

## Effects of benzoic acid and cadmium toxicity on wheat seedlings

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Benzoic acid (BA) and Cd exhibit cumulative effects on plants due to their accumulation in the soil. The present study reports the effects of BA an allelochemical, Cd and their combinations on seed germination, seedling growth, biochemical parameters, and response of antioxidant enzymes in *Triticum aestivum* L. The experiment was conducted in sand supplemented with Hoagland nutrient solution. Benzoic acid was applied at concentrations of 0.5, 1.0, and 1.5 mM with or without Cd (7 mg L<sup>-1</sup>) to observe effects of allelochemical and Cd alone and in combination on wheat. Both stresses exhibited inhibitory effect on growth and metabolism of wheat seedlings. The allelochemical in single and combined treatments with Cd decreased seedling growth as compared to Cd stress. The two stresses significantly enhanced malondialdehyde content of wheat seedlings. The activity of other antioxidant enzymes, viz. superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), and guaiacol peroxidase (POX) were also recorded. SOD increased in seedlings under the two stresses. CAT more prominently ameliorates the toxic effects of H<sub>2</sub>O<sub>2</sub> as compared with APX and POX and protected wheat seedlings from oxidative stress. Allelochemical buttressed the toxic effect of Cd on wheat seedlings.

**Key words:** Antioxidants, allelopathy, lipid peroxidation, oxidative stress, *Triticum aestivum*.

### INTRODUCTION

Biotic and abiotic stresses affect normal physiological processes of plants including crops of economic importance. Under natural conditions plants are hardly subjected to a single stress factor, rather a group of factors. Allelopathy is a chemical interaction caused by allelochemicals produced by plants in both natural and agro-ecosystems (Dakshini et al., 1999). Allelochemicals are compounds with low molecular weight that interfere with metabolic processes of plants. Several allelochemicals such as *p*-hydroxybenzoic acid, *trans-p*-coumaric, *cis-p*-coumaric, syringic, vanillic, *trans*-ferulic and *cis*-ferulic acid, and 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA) have been reported from shoot and root of wheat seedling (Wu et al., 2001). Phenolic derivatives of benzoic acid (BA) extracted from soil suppress growth and development of plants (Vaughan and Ord, 1991). Allelochemicals influence a number of physiological processes (Blum, 1995; Inderjit and Duke, 2003; Singh et al., 2010). In addition to allelopathy contamination of soil with heavy metals poses a serious ecological problem all over the world. Cadmium (Cd) is one of the most harmful heavy metals, because it is readily taken up by plant cell (Liu et al., 2007). Cadmium is released into the environment by power stations,

heating systems, metal industries, Ni-Cd batteries, and phosphate fertilizers (Toppi and Gabbrielli, 1999) as well as from geo-chemical weathering of rocks. It causes morphological, anatomical, and physiological changes in plants including growth inhibition, water imbalance, and reduction in seed germination (Benavides et al., 2005; Mishra et al., 2006). Increased level of malondialdehyde (MDA) and H<sub>2</sub>O<sub>2</sub> are major indicators of Cd induced oxidative stress in plants (Dixit et al., 2001). It induces both enzymatic and non-enzymatic antioxidant systems in plants (Iannelli et al., 2002). Low level of reactive oxygen species (ROS) produced in cell organelles is usually enhanced under stress condition. Oxidative stress caused by ROS damages macromolecules such as pigments, proteins, nucleic acids, and lipids (Apel and Hirt, 2004). In order to cope with environmental stress, plants have developed protective antioxidant enzyme systems. Several ROS scavenging enzymes such as superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), and guaiacol peroxidase (POX) are induced to buttress plant strength (Gratao et al., 2005). Iannelli et al. (2002) reported increased activity of SOD, APX, and CAT in all parts of *Phragmites australis* (Cav.) Trin. ex Steud. induced by Cd toxicity. The studies on allelochemicals combined with Cd are scanty. There are no reports on the effect of allelochemicals with Cd on plants. The objective of this study was to evaluate the interactive effect of two environmental stresses viz. allelopathy and metal stress on *Triticum aestivum* L. Emphasis was laid on how established cross-interactions i.e. adaptation or synergism could be applied in the agricultural practice.

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## MATERIALS AND METHODS

### Seeds, chemicals, and growth and stress treatments

Seeds of wheat (*Triticum aestivum* L.) var. HW2004 were procured from seed agency at Allahabad, India; and the heavy metal CdCl<sub>2</sub>·H<sub>2</sub>O (molecular weight 201.32) and benzoic acid (molecular weight 122.12) from LOBA Chemie, Mumbai. Heavy metal (7 mg L<sup>-1</sup>) and benzoic acid (1.5 mM) were prepared separately in buffer (pH 7.0). The solutions of graded concentrations of benzoic acid were prepared by adding distilled water in output solution before each experiment.

Seeds were surface sterilized with 0.01% HgCl<sub>2</sub> solution and imbibed in water for 3 h. Seeds were sown in plastic pots (height 10 cm and diameter 6 cm) filled with sterilized sand. The sand was treated with 0.2 N H<sub>2</sub>SO<sub>4</sub> to remove organic matter then washed three times with distilled water and sterilized at 170 °C for 24 h. Sand saturated with Hoagland nutrient solution (Hoagland and Arnon, 1950) served as control and supplemented with 7 mg L<sup>-1</sup> CdCl<sub>2</sub> for Cd treatment. Benzoic acid (BA) was applied in pots along with Hoagland solution at concentrations of 0.5, 1.0, and 1.5 mM with and without Cd. Sowing was done at the rate of three seeds per pot. Pots were transferred to a growth chamber (temperature: 28 ± 2 °C; photoperiod: 18:6 h; humidity: 61 ± 5%, and photon flux density: 240 μmol m<sup>-2</sup> s<sup>-1</sup>). Pots were irrigated as and when required. The experiments were performed in triplicate.

Germination started on 2 d after sowing (DAS). Germination was recorded at intervals of 24 h till 8 DAS. Thinning was done to one seedling per pot. Growth of 14 d old seedlings was recorded and first fully expanded leaves were sampled for biochemical analysis. Dry weight (DW) of control and treated seedlings was measured.

### Sugar content and pigment and protein contents

The quantification of total soluble sugars was done following Hedge and Hofreiter (1962). About 0.1 g fresh leaf tissue was homogenized in 5 mL of 95% ethanol. After centrifugation, 1 mL supernatant was mixed with 4 mL anthrone reagent and heated on boiling water bath for 10 min. Absorbance was recorded at 620 nm after cooling. The amount of sugar was determined by the standard curve prepared from glucose.

Chlorophylls and carotenoids from leaves (10 mg) of experimental plants were extracted with 80% acetone and quantified following the method of Lichtenthaler (1987). Protein content was determined according to the method of Lowry et al. (1951). The amount of protein was calculated with reference to standard curve obtained from bovine serum albumin.

### Lipid peroxidation

Lipid peroxidation was measured as the amount of malondialdehyde (MDA) determined by thiobarbituric

acid reactive substance (TBRS) as described by Heath and Packer (1968). Fresh leaf (0.2 g) was ground in 0.1 w/v trichloroacetic acid (TCA) and centrifuged at 10 000 g for 10 min. One milliliter of supernatant was mixed with 4 mL of 0.5% thiobarbituric acid. The mixture was heated at 95 °C for 30 min and it was again centrifuged after cooling. The absorbance of the supernatant was recorded at 532 nm and corrected by subtracting the non-specific absorbance at 600 nm. The MDA concentration was calculated using the extinction coefficient of 155 mM<sup>-1</sup> cm<sup>-1</sup> and expressed as n mol g<sup>-1</sup> FW.

### Extraction and assay of antioxidant enzymes

The fresh leaves (0.25 g) were homogenized with 0.1 M sodium phosphate buffer containing 1% polyvinyl pyrrolidone (pH 7.00) in a pre-cooled mortar and pestle. The extract was centrifuged at 4 °C at 15 000 g for 30 min in cooling centrifuge (Remi instruments C 24). The supernatant was used for the assay of superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), and guaiacol peroxidase (POX).

### Assay of superoxide dismutase and assay of catalase

The superoxide dismutase (EC 1.15.1.1) was estimated by the nitro blue tetrazolium (NBT) photochemical assay according to the method of Beyer and Fridovich (1987). Reaction mixture (4 mL) consisted of 20 mM methionine, 0.15 mM ethylene diamine-tetra acetic acid (EDTA), 0.12 mM NBT, 0.5 mL supernatant. Test tubes were exposed to fluorescent lamp for 30 min and identical unilluminated assay mixture served as blank. One unit of enzyme was measured as the amount of enzyme which caused 50% inhibition of NBT reduction.

Catalase activity (EC1.11.1.6) was assayed following Cakmak and Marschner (1992). Assay mixture (2 mL) contained 25 mM potassium phosphate buffer (pH 7.0), 10 mM H<sub>2</sub>O<sub>2</sub> and 0.5 mL enzyme extract. The rate of H<sub>2</sub>O<sub>2</sub> decomposition for 1 min was monitored at 240 nm and calculated using extinction coefficient of 39.4 mM<sup>-1</sup> cm<sup>-1</sup> and expressed as enzyme unit g<sup>-1</sup> FW. One unit of catalase was determined as the amount of enzyme required to oxidize 1 μM H<sub>2</sub>O<sub>2</sub> min<sup>-1</sup>.

### Assay of ascorbate peroxidase and assay of guaiacol peroxidase

Ascorbate peroxidase (EC1.11.1.11) was assayed following Nakano and Asada (1981). Assay mixture (2 mL) contained 25 mM potassium phosphate buffer (pH 7.0), 0.1 mM EDTA, 0.25 mM ascorbate, 1.0 mM H<sub>2</sub>O<sub>2</sub> and 0.2 mL enzyme extract. H<sub>2</sub>O<sub>2</sub> was the last component to be added. The absorbance was recorded for 1 min at 290 nm (extinction coefficient of 2.8 mM<sup>-1</sup> cm<sup>-1</sup>). Enzyme specific activity was measured as enzyme unit g<sup>-1</sup> FW as the amount of enzyme required to oxidize 1 μM H<sub>2</sub>O<sub>2</sub> min<sup>-1</sup>.

Guaiacol peroxidase (EC 1.11.1.7) was assayed following Hemeda and Klein (1990). The reaction

mixture (2 mL) consisted of 25 mM phosphate buffer (pH 7.0), 0.1 mM EDTA, 0.05% guaiacol, 1.0 mM H<sub>2</sub>O<sub>2</sub> and 0.2 mL of enzyme extract. The increase in absorbance due to oxidation of guaiacol was monitored at 470 nm. The enzyme activity was measured using extinction coefficient of 26.6 mM<sup>-1</sup> cm<sup>-1</sup> and expressed as enzyme unit g<sup>-1</sup> FW.

### Statistical analysis

Treatments were arranged in a randomized block design with three replicates. Standard errors of means were calculated. In addition, ANOVA was carried out for all the data generated from this experiment, employing one way ANOVA test using GPIS software 3.0 (GRAPHPAD, California, USA).

## RESULTS AND DISCUSSION

### Seed germination, seedling growth and sugar contents

Seed germination decreased significantly ( $p < 0.05$ ) in dose dependent manner by application of BA in dose dependent manner. Maximum 37.5% reduction in germination was recorded at highest concentration of BA. Cadmium exhibited adverse effect in single treatment and in combinations with BA. Allelopathic stress caused by BA suppressed seedling height to 14.6% as compared with control. However, no alteration was observed under combined stress when compared with single stress. Dry weight (DW) of seedlings significantly declined under the influence of both stresses. Cadmium decreased DW and proved to be more toxic as compared to BA stress. Combination of two stresses further decreased DW. Sugar content significantly increased under stress. The increase of sugar was higher under allelochemical stress as compared to metal stress. BA at highest concentration caused maximum increase (3.65 times) in sugar content over control. BA+Cd combinations elevated sugar content as compared to single treatments of BA and Cd. Seedlings treated with B<sub>3</sub>+Cd treatment exhibited maximum 5.94 times increase in sugar content as compared with control (Table 1).

Under natural conditions plants are rarely affected by

**Table 1. Effects of benzoic acid and Cd on seed germination, seedling height, dry weight, and sugar content of *Triticum aestivum*.**

Treatments	Seed germination %	Seedling height cm	Dry weight mg plant <sup>-1</sup>	Sugar content mg g <sup>-1</sup> FW
C	80.00 ± 3.85	3.63 ± 0.29	36.83 ± 0.44	14.32 ± 3.06
Cd	61.55 ± 2.19 <sup>a</sup>	3.26 ± 0.35	18.33 ± 1.59 <sup>c</sup>	24.64 ± 1.75
B <sub>1</sub>	56.88 ± 1.93 <sup>b</sup>	3.40 ± 0.56	24.83 ± 0.72 <sup>c</sup>	25.83 ± 6.92
B <sub>2</sub>	54.11 ± 0.48 <sup>b</sup>	3.26 ± 0.03	23.66 ± 1.45 <sup>c</sup>	44.03 ± 2.19 <sup>ef</sup>
B <sub>3</sub>	50.00 ± 1.92 <sup>b</sup>	3.10 ± 0.15	22.83 ± 1.01 <sup>c</sup>	52.35 ± 0.89 <sup>efx</sup>
B <sup>1</sup> +Cd	57.78 ± 3.93 <sup>b</sup>	3.03 ± 0.08	24.83 ± 0.88 <sup>c</sup>	54.36 ± 2.05 <sup>efx</sup>
B <sub>2</sub> +Cd	57.33 ± 3.00 <sup>b</sup>	3.00 ± 0.11	21.83 ± 0.72 <sup>c</sup>	58.79 ± 2.89 <sup>efx</sup>
B <sub>3</sub> +Cd	47.78 ± 4.84 <sup>b</sup>	2.90 ± 0.10	20.66 ± 1.16 <sup>c</sup>	85.09 ± 1.20 <sup>efxyz<sup>2</sup>5</sup>

Data are mean of three replicates ± standard error of the mean (SEM).

<sup>a</sup> $P < 0.05$ , <sup>b</sup> $P < 0.01$ , <sup>c</sup> $P < 0.001$  versus C, <sup>d</sup> $P < 0.001$  versus Cd, <sup>e</sup> $P < 0.001$  versus B<sub>1</sub>, <sup>f</sup> $P < 0.001$  versus B<sub>2</sub>, <sup>g</sup> $P < 0.001$  versus B<sub>3</sub>, <sup>h</sup> $P < 0.001$  versus B<sub>1</sub>+Cd, <sup>i</sup> $P < 0.001$  versus B<sub>2</sub>+Cd.

FW: fresh weight, C: control, Cd: 7 mg L<sup>-1</sup>, B<sub>1</sub>, B<sub>2</sub>, and B<sub>3</sub>: 0.5, 1.0, and 1.5 mM benzoic acid, respectively.

a single stress, rather subjected to combination of various environmental stresses. The present study was aimed to explore the impact of two important environmental stresses on plants and efficiency of defense system to mitigate the effect. Seed germination and seedling growth decreased with increase in concentration of allelochemical applied with and without Cd. BA reduced seed germination and seedling growth as reported by Yu and Matusi (1997). Cadmium exhibited toxic impact on germination and seedling growth (Rascio et al., 1993; Asgharipour et al., 2011). Variation of plant growth at germination and seedling stage under stress is often regarded as an important index to investigate plant tolerance to stress. Cadmium is known to inhibit cell growth by formation of stronger cross-binding between the pectin molecules in the cell wall and by decrease in size of intercellular spaces (Prasad, 1995). Wheat seedlings DW decreased under stress and more prominently in metal stress. Decrease in DW corresponded to level of chlorophyll content in respective treatments (Buttery and Buzzell, 1977; Dube et al., 2002). Photosynthesis is proportional to chlorophyll content which decreased plant growth. In combined treatments, inhibition of germination rates, seedling growth and DW revealed that BA and Cd worked antagonistically because reduction of all the above parameters was lesser than sum of inhibition caused by BA and Cd separately. The stimulatory effect of BA and Cd on sugar content was recorded. Stress conditions which cause accumulation of ROS, directly or indirectly, are associated with soluble sugar accumulation, which has generally been associated to be an adaptive response to the stress situation (Roitsch, 1999). Asgharipour et al. (2011) found that Cd increased the sugar content of wheat seedlings. The elevated sugar level observed in stressed seedlings can be explained by less utilization of sugar for growth of seedlings subjected to stress (Asgharipour et al., 2011). Soluble sugars contribute to defense and act as signal molecule that benefits the plant. It sense and control photosynthetic activity and ROS balance (Couée et al., 2006).

### Pigment and protein contents

Allelochemical and Cd did not significantly influence total pigment content of wheat seedlings. However chlorophyll decreased significantly under combined treatments. Maximum 29.32% reduction in total chlorophyll was observed in B<sub>3</sub>+Cd treatment as compared with control. BA in highest concentrations decreased carotenoid content. All combinations decreased carotenoid content when compared with single stress treatment. B<sub>3</sub>+Cd exhibited most inhibitory effect on carotenoid content. Protein content of wheat seedlings decreased significantly under Cd stress. BA at highest concentration (1.5 mM) caused maximum 15.03% reduction of protein content as compared with control. Cadmium with higher concentrations of BA decreased amount of protein (Table 2).

**Table 2. Effects of benzoic acid and Cd on pigment and protein contents of *Triticum aestivum*.**

Treatments	Chlorophyll a	Chlorophyll b	Total chlorophyll	Carotenoid	Protein
	mg g <sup>-1</sup> FW				
C	9.68 ± 0.272	3.61 ± 0.052	13.30 ± 0.324	23.91 ± 3.24	15.23 ± 0.62
Cd	9.04 ± 0.353	3.52 ± 0.081	12.56 ± 0.434	23.13 ± 1.36	13.11 ± 0.49 <sup>a</sup>
B <sub>1</sub>	9.04 ± 0.336	3.47 ± 0.112	12.52 ± 0.449	16.67 ± 1.05 <sup>b</sup>	14.07 ± 0.25
B <sub>2</sub>	8.83 ± 0.186	3.40 ± 0.040	12.24 ± 0.226	16.36 ± 1.40 <sup>b</sup>	14.3 ± 0.72
B <sub>3</sub>	8.90 ± 0.029	3.35 ± 0.227	12.26 ± 0.249	14.09 ± 1.92 <sup>c</sup>	12.94 ± 0.39 <sup>a</sup>
B <sub>1</sub> +Cd	8.43 ± 0.017 <sup>a</sup>	3.39 ± 0.302	11.83 ± 0.310	13.95 ± 0.78 <sup>c</sup>	13.26 ± 0.45
B <sub>2</sub> +Cd	7.99 ± 0.035 <sup>c</sup>	3.08 ± 0.049	11.07 ± 0.085 <sup>b</sup>	13.46 ± 1.52 <sup>c</sup>	13.21 ± 0.16 <sup>c</sup>
B <sub>3</sub> +Cd	6.85 ± 0.067 <sup>cdhxyz</sup>	2.20 ± 0.409 <sup>b</sup>	8.87 ± 0.471 <sup>cdhxyz</sup>	10.37 ± 1.49 <sup>cd</sup>	12.94 ± 0.50 <sup>c</sup>

Data are mean of three replicates ± standard error of the mean (SEM).

<sup>a</sup>*P* < 0.05, <sup>b</sup>*P* < 0.01, <sup>c</sup>*P* < 0.001 versus C, <sup>d</sup>*P* < 0.001 versus Cd, <sup>e</sup>*P* < 0.001 versus B<sub>1</sub>, <sup>f</sup>*P* < 0.001 versus B<sub>2</sub>, <sup>g</sup>*P* < 0.001 versus B<sub>3</sub>, <sup>h</sup>*P* < 0.001 versus B<sub>1</sub>+Cd.

FW: fresh weight, C: control, Cd: 7 mg L<sup>-1</sup>, B<sub>1</sub>, B<sub>2</sub>, and B<sub>3</sub>: 0.5, 1.0, and 1.5 mM benzoic acid, respectively.

Allelochemical and metal decreased chlorophyll contents which may be due to inhibition of biosynthesis of chlorophyll (Baziramakenga et al., 1994). In the present study BA inhibited chlorophyll synthesis which was evident from decreased biosynthesis of chlorophyll a which influenced total chlorophyll. Cadmium also reduced chlorophyll content. Oncel et al. (2000) found that Cd reduced chlorophyll in wheat seedlings. Cadmium severely inhibits plant growth and even causes plant death by disturbing the uptake of nutrients and inhibiting photosynthesis via degradation of chlorophyll (Zhang et al., 2007). Carotenoid was more sensitive towards stress as compared with chlorophyll in respective treatments. Single and combined stress treatment exhibited negative impact on carotenoid content of wheat seedling (Ünyayar et al., 2005; Mishra and Agrawal, 2006). The decrease in carotenoid content is harmful to chlorophyll because carotenoid protects chlorophyll from photooxidative destruction (Middleton and Teramura, 1993). Both allelochemical and metal stresses reduced protein content. BA (Baziramakenga et al., 1994) and Cd (Gupta et al., 2003) caused reduction of protein content. Both stresses are known to produce ROS (Zhang et al., 2010; Nahakpam and Shah, 2010) which caused modification/degradation of proteins (Pacifice and Davies, 1990). The inhibition of chlorophyll biosynthesis by BA and Cd treatments influenced photosynthesis and ultimately decreased protein contents. The counteractive effect of BA and Cd was observed in combinations as the percentage decrease

of protein content was lesser than sum of decrease caused by both stress.

### Lipid peroxidation and antioxidant enzymes activity

Lipid peroxidation (LP) measured in terms of MDA content exhibited high reactivity with thiobarbituric acid. The level of MDA increased under allelochemical stresses with maximum 42.72% increase in B<sub>3</sub> treatment. BA+Cd stimulated LP as compared to single stress. Application of BA with Cd synergistically increased MDA content in graded manner in comparison to sum of increase caused by BA and Cd (Table 3).

Increased level of MDA in response to metal and allelochemical stress indicated the oxidative stress in wheat seedling through the formation of free radicals. MDA accumulation was more prominent in BA. It reveals that BA is more toxic than Cd. Cadmium caused oxidative stress resulting into membrane damage (Sharma et al., 2004) by stimulating LP in Cd stressed seedlings as reported by several authors (Finkemeier et al., 2003; Metwally et al., 2003). BA synergized the impact of Cd by accelerating LP. The elevation of LP was more as compared to sum of increase caused by individual stress in respective treatments. Lipid molecules are very sensitive to oxidation by ROS generated under BA stress (Baziramakenga et al., 1995). Lipid peroxidation resulted in the formation of lipid radicals (lipid peroxides). Thus elevated level of lipid peroxides is generally accepted as an indicator of severe oxidative stress (El-Tayeb, 2005).

**Table 3. Effects of benzoic acid and cadmium on lipid peroxidation and antioxidant enzymes activity of *Triticum aestivum*.**

Treatments	Lipid peroxidation	Superoxide dismutase	Catalase	Ascorbate peroxidase	Guaiacol peroxidase
	n mol g <sup>-1</sup> FW	EU g <sup>-1</sup> FW			
C	48.38 ± 0.18	32.33 ± 1.67	0.27 ± 0.039	0.32 ± 0.10	0.83 ± 0.14
Cd	50.36 ± 0.68	38.34 ± 0.97	0.30 ± 0.019	0.67 ± 0.14	0.67 ± 0.07
B <sub>1</sub>	50.87 ± 0.52	48.59 ± 2.24 <sup>b</sup>	0.25 ± 0.009	1.17 ± 0.26	0.90 ± 0.09
B <sub>2</sub>	57.35 ± 1.08 <sup>cdh</sup>	51.90 ± 4.42 <sup>c</sup>	0.27 ± 0.011	2.49 ± 0.32	1.02 ± 0.02
B <sub>3</sub>	62.58 ± 0.37 <sup>cdhxyz</sup>	53.08 ± 0.55 <sup>c</sup>	0.42 ± 0.068	1.89 ± 0.26	1.07 ± 0.01
B <sub>1</sub> +Cd	65.15 ± 0.22 <sup>cdhxyz</sup>	61.77 ± 1.11 <sup>cd</sup>	0.59 ± 0.029 <sup>ghxyz</sup>	1.32 ± 0.02	1.38 ± 0.01 <sup>gh</sup>
B <sub>2</sub> +Cd	66.48 ± 0.50 <sup>cdhxyz</sup>	64.96 ± 1.75 <sup>cd</sup>	0.64 ± 0.019 <sup>ghxyz</sup>	2.28 ± 0.94	1.75 ± 0.05 <sup>ghxyz</sup>
B <sub>3</sub> +Cd	69.05 ± 0.33 <sup>cdhxyz</sup>	70.15 ± 1.69 <sup>cdhxyz</sup>	0.69 ± 0.048 <sup>ghxyz</sup>	3.42 ± 0.53 <sup>b</sup>	1.84 ± 0.15 <sup>ghxyz</sup>

Data are mean of three replicates ± standard error of the mean (SEM).

<sup>b</sup>*P* < 0.01, <sup>c</sup>*P* < 0.001 versus C, <sup>d</sup>*P* < 0.001 versus Cd, <sup>e</sup>*P* < 0.001 versus B<sub>1</sub>, <sup>f</sup>*P* < 0.001 versus B<sub>2</sub>, <sup>g</sup>*P* < 0.001 versus B<sub>3</sub>.

C: control, Cd: 7 mg L<sup>-1</sup>, B<sub>1</sub>, B<sub>2</sub>, and B<sub>3</sub>: 0.5, 1.0, and 1.5 mM benzoic acid, respectively.

We quantified the activity of antioxidant enzymes viz. SOD, CAT, APX, and POX to compare the oxidative damage caused by metal and allelochemical stresses on wheat seedlings. The activity of SOD increased significantly ( $p < 0.05$ ) in response to allelochemical stress. Elevation of SOD activity was more prominent in BA as compared to Cd stress with maximum 1.64 fold increase in B<sub>3</sub> treatment. Seedlings in Cd treatment showed no effect on SOD activity. The seedlings under combined stress exhibited graded increase in SOD activity and comparatively higher when compared with sum of BA and Cd stress. BA exhibited no effect on CAT activity as compared to control. When compared to single stress, BA, and Cd in combinations stimulated CAT activity. Significant elevation of APX activity was evident in higher concentrations of BA while POX activity did not increase significantly. As compared to single treatment, higher level of POX was recorded in the seedlings treated with combination of allelochemical and Cd. Single stress exhibited no influence on POX activity, however in combined treatments it increased significantly in dose dependent manner with maximum 121.68% increase in B<sub>3</sub>+Cd treatment (Table 3).

The two stresses induced antioxidant enzyme in wheat seedlings. Lipid peroxidation corresponded to SOD activity. A significant ( $p < 0.05$ ) elevation of SOD activity in seedlings subjected to BA+Cd stress suggested synergistic effect. Our results with increased activity of SOD and CAT under different allelochemical stress in wheat seedlings are in agreement with studies on several crops viz. *Lycopersicon esculentum* L. (Macias et al., 2002), *Cucumis sativus* L. (Romero-Romero et al., 2005), *Brassica campestris* L. (Oracz et al., 2007) and *Zea mays* L. (Singh et al., 2009). Cd toxicity is reported to enhance SOD activity in leaves and roots of *Phragmites australis* (Iannelli et al., 2002). Elevation in SOD activity as recorded in BA+Cd treatments leads to the accumulation of H<sub>2</sub>O<sub>2</sub>. It is quite pertinent that the net oxidative stress is the result of overall alteration in the ratio of O<sub>2</sub>•<sup>-</sup> scavenging enzyme (SOD) and H<sub>2</sub>O<sub>2</sub> scavenging enzymes (CAT, APX, and POX) (Kanazawa et al., 2000; Shah et al., 2001). APX present in chloroplasts involves in ascorbate glutathione cycle while POX present in cytoplasm binds with cell wall. It seems that increased and decreased activity of CAT in Cd and B<sub>1</sub> treatments respectively compensated by altered activity of other two H<sub>2</sub>O<sub>2</sub> scavenging enzymes viz. APX and POX in respective treatments. The allelochemical with Cd played protective role by stimulating the activity of antioxidant enzymes. The stimulation of CAT activity in allelochemical stress has been shown in earlier studies (Batish et al., 2006; Singh et al., 2010). Ferulic acid and BA are known to elevate CAT activity in maize seedling (Devi and Prasad, 1996) and cucumber cotyledons (Maffei et al., 1999) respectively. Our results are in agreement with

these findings. Cd toxicity reduces activity of H<sub>2</sub>O<sub>2</sub> scavengers causing H<sub>2</sub>O<sub>2</sub> accumulation in plants (Hatata and Abdel-Aal, 2008). Allelochemical with Cd increased APX activity over Cd treatment. Ünyayar et al. (2005) also reported that SOD and APX activity increased in response to combined stress (drought+Cd) as compared to single stress. Our results reveal better performance of antioxidant enzymes under BA stress as compared to Cd and also in combination of BA+Cd when compared with single stress. Single stress has no influence on POX activity, however in combined treatment POX activity increased significantly. Allelochemical and metal work synergistically and increase antioxidant enzymes activity under combined treatments as compared to sum of elevation caused by single stress.

## CONCLUSIONS

The present investigation showed that Cd toxicity creates a stressed condition in wheat plants that required protection of defense system. Seed germination, seedling growth, and biochemical parameters varied with benzoic acid, Cd, and combined treatments. Under combined treatments, toxicity reduced antagonistically as compared to sum of toxicity caused by Cd and allelochemical. Allelochemical appear to mitigate the effect of Cd and buttress antioxidant defense system in presence of Cd. An involvement of cellular antioxidant defense system against oxidative stress broadens the understanding of impact of two different stresses on wheat plants. The cross-adaptation or synergism can be induced. This study reveals new possibilities for deliberate and predictable approach for induced adaptation in crop plants.

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