metal stress has emerged as a major threat to agriculture in large area across the globe. Heavy metals are defined as the group of elements that have specific weights higher than about 5 g×cm^{-3}. Metals which are considered nonessential (Pb, Cd, Cr, Hg, etc.) are potentially highly toxic for plants [3].

Cadmium (Cd) has been shown to cause delay in germination, induce membrane damage, impair food reserve mobilization by increased cotyledon/embryo ratios of total soluble sugars, glucose, fructose and amino acids, mineral leakage leading to nutrient loss,[4] accumulation in seeds and over-accumulation of lipid peroxidation production seeds[5]. Inhibition of root elongation is one of the most sensitive responses to cadmium stress and this occurs faster than most of the other physiological reactions and proceeds cell death in different systems [6].

Salicylic acid (SA) (2-hydroxybenzoic acid), as a natural plant hormone is an internal regulator of phenolic nature which regulates and different physiological processes in crops and modulates plant reaction to different stresses such as drought, salt, chilling, heat, ultraviolet and pathogens and disease resistance[7]. Salicylic acid enhances plant capacity and resistance to different stresses. It ameliorates the growth of crop, enzyme activities, ion uptake and transport ethylene synthesis, seed germination, fruit yield, glycolysis, the growth-inhibitory effect of abscisic acid [8].

Another plant hormone which has emerged as a potential tool against abiotic stresses is paclobutrazol. Paclobutrazol (PBZ), a member of the triazole plant growth regulators is an inhibitor of gibberellic acid (GA) biosynthesis. PBZ is a cell elongation and internodes extension inhibitor that retards plant growth by inhibition of gibberellins biosynthesis. Gibberellins stimulate cell elongation. When gibberellins production is inhibited, cell division still occurs, but the new cells do not elongate. Treatments with paclobutrazol ((αR,βR)-rel-β-[(4-chlorophenyl) methyl]-α-(1,1-dimethylethyl)-1H-1,2,4-triazole-1-ethanol) and Salicylic acid (2-Hydroxybenzoic acid) proved effective in reducing the adverse effect of stress in plants[9].

Keeping the above information in view, the present investigation was undertaken to understand the morpho-physiological basis of heavy metal tolerance in Mungbean treated with paclobutrazol and salicylic acid.

Materials and Methods

Pot experiments were carried out at the net house of the Department of Horticulture, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi with Mungbean variety (HUM-1) consisting of ten treatments and three replications during kharif season

Observations on different morpho-physiological parameters were done and recorded at regular intervals (20 and 50 days after sowing). The 18 (9 with PBZ and 9 with SA denoted as same) treatments were C0T0 = No cadmium, irrigation with distilled water; no PBZ, SA treatment; C0T1 = No cadmium treatment, PBZ @ 50µM or SA application @ 0.5; C0T2 = No cadmium treatment PBZ @ 100µM or SA application@ 1mM; C1T0 = CdSO4 @75µM, No PBZ and salicylic acid application; C1T1 = CdSO4 @75µM, PBZ @ 50µM PBZ or SA @ 0.5mM application; C1T2= CdSO4 @75µM PBZ @ 100µM or SA @1mM application; C2T0 = CdSO4 @ 150µM No PBZ and SA application; C2T1 = CdSO4 @ 150µM PBZ application @ 50µM or 1mM SA application; C2T2= CdSO4 @ 150µM, PBZ @ 100µM or SA @ 1mM application.

Plant height (cm) was measured from the base of the plant to the top of the main stem with meter scale and expressed in centimeter. The total leaf area of all the counted leaves was measured with the help of leaf area meter (Systronics 211). The total dry weight (g) of the whole plant is recorded by gently removing the whole plant from soil without damaging the roots. Cell membrane injury was calculated in leaf samples after 20 and 50 days of sowing in normal and cadmium stressed plants as per the protocol of Zhu et al. (1999)[10]. The electrolyte leakage was determined to assess membrane permeability as described by Zwiazel and Blake (1991)[11].

Results and Discussion

The study was performed to attain the response foliar sprayed paclobutrazol and salicylic acid on morphological physiological attribute in grain legume mungbean (*Vigna radiata L.*) under cadmium stress. The morpho-physiological parameters of mungbean were critically observed and recorded at various growth stages, which include germination percentage, number of leaves plant^{-1}, plant height, root length, total leaf area plant^{-1}, and total dry weight of seedlings under control and Cd stressed conditions. A progressive decrease in plant height, leaf number, and dry matter partitioning and leaf area was observed in stressed plants in comparison to normal plants. Growth changes were the first most obvious reactions of plant under metal stress. Germination and early seedling growth have been regarded as critical phases in plant growth and are greatly influenced by stressful conditions [2]. A number of observations were recorded at various growth stages of the crop that continued up to the 50 days of growth only. The study is based on long duration analysis, i.e., at 20 DAS and 50 DAS.

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Seed soaking treatment with PBZ @ 50 µM was found to have an enhanced effect on germination and helped in better establishment of seedlings. A highest germination percentage of 93.33 were observed with PBZ application @ 50 µM, accounting for 167% increment when compared to that of control, both at no stress condition (C0) and @ 75 µM CdSO4 (C1) applications (Table 1). The above observations were found contrary to the previous studies, related to effect of PBZ on germination. Zhu *et al.* (2010)[12] studied PBZ soaking effects on seeds germination and seedling growth of *Cassia obtusifolia L.* where PBZ delayed the germination of seeds reduced the seed germination percentage. Whereas salicylic acid (SA) did not show positive effect upon germination percentage and in plants treated with SA there was a progressive decline in germination percentage at an increased level of Cd stress.

Table 1: Influence of paclobutrazol and salicylic acid on germination (%) under Cadmium stress in grain legume mungbean at 5 DAS.

		Germination %	
Metal	**Treatments**	**Paclobutrazol**	**Salicylic acid**
C0 ($CdSO_4$=0 µM)	C0T0	80.00	80.00
	C0T1	93.33(+.167)	76.66(-.04)
	C0T2	90.00(.125)	80.00(0.00)
C1 ($CdSO_4$=75µM)	C1T0	53.33(-.333)	53.33(-.333)
	C1T1	93.33(.167)	70.00(-.125)
	C1T2	76.67(-.04)	60.00(-.25)
C2($CdSO_4$=150 µM)	C2T0	46.67(-.04)	46.67(-.41)
	C2T1	73.33(-.083)	73.33(-.083)
	C2T2	86.67(.083)	63.33(-.208)
	Mean	77.03(-.903)	67.70(-.916)

A perusal Table 2 visualizes significant differences among the various treatments and when plant height compared with control, it was found to be significantly reduced under cadmium stress at all the three days of intervals. The PBZ was found to be accounted for the highest plant height as well as increased plant height even under Cd stress conditions. PBZ treatment at 75 µM was found to show a per cent increase in plant height by 16.67, 0.71 and 11.68 respectively as compared with the mean values, while SA exhibited a progressive decline of 24.46, 21.03 and 19.87 per cent respectively, over the mean control values at the same stages (50 DAS).

Root length performed in the similar manner as above in the long duration analysis too. Highest root length was observed with PBZ application at 50µM at both the levels of Cd stress, being 17.3cm and 17.03cm at 75 µM and 150 µM CdSO4 respectively (Table 2). It elicited that highest root length was observed with PBZ application at 50µM with both the levels of Cd stress; 75 µM (C1T1) and 150 µM CdSO4 (C2T1). The plant height was found to be significantly enhanced with PBZ treatment @ 100 µM, with 150 µM CdSO4 (C2T2). Similar results were earlier reported by Fletcher and Arnold (1986) who noticed that total root length increased significantly after triazole treatment. They also postulated that the stimulatory effect of triazole compounds in rooting may be due to inhibition of GA synthesis [13].

Table 2: Influence of Paclobutrazol and salicylic acid on root length (cm) and plant height (cm) of mungbean at different days after sowing under cadmium stress.

		Root length(cm)				Plant height (cm)			
		20 DAS		50 DAS		20 DAS		50 DAS	
METAL	**Treatments**	**PBZ**	**SA**	**PBZ**	**SA**	**PBZ**	**SA**	**PBZ**	**SA**
C0=$CdSO_4$	C0T0	7.3	7.3	8.33	8.33	19.03	19.03	22.77	22.77
	C0T1	7.23	7.2	8.87	8.97	19.03	18.13	26.57	17.27

		(-.91)	(-1.37)	(+6.44)	(+7.64)	(0.000	(-4.71)	(+16.67)	(-24.46)
	C0T2	7.03 (-3.65)	7.1 (-2.74)	9.03 (+8.44)	8.87 (+6.42)	19.279 (1.24)	18.97 (-.33)	24.27 (+6.57)	24.43 (+7.30)
C1	C1T0	4.87 (-33.3)	4.9 (-32.8)	7.83 (-5.96)	7.83 (-5.96)	16.2 (-14.87)	16.33 (-14.7)	18.27 (-19.77)	18.27 (-19.77)
	C1T1	5.67 (-22.37)	3.53 (-51.59)	17.3 (+107.68)	8.13 (-2.36)	19.97 (+4.59)	15.43 (-18.9)	22.93 (+.71)	21.03 (-7.62)
	C1T2	6.57 (-10.04)	4.37 (-40.18)	10 (+20.04)	8.23 (-1.16)	23.4 (+22.96)	14 (-26.43)	22.33 (-1.91)	19.73 (-13.33)
C2	C2T0	6.2 (-15.06)	6.1 (-16.43)	8.8 (+5.64)	8.8 (+5.64)	16.87 (+11.36)	16.87 (-11.36)	20.17 (-11.43)	20.17 (-11.43)
	C2T1	7.37 (+.91)	5.87 (-19.63)	17.03 (+104.48)	10.83 (30.05)	20.2 (+6.14)	17.63 (-7.33)	25.43 (+11.68)	19.87 (-12.75)
	C2T2	7.6 (+4.10)	7.13 (-2.28)	9.87 (+18.44)	7.93 (-4.76)	15.33 (+19.42)	18.5 (-2.78)	23.33 (+2.47)	19.03 (-16.41)
	MEAN	6.65 (-8.92)	5.94 (-18.5)	10.79 (+29.47)	8.66 (+3.95)	18.81 (+1.15)	17.21 (-9.55)	22.9 (+.55)	20.29 (-10.91)
		SEM	**CD 5%**	**SEM**	**CD 5%**	**SEM**	**CD 5%**	**SEM**	**CD 5%**
	M	1.06	3.04	2.31	6.63	2.98	8.55	3.97	11.37
	H	1.06	3.04	2.31	6.63	2.98	8.55	3.97	11.37
	T	1.06	3.04	2.31	6.63	2.98	8.55	3.97	11.37
	M×H	1.5	4.3	3.27	9.38	4.22	12.1	5.61	16.08
	M×T	1.84	5.27	4	11.48	5.17	14.82	6.87	19.7
	H×T	1.5	4.3	3.27	9.38	4.22	12.1	5.61	16.08
	M×H×T	2.6	7.45	5.66	16.24	7.31	20.95	9.71	27.86

***values in parenthesis indicate percent increase(+) or decrease (-) with respect to control under Cadmium stress. M = Metal, H = Hormone,T = Treatment ,M×H = Metal into hormone interaction, M×T = Metal into treatment interaction, H×T = Hormone into treatment interaction, M×H×T = Metal, hormone and treatment interaction, DAS = Days after sowing, C0(0 µM $CdSO_4$); C1(75µM $CdSO_4$); C2(150 µM $CdSO_4$; T0 (Control; no $CdSO_4$ and hormone treatment) ; T1 (50 µM Paclobutrazol; 0.5Mm Salicylic acid) ; T2 (100 µM Paclobutrazol; 1.0 mM Salicylic acid)*

Table 3 represented number of leaf and total leaf area per plant are directly correlated to each other and increased from 20 DAS to 50 DAS. Plants exposed to both the levels of Cd stress and devoid of any plant growth regulator treatment were found to exhibit lowest number of leaves at all the growth stages. Maximum number of leaves was found to occur at 50 DAS, obtained with the PBZ treatment @ 75µM and exhibited a significant increase of 54.24 per cent over control. As compared to control, leaf area was found to increase significantly with both the PBZ and SA treatments during the early period of growth, in the plants, which were not exposed to Cd stress (C0).

Table 3: Influence of Paclobutrazol and salicylic acid on no. of leaves and leaf area (sq.cm) of mungbean at different days after sowing under cadmium stress.

		No. of leaves				**Leaf area(sq.cm)**			
		20 DAS		**50 DAS**		**20 DAS**		**50 DAS**	
METAL	**Treatments**	**PBZ**	**SA**	**PBZ**	**SA**	**PBZ**	**SA**	**PBZ**	**SA**
C0 ($CdSO_4$)	C0T0	8	8	11.67	11.67	65.92	65.92	105.23	105.23
	C0T1	7.33 (-8.33)	7.67 (-4.1)	10.67 (-8.59)	11 (-5.74)	66.9 (+1.4)	65.8 (-.18)	109.03 (+3.60)	101.71 (-3.34)
	C0T2	9 (+12.5)	8 (0.00)	11.33 (-2.88)	10.67 (-8.59)	65.83 (-.13)	68.13 (+3.35)	117.38 (+11.54)	106.2 (+0.92)
C1	C1T0	5.33 (-33.33)	5.339 (-33.3)	10.33 (-11.45)	10.33 (-11.45)	65.6 (-.48)	65.6 (-.48)	72.82 (-30.79)	72.82 (-30.79)
	C1T1	9.33 (+16.66)	79 (-12.50)	12 (+2.82)	12.67 (+8.54)	73.15 (+10.96)	41.81 (-36.57)	133.12 (+76.50)	90.42 (-14.07)
	C1T2	7.67 (-4.1)	6 (-25.00)	18 (+54.24)	12 (+2.82)	83.83 (+2.16)	33.33 (-49.43)	107.17 (+1.80)	84.44 (-19.75)
C2	C2T0	4.67 (-41.66)	4.67 (-41.66)	8.33 (-28.59)	8.67 (-25.73)	61.5 (-6.70)	61.5 (-6.70)	68.13 (-35.25)	68.13 (-35.25)
	C2T1	9.33 (+16.66)	7.33 (-8.33)	11.33 (-2.88)	10.67 (-8.59)	61.63 (6.51)	61.66 (-6.46)	112.22 (+6.64)	33.83 (-67.84)
	C2T2	8.67 (+8.33)	6.33 (-20.830	11.67 (0.00)	9.67 (-17.16)	49.62 (-24.71)	55 (-16.57)	136.67 (+29.74)	86.1 (-18.17)
	MEAN	7.7 (-3.7)	8 (0.00)	11.70 (+0.01)	10.81 (-7.32)	66 (.11)	57.64 (-12.56)	106.86 (+1.59)	83.21 (-20.92)
		SEM	**CD 5%**	**SEM**	**CD 5%**	**SEM**	**CD 5%**	**SEM**	**CD 5%**
	M	0.38	1.1	1.64	4.71	3.98	11.41	1.27	3.65
	H	0.38	1.1	1.64	4.71	3.98	11.41	1.27	3.65
	T	0.38	1.1	1.64	4.71	3.98	11.41	1.27	3.65
	M×H	0.54	1.55	2.32	6.66	5.63	16.14	1.8	5.16
	M×T	0.66	1.9	2.85	8.16	6.89	19.77	2.21	6.32
	H×T	0.54	1.55	2.32	6.66	5.63	16.14	1.8	5.16
	M×H×T	0.94	2.69	4.02	11.54	9.75	27.95	3.12	8.94

***values in parenthesis indicate percent increase(+) or decrease (-) with respect to control under Cadmium stress. M = Metal, H = Hormone,T = Treatment ,M×H = Metal into hormone interaction, M×T = Metal into treatment interaction, H×T = Hormone into treatment interaction, M×H×T = Metal, hormone and treatment interaction, DAS = Days after sowing, C0(0 µM $CdSO_4$); C1(75µM $CdSO_4$); C2(150 µM $CdSO_4$; T0 (Control; no $CdSO_4$ and hormone treatment) ; T1 (50 µM Paclobutrazol; 0.5Mm Salicylic acid) ; T2 (150 µM Paclobutrazol; 1.0 mM Salicylic acid)*

The results of dry weight seen in the light of the comparative better performance of the treatment PBZ application @ 50 µM, with 150 µM CdSO4 (C1T2) at both early as well as the later growth stages point out to a possibility of an effective means to combat Cd stress but total dry weight was found to increase thereafter up to 50 DAS. The treatment which caused minimum dry weight in the short duration observations was salicylic acid application to the plants exposed to 150 µM CdSO4 (C2T2), showing a significant decrease of 58.03 per cent over the control. The treatment which had the best effect upon the dry matter accumulation was the higher level of PBZ combined with the lower level of Cd stress C1T2 with a massive 72.18 per cent rise than the control. The same observations were recorded in the long duration analysis at 50 DAS with C1T2, which accounted for a dry weight of 1352.17 mg, and showing a stark per cent increase of 46.70 over control.

Plants exposed to both the levels of Cd stress and without any growth regulator's application; C1T0 and C2T0 were found to exhibit consistent decline in total dry weight at every growth stage, which matches the findings of various other scientists, Melo *et al.* (2011) who concluded that Cd uptake and accumulation in plants results in growth inhibition and reduction in biomass production [14].

The results were seen in the light of the comparative better performance of the treatment C1T2 at both early as well as the later growth stages point out to a possibility of an effective means to combat Cd stress and thus protect the plants against the deleterious effects of Cd stress, and may prove to be a major tool in sustaining plant growth and development.

Table 4: Influence of Paclobutrazol and salicylic acid on total dry weight and cell membrane injury (%) of mungbean at different days after sowing under cadmium stress

		Total dry weight (gm)				**Cell membrane injury (%)**			
		20 DAS		**50 DAS**		**20 DAS**		**50 DAS**	
METAL	**Treatments**	**PBZ**	**SA**	**PBZ**	**SA**	**PBZ**	**SA**	**PBZ**	**SA**
C0 ($CdSO_4$)	C0T0	360.57	360.57	928.50	928.50	67.34	67.34	52.31	52.31
	C0T1	277.03 (-23.16)	365.8 (+1.45)	940.1 (+2.32)	900.97 (-2.96)	58.44 (-13.21)	70.64 (+4.89)	59.56 (+13.81)	64.60 (+23.50)
	C0T2	267.83 (-25.71)	265.27 (-26.43)	874.16 (-4.77)	827.13 (-10.91)	65.06 (-3.37)	66.67 (-.99)	54.63 (+4.44)	65.66 (+25.53)
C1	C1T0	240.63 (-33.260	240.63 (-33.26)	754.67 (-17.64)	754.67 (-18.72)	76.95 (+14.27)	76.95 (+14.27)	62.67 (+19.81)	62.67 (+35.18)
	C1T1	251.6 (-30.22)	303.53 (-15.81)	993.73 (+8.10)	900.77 (-2.98)	58.48 (-13.15)	77.44 (+14.98)	66.87 (+27.84)	70.71 (+35.18)
	C1T2	230.17 (-36.16)	154.43 (-57.16)	1352.17 (+46.70)	763.63 (-17.75)	48.06 (-28.62)	64.21 (-4.64)	55.34 (+5.79)	72.61 (+38.81)
C2	C2T0	246.07 (-31.75)	246.07 (-31.75)	756.37 (-17.4)	756.37 (-18.58)	80.60 (+19.70)	80.60 (+19.70)	62.97 (+20.37)	62.97 (+20.37)
	C2T1	282.07 (-21.77)	304.6 (-15.52)	1255.53 (+36.29)	903.3 (-2.71)	62.53 (-7.13)	92.38 (+37.18)	56.39 (+7.79)	55.19 (+5.50)
	C2T2	239.73 (-33.51)	265.4 (-26.39)	871.2 (-5.09)	772.33 (-16.81)	65.43 (-2.83)	78.90 (17.17)	54.29 (+3.79)	69.70 (+33.81)
	MEAN	**266.18 (-26.17)**	**278.48 (-22.76)**	**969.60 (+5.50)**	**834.18 (-10.15)**	64.77 (-3.81)	75.01 (+11.3)	58.34 (+11.51)	64.08 (+22.50)
		SEM	**CD 5%**	**SEM**	**CD 5%**	**SEM**	**CD 5%**	**SEM**	**CD 5%**
	M	13.84	39.71	NS	NS	10.01	28.71	4.43	12.70
	H	13.84	39.71	NS	NS	10.01	28.71	4.43	12.70
	T	13.84	39.71	NS	NS	10.01	28.71	4.43	12.70
	M×H	19.58	56.16	NS	NS	14.16	40.61	6.26	17.97
	M×T	23.98	68.78	NS	NS	17.34	49.73	7.67	22.00
	H×T	19.58	56.16	NS	NS	14.16	40.61	6.26	17.97
	M×H×T	33.91	97.26	NS	NS	24.52	70.33	10.85	31.12

***values in parenthesis indicate percent increase(+) or decrease (-) with respect to control under Cadmium stress. M = Metal, H = Hormone,T = Treatment ,M×H = Metal into hormone interaction, M×T = Metal into treatment interaction, H×T = Hormone into treatment interaction, M×H×T = Metal, hormone and treatment interaction, DAS = Days after sowing, C0(0 µM $CdSO_4$); C1(75µM $CdSO_4$); C2(150 µM $CdSO_4$; T0 (Control; no $CdSO_4$ and hormone treatment) ; T1 (50 µM Paclobutrazol; 0.5Mm Salicylic acid) ; T2 (100 µM Paclobutrazol; 1.0 mM Salicylic acid)*

Cell membrane injury showed significant differences among different treatments at observations as presented in Table 4. Cell membrane injury was found maximum for C2T1, higher level of Cd stress with PBZ treatment at 75 µM at 20 DAS showing 37.18 percent increase over control respectively. The minimum cell membrane injury was recorded in with the PBZ treatment at early growth stages of 20 DAS. There was significant decrease in cell membrane injury recorded on treatment with PBZ under both normal control and Cd stress conditions. In all the observations at all the growth stages; 20, 35 and 50 DAS, a significant increase was found in the plants which were not given any plant growth regulator treatment (C2T0), showing a stark percent increase of 19.70 and 7.79 over normal at 20 and 50 DAS respectively. At higher growth stages, SA treatment could be accounted for the decrease in membrane injury, exhibiting a 4.64 per cent decrease in membrane injury over control at 20 and (Table 4). These observations support the findings of earlier research in which SA pre-treatment alleviated Pb-induced membrane damage in rice [15], and Cd toxicity in barley and maize [16].

Electrolytic Leakage (EL) showed significant differences among treatments at different day after sowing as presented in Table 5. Maximum electrolytic leakage was found to be associated with both, high and low levels of Cd stress, devoid of both SA and PBZ application; C1T0 and C2T0 at all growth stages at which observations were recorded in the present investigation, and shown noteworthy per cent increase over control. The treatment C1T0 has shown per cent increase of 19.43 and 19.15, while C2T0 exhibited a per cent increase of 29.26 and 17.70 over control at 20 and 50 DAS respectively (Table 5). A significant decrease in electrolytic leakage was recorded with treatment of PBZ and Salicylic acid under both control and Cd stress conditions. It is interesting to note that Salicylic acid performs better than PBZ in protecting the plants against the loss of membrane integrity. Salicylic acid exhibited a consistent per cent decrease in EL over normal; SA application @ 0.5mm, was also found to be associated with highest per cent decrease in EL at all growth stages, showing a per cent decrease of 36.51, 20.66 and 19.81 as compared to 21.24, 7.36 and 16.71 as compared to PBZ treatment @50 µM at 20, 35, and 50 DAS. Similar results were reported by Metwally *et al.* (2003) [16]. Above observations summarized that electrolytic leakage was found to be considerably higher in plants under both, high and low levels of Cd stress and devoid of SA and PBZ application at all growth stages, as a result of membrane damage and deterioration of membrane integrity. ; SA application @ 0.5mM, helped to maintain membrane function thus reduced electrolytic leakage significantly.

Table 5: Influence of Paclobutrazol and electrolyte leakage of mungbean at different days after sowing under cadmium stress

		20 DAS		50 DAS	
METAL	**Treatments**	**PBZ**	**SA**	**PBZ**	**SA**
C0	**C0T0**	42.22	42.22	77.00	77.00
	C0T1	33.25(-21.24)	26.80(-36.51)	64.14(-16.71)	61.74(-19.81)
	C0T2	37.57(-10.99)	44.28(+4.87)	77.97(+1.25)	70.97(-7.83)
C1	**C1T0**	50.42(+19.43)	50.43(+19.43)	91.75(+19.15)	91.74(+19.15)
	C1T1	36.60(-13.32)	24.16(-42.67)	76.24(-.99)	66.33(-13.85)
	C1T2	41.77(-1.07)	44.15(+4.57)	74.47(-3.29)	70.97(-7.83)
C2	**C2T0**	54.58(+29.26)	54.58(+29.26)	90.63(+17.70)	90.64(+17.70)
	C2T1	40.12(-4.96)	36.61(-13.26)	41.06(-46.66)	79.47(+3.20)
	C2T2	38.19(-9.56)	27.78(-13.27)	80.24(+4.20)	54.70(-28.95)
	Mean	41.6(-1.38)	39.00(-7.6)	74.83(-2.81)	73.73(-4.24)
		SEM	CD 5%	**SEM**	**CD 5%**
	M	1.18	3.40	NS	NS
	H	1.18	3.40	NS	SIG**
	T	1.18	3.40	NS	SIG**
	M×H	1.68	4.81	NS	NS
	M×T	2.05	5.89	NS	NS
	H×T	1.68	4.81	NS	NS
	M×H×T	2.90	8.32	NS	NS

***values in parenthesis indicate percent increase(+) or decrease (-) with respect to control under Cadmium stress. M = Metal, H = Hormone,T = Treatment ,M×H = Metal into hormone interaction, M×T = Metal into treatment interaction, H×T = Hormone into treatment interaction, M×H×T = Metal, hormone and treatment interaction, DAS = Days after sowing, C0(0 µM $CdSO_4$); C1(75µM $CdSO_4$); C2(150 µM $CdSO_4$; T0 (Control; no $CdSO_4$ and hormone treatment) ; T1 (50 µM Paclobutrazol; 0.5Mm Salicylic acid) ; T2 (100 µM Paclobutrazol; 1.0 mM Salicylic acid)*

Conclusions: The overall findings indicate significance of the present investigation, which was especially planned to evaluate the mitigating effects of PBZ and salicylic acid under cadmium stress. These findings up to 50 DAS are of relevance that develops insight that salicylic acid and PBZ could be utilized effectively for combating Cd stress. These findings are of great relevance in present times, where arable land area is decreasing day by day, while population is increasing at an alarming rate, so only option remains is of enhancing crop productivity. This experiment could have been extended up to yield studies, which could not become possible due to making critical studies on a number of aforementioned morpho-physiological for arriving at few conclusive notes of significance and/or suggestions for future research and for our farmers who are ignorant up to a great extent regarding the ill effects and impact of Cd stress. Therefore, it may be suggested that PBZ and SA command a great significance in mitigating Cd stress.

References

1. Anonymous. (2001). Statistical yearbook of Bangladesh. Bur.Stat. Div. Min. Plan. Govt. People's Repub. Bangladesh, Dhaka. pp. 372.
2. Al- Rumaih, M.M and Al-Rumaith, M.M. (2008). Influence of ionizing radiation on antioxidant enzymes in three species of Trigonella. *American Journal of Environmetal Sciences* 4 (2): 151.
3. Sebastiani, L., Scebba, F., & Tognetti, R. (2004). Heavy metal accumulation and growth responses in poplar clones Eridano (*Populus deltoides* × *maximowiczii*) and I-214 (*P.* × *euramericana*) exposed to industrial waste. *Env. Exp. Bot.*, 52:79-84.
4. Sfaxi-Bousbih, A., Chaoui, A., El Ferjani, E. (2010). Cadmium impairs mineral and carbohydrate mobilization during the germination of bean seeds. *Ecotoxicol Environ Saf.* 73:1123–9.
5. Smiri, M., Chaoui, A., Rouhier, N., Gelhaye, E., Jacquot, J.P., El Ferjani, E. (2011). Cadmium affects the glutathione/glutaredoxin system in germinating pea seeds. *Biol Trace Elemental Research*, 142:93–105.
6. He, J. Ren, Y. Pan, X. yan, Y., Zhu, C. and Jiang, D. (2010). Salicylic acid alleviates the toxicity effects of cadmium on germination seedlings growth and amylase activity of rice. Z. *Pflanzenrnahr Bodenk*, 173: 300-305.
7. Khan, W., Prithiviraj B, Smith D.L. (2003). Photosynthetic responses of corn and soybean to foliar application of salicylates. Journal of Plant Physiology, 160:485–92.
8. Nazar, R., Iqbal, N., Masood, A., Iqbal R. Khan, Syeed,S., Khan, N.A. (2012). Cadmium Toxicity in Plants and Role of Mineral nutrients in Its Alleviation, *American Journal of Plant Sciences*, 3:1476-1489.
9. David, T., Curry, E. (1991). Chemical regulation of vegetative growth and yield attributed of chick pea. *Ann. Bio* 3: 27-188.
10. Zhu, Y.L., Pilon-Smiths, E.A.H, Tarun, A.S., Weber, S.U., Jouanin, L., Terry, N. (1999). Cadmium tolerance and accumulation in Indian mustard is enhanced by over expressing γ-glutamylcysteine synthetase. *Plant Physiol.* 121:1169–1177.
11. Zwiazek J.J. and Blake T.J. (1990). Effects of preconditioning on electrolyte leakage and lipid composition in black spruce (Pi-cea mariana) stressed with polyethylene glycol. Physiol. *Plant*, 79: 71–77.
12. Zhu, Xia *et al.* (2010). Effects of Soaking with Paclobutrazol on Seeds Germination and Seelding Growth of Cassia obtusifolia L. *Journal of agricultural sciences*, 2010-17.
13. Fletcher, R.A. and Arnold, V. (1986). Stimulation of cytokinins and chlorophyll synthesis in cucumber cotyledons by Triadimefon. *Physiol. Plant.*, 66: 197-201.
14. Melo, E.E.C., Costa, E.T.S., Guilherme, L.R.G., Faquin, V. and Nas-cimento, C.W.A. (2011). Accumulation of arsenic and nutrients by castor bean plants grown on an As-enriched nutrient solution. *J. Hazard. Mater.* 168:479– 483.
15. Mishra, A., Choudhuri, M.A. (1997). Ameliorating effects of salicylic acid on lead and mercury induced inhibition of germination and early seedling growth of two rice cultivars, *Seed Sci. Technol.* 25: 263–270.
16. Metwally, A., I. Finkermeier, M. Georgi, K.J. Dietz, (2003). Salicylic acid alleviates the cadmium toxicity in barley seedlings, *Plant Physiol.*, 132:272-281.