

Interactive Effects of Cinnamic Acid and Water Stress on Wheat Seedlings

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Abstract

In the present study we compared the effects of allelochemical with water stress (WS) on growth, biochemical parameters and responses of antioxidative enzymes in wheat seedlings. The wheat seedlings were treated with 0.5, 1.0 and 1.5 mM concentrations of cinnamic acid (CA) with and without water stress by withholding water supply for 5 days. Leaf water status, photosynthetic pigments, protein content, amount of proline and nitrate reductase (NR) and antioxidant enzymes activities were examined. CA resulted in reduction of seedling height with drastic decrease in stressed seedlings. The combined treatments CA+WS further decreased the seedling height. The same result was registered for seedlings dry weight, relative water content and pigment and protein contents. Total soluble sugar content and nitrate reductase activity were variedly affected under all treatments. Proline content and lipid peroxidation increased. Activity of superoxide dismutase increased significantly ($P < 0.05$) while catalase activity was lower in all treatments. Ascorbate peroxidase and guaiacol peroxidase activities were higher as compared with catalase which showed protection of wheat seedlings from oxidative stress. Water stress elevated the toxic effect of allelochemical.

1. Introduction

Allelopathy includes both interspecific and intraspecific interactions among plant-plant, plant-microorganisms, plant-virus, plant-insect, and plant-soil-plant via chemicals. Lambers *et al.* (1998) described allelopathy as "suppression of plant growth due to release of chemicals from another species". A varied range of natural products are synthesized and released from plants.

Secondary metabolites have been associated with plant defense mechanism against pathogen, micro-organism, etc. (Silva *et al.*, 2006). However, toxicity of allelochemicals against plants resulting into reduced growth, development and yield has been established by several workers. Cinnamic acid (CA) and its derivatives (ferulic acid, coumaric acid etc.) are major phenolic acids present in wheat plants. They are the most common phenolic acids compounds found in natural and agricultural ecosystems. Cinnamic acids are derived from phenylalanine through phenylpropanoid pathway (Einhellig, 2004). CA is released in soil through root exudation, leaching and residue decomposition of plants (Yu and Matsui, 1997). Toxicity of CA has been established (Einhellig, 1995; Politycka, 1996; Hussain and Regiosa, 2011; Li *et al.*, 2011). Effects of CA on growth, photosynthesis and ion leakage due to altered membrane permeability have been experimentally demonstrated (Yu *et al.*, 2003; Ding *et al.*, 2007). Allelochemicals inhibit seed germination and root and

shoot growth by triggering a wave of reactive oxygen species (ROS) which cause Ca^{+2} signaling cascade triggering genome wide changes leading to death of root system (Bais *et al.*, 2003).

In natural ecosystems, plants encounter variety of environmental stresses viz., heat drought, flood, metal toxicity, etc. Water stress (WS) is one of the major stresses affecting the agriculture and crop productivity. WS is a limiting factor influencing the growth and productivity and affects most of the plant functions (Yamaguchi-Shinozaki *et al.*, 2002).

The production of reactive oxygen species (ROS) accelerates in plants in response to environmental stresses. Increased ROS formation activates the protective response in plants. Superoxide dismutase (SOD) scavenges superoxide radical (O_2^-) by dismutating it into H_2O_2 . Intercellular H_2O_2 level is mediated with help of wide range of enzymes with catalase, ascorbate and guaiacol peroxidase are frontrunner (Blokhina *et al.*, 2003).

The aim of present investigation was to evaluate the interactive effects of biotic and abiotic stresses. The effects of CA with and without water stress on growth and development of wheat seedlings were observed.

2. Materials and Methods

2.1. Plant material and chemicals

Certified and pure line seeds of wheat (*Triticum*

aestivum L. cv HW 2004) were procured from a seed agency in Allahabad. Cinnamic acid (Molecular weight 148.16) extra pure quality was procured from CDH, New Delhi. Stock solution (1.5 mM) of CA was prepared by mixing requisite amount in 250 ml double distilled water (DDW). The stock solution was further diluted with DDW to get 0.5 and 1.0 mM concentrations of CA. Concentrations 0.5, 1.0 and 1.5 mM were labeled as C_1 , C_2 and C_3 , respectively.

2.2. Experimental design

Experimental trays (length 30 cm; width 30 cm; height 10 cm) each filled with 2 kg homogeneous soil (soil:sand, 2:1) were prepared and divided into 2 sets and 3 subsets of each as per treatments. The seeds were surface-sterilized with 0.01% $HgCl_2$ solution for 1 min and washed for three times with DDW and then soaked in distilled water. The seeds of uniform size were sown at equal distance in five rows in each tray. Seedlings were maintained in the growth chamber (temperature: $28 \pm 2^\circ C$; photoperiod: 18 h; humidity: $65 \pm 5\%$ and photon flux density: $240 \mu mol m^{-2} s^{-1}$). The experiment was performed in triplicate. Ten days old seedlings were irrigated with different concentrations of cinnamic acid solution ($C_1=0.5$, $C_2=1.0$ and $C_3=1.5$ mM) accordingly. Untreated seedlings were taken as control. Fifteen days old seedlings of one set was subjected to water stress (WS) by withholding water supply for 5 days while other set was regularly irrigated with water as and when required. Twenty days old seedlings were harvested for measurement of biophysical and analyses of biochemical parameters.

2.3. Plant growth parameters

Seedling height of 20 days old plants treated with CA with and without WS was recorded. Seedlings biomass was measured on the basis of dry weight.

2.4. Relative water content

The first fully expanded leaves were collected and cut into discs of uniform size. Fresh weight (FW) of 10 discs from each treated and control seedlings, was recorded and then discs were immediately floated in double distilled water at $25^\circ C$ in dark. After 24 h turgid weight (TW) of discs was recorded and then oven dried at $80^\circ C$ for dry weight (DW). Relative water content (RWC) was calculated following Bars and Weatherly (1962).

$$RWC (\%) = (FW - DW) / (TW - DW) \times 100$$

2.5. Sugar content

Sugar content was determined following method of Hedge and Hofreiter (1962). Plant leaves (100 mg)

were homogenized in 5 mL 95% ethanol. The homogenate was centrifuged at 4000 *g* for 15 min. The supernatant (0.1 mL) was mixed with 0.9 mL distilled water and 4 mL anthrone solution (0.2% Anthrone and concentrated H_2SO_4). The reaction mixture was boiled in water bath for 15 min. Absorbance was recorded at 620 nm. The amount of total soluble sugar was calculated using standard curve obtained from glucose as reference.

2.6. Protein and pigment contents

The protein content was determined following Lowry *et al.* (1951). The amount of protein was calculated using standard curve obtained from bovine serum albumin as reference. The pigments (chlorophyll *a*, chlorophyll *b* and carotenoids) from the leaves of experimental plants were extracted with 80% acetone and estimated following Lichtenthaler (1987).

2.7. Nitrate reductase activity

Nitrate reductase (EC 1.6.6.1) activity was measured according to the modified procedure of Jaworski (1971) based on incubation of fresh wheat leaves (250 mg) in 4.5 ml medium containing 0.1 phosphate buffer (pH 7.5), 3% KNO_3 and 5% propanol. Aliquot 0.4 mL was treated with 3% sulphanilamide in 3N HCl and 0.02% N-(1-naphthyl)-ethylene diamine dihydrochloride (NEDD). The absorbance was recorded at 540 nm. Activity of NR was calculated using standard curve prepared from $NaNO_2$ as reference. The activity was expressed in term of $\mu mol NaNO_2 g^{-1} FW h^{-1}$.

2.8. Free proline content

Free proline content was determined by following the method described by Bates *et al.* (1973). Plant leaves (250 mg) were homogenized in 3% sulphosalicylic acid and centrifuged at 4000 *g* for 15 min. The supernatant was mixed with acid- ninhydrin reagent prepared by mixing 1.25 g ninhydrin in 30 mL glacial acetic acid and 20 mL of 6 M orthophosphoric acid and acetic acid. The reaction mixture was boiled for 1h and extracted with 4mL toluene. The absorbance of chromophore containing toluene was determined at 520 nm. Amount of free proline was expressed in term of $\mu mol g^{-1} FW$.

2.9. Lipid peroxidation

Lipid peroxidation was measured by estimating malondialdehyde content (MDA) following Heath and Packer (1968). Wheat leaves (200 mg) were homogenized in 5 mL of 0.01% w/v of trichloroacetic acid (TCA) and centrifuged at 10,000 *g* for 10 min. One mL of supernatant was mixed with 4 mL of 0.5% thiobarbituric acid (TBA) prepared in 20% TCA. The

mixture was heated in water bath at 95°C for 30 min followed by quick cooling and centrifuged at 10,000 g for 10 min. The absorbance of supernatant was recorded at 532 nm and corrected by subtracting the non-specific absorbance at 600 nm. MDA content was determined by using extinction coefficient of 155 mM⁻¹ cm⁻¹ and expressed as µmol g⁻¹ FW.

2.10. Preparation of antioxidant enzymes extract

Enzyme extract was prepared by homogenizing 500 mg of plant leaves from each treatment in 10 mL of sodium phosphate buffer (0.1M, pH 7.0, 1% PVP). The homogenate was filtered through cheese cloth and centrifuged at 15,000 g for 30 min in cooling centrifuge (Remi Instruments C 24). The supernatant was collected, stored at 4°C and used as enzyme extract for determining the activities of superoxide dismutase, catalase, ascorbate peroxidase and guaiacol peroxidase.

2.11. Assay of antioxidant enzymes

The superoxide dismutase (EC 1.15.1.1) was assayed according to the method of Beyer and Fridovich (1987) by measuring the activity of superoxide dismutase to inhibit photochemical reduction of nitroblue tetrazolium (NBT). The 4 mL reaction mixture consisted of 20 mM methionine, 0.15 mM ethylene diamine-tetra acetic acid (EDTA), 0.12 mM NBT and 0.5 mL supernatant. Riboflavin 11.96 µM was administered at the end. Test tubes were exposed to fluorescent lamps for 30 min and identical un-illuminated assay mixture served as blank. The absorbance was recorded at 560 nm. One unit of enzyme was expressed 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 in a total volume of 2 mL contained 25 mM potassium phosphate buffer (pH 7.0), 10 mM H₂O₂ and

0.2 mL enzyme extract. The activity was measured by determining the rate of disappearance of H₂O₂ per min at 240 nm and calculated using an extinction coefficient of 39.4 mM⁻¹ cm⁻¹ and expressed as enzyme unit g⁻¹FW. One unit of catalase was determined as the amount of enzyme required to oxidise 1 µM H₂O₂ min⁻¹.

Ascorbate peroxidase (EC1.11.1.11) was assayed on the method based on 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 H₂O₂ and 0.2 mL enzyme extract. The absorbance was recorded for 1 min at 290 nm and calculated using extinction coefficient of 2.8 mM⁻¹ cm⁻¹. Enzyme specific activity was measured as enzyme unit g⁻¹ FW as the amount of enzyme required to oxidise 1 µM ascorbate min⁻¹.

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 H₂O₂ 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 calculated using extinction coefficient of 26.6 mM⁻¹ cm⁻¹ and expressed as enzyme unit g⁻¹ FW.

2.12. Statistical analysis

Statistical significance was assessed at the $P < 0.05$ level using one way ANOVA and means were separated by Duncan's multiple range test ($P < 0.05$) with the help of SPSS 10 software. Mean (\pm SE) were calculated from 3 replicates.

3. Results and Discussion

3.1. Seedling height and dry weight

CA with or without water stress caused significant

Table 1: Seedling height (cm), dry weight (DW) (mg), relative water content (RWC) (%) and protein and sugar content (mg g⁻¹ FW) of wheat seedlings grown in the presence of three concentrations ($C_1=0.5$, $C_2=1$ and $C_3=1.5$ mM) of cinnamic acid with and without water stress (WS).

Treatments	Seedling height	DW	RWC	Protein	Sugar
Control	6.29±0.617 ^a	27.59±0.239 ^a	87.09±2.020 ^a	37.37±1.426 ^a	54.73±1.575 ^e
WS	4.58±0.473 ^{bc}	19.57±1.094 ^{cd}	50.31±1.496 ^e	24.95±0.767 ^b	79.61±0.962 ^b
C ₁	5.02±0.280 ^b	23.75±0.144 ^b	74.23±0.924 ^b	21.20±1.223 ^{bc}	43.46±1.762 ^f
C ₂	4.04±0.118 ^{bcd}	21.05±0.317 ^c	68.26±1.798 ^c	17.28±0.993 ^{cd}	65.32±1.532 ^d
C ₃	3.58±0.256 ^{cd}	17.93±0.156 ^e	61.70±1.044 ^d	14.16±1.385 ^e	73.59±0.777 ^{bc}
C ₁ + WS	3.98±0.129 ^{bcd}	22.75±0.433 ^b	37.56±2.049 ^f	23.28±1.870 ^b	71.54±0.693 ^c
C ₂ + WS	3.25±0.202 ^d	18.81±0.279 ^{de}	32.61±1.772 ^g	18.92±1.293 ^{cd}	77.41±1.568 ^{ab}
C ₃ + WS	3.05±0.144 ^d	15.62±0.649 ^f	30.51±1.627 ^g	16.30±1.050 ^{de}	81.74±2.422 ^a

Mean±SE values followed by same letters within each column are not significantly different at 0.05 (ANOVA and Duncan's multiple range test) n=3. Control=untreated seedlings and WS=withholding water supply for 5 days

decrease in seedling height. Maximum inhibition of 1.75 and 2.06 folds was observed in C_3 and C_3 +WS, respectively. Dry weight (DW) of the seedlings was variedly affected under all treatments. WS caused 29.06% decrease in DW. CA decreased DW of the seedlings and water stress further enhanced the toxicity of CA (Table 1).

Plants compete among themselves for light, space and nutrients. In natural or manipulated ecosystems, biotic and abiotic factors play major role in alteration of normal physiological processes causing stress. Stress is result of environmentally induced changes adversely affecting growth of plants, among others allelopathic stress. Indeed, root elongation, cell division, and cellular structure are altered by phenolic acids. Allelochemicals delay seed germination and seedling growth. In the present study, CA inhibited wheat seedlings growth, a similar result was recorded with *Lactuca sativa* (Hussain *et al.*, 2010) and cucumber (Ding *et al.*, 2007) under high concentrations of CA. Growth was reduced also by water stress, and combined allelochemicals and water stress caused reduction in growth, resulting in loss rate of photosynthesis and decreased dry weight. Reduced biomass was reported in soybean (Specht *et al.*, 2001) and green gram (Webber *et al.*, 2006). Gershenson (1984) has reviewed effect of water deficit on the secondary metabolites and reported that water deficit increase concentration of secondary metabolites.

3.2. Relative water content (RWC) and sugar content

RWC and sugar content were variedly affected under all treatments. WS caused 42.2% decrease in RWC of the seedlings. The effect of combined CA+WS treatment was severe than single WS and CA treatments. A gradual increase in sugar content was

recorded in the seedlings under CA with or without WS, with maximum 49.35% in C_3 +WS except C_1 in which 20.59% inhibition was recorded (Table 1).

Phenolic compounds like caffeic, ferulic, cinnamic and p-coumaric acids have been reported to cause water stress in plants (Einhellig, 1995; Barkosky and Einhellig, 2003; Hussain and Regiosa, 2011). The phenolic compounds and water stress primarily effect root cell membrane by changing configurations, causing ion efflux, inhibition of water and nutrient uptake (Baziramkenga *et al.*, 1995). These changes result in varied plant water relations. Lower water content and growth inhibition have been suggested as main cause of sugar accumulation in plants under stress. Prado *et al.* (2000) determined the enhanced accumulation of sugar in different parts of *Chenopodium quinone* under stress. It is possible that sugars may function as typical osmoprotectant resulting in cell membrane stabilization and maintenance of turgor pressure (Gupta and Kaur, 2005).

3.3. Protein and pigment content

Protein content decreased significantly in all treatments in comparison to control. Maximum 2.63 times decrease was observed in C_3 . CA+WS treatments recorded a small increase in protein content when compared to the seedlings under single CA treatments (Table 1). Pigment content of wheat seedlings decreased under all CA treatments. Combined CA+WS treatments further decreased the pigment content with maximum loss of 69.2% in C_3 +WS (Table 2).

Venkateshwarlu *et al.* (2001) and Rahman *et al.* (2004) reported reduction in protein content due to allelochemicals and water stress respectively. Allelochemicals and water stress have been known to

Table 2: Pigment content (mg g^{-1} FW) and nitrate reductase activity (NRA) ($\mu\text{mol NO}_2 \text{ g}^{-1} \text{ FW h}^{-1}$) of wheat seedlings grown in the presence of cinnamic acid (applied at C_1 , C_2 and C_3 concentrations) with and without water stress (WS).

Treatments	Chlorophyll a	Chlorophyll b	Total Chlorophyll	Carotenoid	NRA
Control	3.67±0.034 ^a	1.22±0.062 ^a	4.89±0.028 ^a	2.62±0.017 ^a	43.42±0.338 ^a
WS	2.38±0.169 ^c	1.06±0.028 ^b	3.44±0.197 ^b	1.21±0.162 ^d	26.40±0.690 ^c
C_1	2.70±0.015 ^b	0.84±0.018 ^c	3.53±0.034 ^b	1.69±0.052 ^c	29.24±0.478 ^b
C_2	1.68±0.039 ^d	0.70±0.027 ^{de}	2.38±0.067 ^c	1.19±0.004 ^d	26.08±0.658 ^c
C_3	1.22±0.010 ^{ef}	0.62±0.013 ^{ef}	1.84±0.023 ^d	1.17±0.011 ^d	21.18±0.207 ^e
C_1 + WS	1.78±0.042 ^d	0.73±0.021 ^d	2.50±0.063 ^c	1.97±0.021 ^b	25.09±0.649 ^{cd}
C_2 + WS	1.40±0.039 ^e	0.59±0.029 ^f	1.99±0.068 ^d	1.49±0.219 ^c	24.45±0.232 ^d
C_3 + WS	1.13±0.005 ^f	0.64±0.032 ^{def}	1.77±0.026 ^d	1.18±0.096 ^{dc}	20.72±0.047 ^e

Mean±SE values followed by same letters within each column are not significantly different at 0.05 (ANOVA and Duncan's multiple range test) n=3. Control=untreated seedling, WS=withholding water supply for 5 days and C_1 =0.5, C_2 =1 and C_3 =1.5 mM of cinnamic acid

alleviate ROS production causing oxidative damage leading to protein degradation. Phenolic acids have been reported to inhibit incorporation of certain amino acids in protein causing decreased rate of protein synthesis (Baziramkenga *et al.*, 1997). Photosynthetic pigment reduction has been associated with decreased biomass (Einhellig and Rasmussen, 1997) due to allelochemicals. Allelochemicals have been reported to reduce chlorophyll synthesis (Yang *et al.*, 2002) or enhance degradation of chlorophyll (Kanchan and Jayachandra, 1980). Combined treatment of allelochemicals and water stress decreased chlorophyll and carotenoids content (Singh *et al.*, 2010). Reduction in carotenoids content clearly indicates the occurrence of oxidative damage resulting in degradation of chlorophyll in wheat plants due to stress treatment.

3.4. Nitrate reductase activity and free proline content

Nitrate reductase activity (NRA) decreased up to 39.1% in the seedlings subjected to water stress. A gradual decrease in NRA was recorded in CA treated seedlings. Combined CA+WS treatment was inhibitorier with 56.3% decrease in activity under C₃+WS (Table 2). A small amount of increase in proline content was observed in CA treated seedlings; however, WS and CA+WS resulted in greater elevation. Maximum increase of 496.7% in C₃+WS followed by 403.2% in WS was recorded in comparison to control (Table 3).

NRA has been known to be affected in response environmental and metabolic signals (Kaiser *et al.*, 1999). NRA reduction might be due to decreased photosynthesis or due to lower induction or synthesis of enzymes (Chen and Sung, 1983). NRA is sensitive to water stress (Foyer *et al.*, 1998). Roots are often

damaged under allelochemicals and water stress consequently decreasing nitrate absorption and foliar availability (Abd-El Baki *et al.*, 2000). Bagavathy and Xavier (2007) and Singh *et al.* (2009) reported decreased NRA due to allelopathins and water stress respectively. Nitrate reductase is substrate induced enzyme. Absorption of nitrate under the influence of allelochemical and water stress may regulate NRA.

Excessive accumulation of ROS and damage due to other stress is capable of inducing proline accumulation (Smirnoff, 1993). Proline accumulation has been correlated to drought (Reddy *et al.*, 2004) as it acts as osmolyte as well as compatible solute. It plays protective role in adaptation of plant cell to water scarcity (Ueda *et al.*, 2008). The accumulation of proline may be due to increased synthesis or decreased degradation (Hare *et al.*, 1999).

3.5. Lipid peroxidation (LP)

The extent of LP varied in response to treatments. LP was measured in terms of malondialdehyde (MDA). The lowest amount of MDA content was recorded in controlled seedlings. WS showed 1.66 folds increase in MDA. A dose dependant increase in MDA was observed in CA treated seedlings. WS in combination with CA further enhanced the extent of LP with the highest 1.92 times increase in C₃+WS over control (Table 3).

Membrane lipid peroxidation is considered as indicator of oxidative stress (Smirnoff, 1993). Membrane lipids are sensitive to ROS that are produced under stress. Membrane damage and lipid peroxidation are common indicator of allelochemical stress (Batish *et al.*, 2006), as it was reported for soybean where CA enhanced MDA content (Baziramkenga *et al.*, 1995).

Table 3: Proline content ($\mu\text{ mol g}^{-1}\text{ FW}$), lipid peroxidation (as $\text{n mol g}^{-1}\text{ FW}$ of malondialdehyde (MDA) and antioxidant enzymes activity ($\text{EU g}^{-1}\text{ FW}$) of wheat seedlings grown in the presence of cinnamic acid (applied at C₁, C₂ and C₃ concentrations) with and without water stress (WS).

Treatments	Proline	LP	SOD	CAT	APX	GPX
Control	0.245±0.006 ^c	58.40±1.037 ^g	63.410±1.326 ^f	0.269±0.015 ^b	1.228±0.075 ^c	0.837±0.042 ^d
WS	1.233±0.048 ^b	97.16±0.595 ^b	82.907±1.672 ^c	0.305±0.005 ^a	2.281±0.085 ^a	0.403±0.008 ^e
C ₁	0.281±0.019 ^c	62.93±2.085 ^f	64.438±1.272 ^f	0.110±0.005 ^d	0.285±0.017 ^d	1.477±0.190 ^b
C ₂	0.289±0.015 ^c	72.58±1.191 ^{de}	71.870±0.993 ^e	0.131±0.010 ^d	0.392±0.020 ^d	1.139±0.103 ^c
C ₃	0.329±0.003 ^c	85.80±1.303 ^c	89.865±0.523 ^b	0.303±0.008 ^a	0.464±0.020 ^d	0.785±0.026 ^d
C ₁ + WS	0.551±0.013 ^d	69.03±0.968 ^c	75.550±1.417 ^d	0.247±0.005 ^b	0.479±0.036 ^d	1.744±0.058 ^a
C ₂ + WS	0.914±0.011 ^c	73.74±0.726 ^d	80.767±1.264 ^c	0.181±0.005 ^c	1.892±0.301 ^{ab}	0.496±0.026 ^c
C ₃ + WS	1.462±0.052 ^a	112.58±1.229 ^a	103.336±0.761 ^a	0.115±0.008 ^d	1.607±0.297 ^{bc}	0.458±0.068 ^c

Mean±SE values followed by same letters within each column are not significantly different at 0.05 (ANOVA and Duncan's multiple range test) n=3. Control=untreated seedling, WS=withholding water supply for 5 days C₁=0.5, C₂=1 and C₃=1.5 mM of cinnamic acid

3.6. Antioxidant enzyme activity

The antioxidant system was activated in response to CA and WS. A dose dependant increase in superoxide dismutase (SOD) activity was observed in CA treated seedlings. In combined treatments WS enhanced activity further with maximum 61.3% stimulation in C_3 +WS. A significant ($P<0.05$) decrease in CAT activity was recorded in all treatments except control and WS. APX activity was variously affected in response to all treatments. Lower activity was observed in all treatments except WS, C_2 +WS and C_3 +WS. GPX activity was inversely proportional to that of APX as the lowest was recorded in WS and C_2 +WS (Table 3).

Biotic and abiotic stresses disrupt the equilibrium between ROS production and degradation. Oxidative damage is caused by accumulation of superoxide and hydroxyl radicals. Superoxide radicals have been reported to increase under allelochemicals and water deficiency and leads to lipid peroxidation resulting into membrane damage (Halliwell and Gutteridge, 1999). Increased SOD activity under allelochemicals was reported in tomato (Macias *et al.*, 2002) and mung bean (Singh *et al.*, 2010). Drought stress in combination with allelochemicals increased SOD in maize (Singh *et al.*, 2009) and mung bean (Singh *et al.*, 2010). H_2O_2 accumulation is toxic to cells and is removed by CAT, APX and GPX. CAT has lower affinity towards H_2O_2 than APX, GPX and is activated in presence of light with final degradation. APX and GPX are one of the most common peroxidases which convert H_2O_2 to H_2O using ascorbate and guaiacol as electron donors to decompose H_2O_2 (Madhusudhan *et al.*, 2003). CAT activity decreased in rice under drought conditions (Sharma and Dubey, 2005). CA singly and in combination with chilling caused increase in APX and GPX activity in cucumber (Li *et al.*, 2011). Increased GPX under drought have been reported in maize (Zhang *et al.*, 1995).

4. Conclusions

It can be concluded from the present study that CA with or without water stress affected the relative water content, growth and plant biomass. All treatments caused decrease in protein and pigment content. Water stress enhanced the level of sugars. CA and water stress induced oxidative stress as evinced by increased LP and accumulation of proline. Activities of antioxidant enzymes increased in response to allelochemical and water stress. Activation of cellular antioxidant defense system against oxidative stress broadens the understanding of impact of two different stresses on wheat plants. The plants were not able to overcome the damage caused by combination of the two stresses

reflecting the injurious nature of biotic and abiotic stresses.

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