

## MODIFICATION OF PHYSIOLOGICAL RESPONSES OF WATER STRESSED *ZEA MAYS* SEEDLINGS BY LEACHATE OF *NICOTIANA PLUMBAGINIFOLIA*

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**Summary.** The present study deals with the effects of allelochemicals present in leachate on the drought response of maize plants. *Nicotiana plumbaginifolia* was selected as a donor plant. 12-d-old maize (*Zea mays* L. cv. Uttam) seedlings with and without leachate were subjected to water stress (WS) by withholding water supply for 6 days. Leaf water status, photosynthetic pigments, protein content, proline, nitrate reductase activity (NRA) and activities of antioxidant enzymes were examined in 18-d-old seedlings. Root and shoot lengths of seedlings decreased in all treatments except in the water stressed seedlings where an increase in root length was recorded. WS decreased relative water content (RWC), pigments and protein content. The decrease was significant when the plants were subjected to both WS as well as allelochemical stress (AS). The higher concentration of leachate caused a drastic decrease in NRA under WS. Superoxide dismutase (SOD) and catalase (CAT) activities were significantly increased under WS. POX activity was not significantly increased under WS alone, but it was significant for the combined stress. However, the higher concentration of leachate (N75 and N100) caused a decrease of these antioxidants. Accumulation of proline was stimulated in plants under WS and WS+AS. Root proline was higher than shoot proline. Impairment of metabolic activities due to WS and the combined effect of WS+AS resulted in inhibited plant growth. The effect of WS was more pronounced in seedlings already experiencing allelopathic condition.

**Keywords:** allelochemical stress, antioxidants, maize, *Nicotiana plumbaginifolia*, water stress.

**Abbreviations:** AS – allelochemical stress; CAT – catalase; NRA – nitrate reductase activity; POX – peroxidase; SOD – superoxide dismutase; WS – water stress.

### INTRODUCTION

Both beneficial and detrimental effects of biochemical interactions on plants caused by secondary metabolites are known as allelopathy (Inderjit, 1996). Leaching,

mulching, volatilization and exudation are the usual phenomena involved in release of allelochemicals in the surroundings (Inderjit and Keating, 1999). Precipitation

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aids in leaching of chemicals from foliar parts of donor plants into the environment. Allelochemicals influence the growth and development of recipient plants in natural ecosystems and agro ecosystems (Inderjit and Duke, 2003). These allelochemicals belong to diverse chemical groups and have different sites and mode of action. In general, when the allelochemicals released from donor plants adversely affect the growth of recipient plants, it is considered as a biotic stress called allelochemical stress (Cruz-Ortega et al., 2002; Romero-Romero et al., 2002). Abiotic stresses play a key role in accumulation and transportation of allelochemicals in recipient plants (Luo Simming, 2005). These stresses have been shown to affect the amount of allelochemicals produced in the donor plants (Mwaja et al., 1995). Stresses like drought, salinity and freezing cause water deficit in the recipient plants (Ingram and Bartels, 1996).

Water deficit causes low photosynthetic rate and impaired metabolic activities (Lawlor and Cornic, 2002), thus leading to decreased growth of plants. Nitrate reductase (NR) is an important cytosolic enzyme and its activity is related to crop productivity (Lee and Stewart, 1978). Its activity is affected by allelochemicals (Rice, 1984) and water stress (Hsiao, 1973). Both allelochemicals and water stress cause oxidative stress independently and lead to the generation of reactive oxygen species (ROS) which cause lipid peroxidation (Giortti, 1990; Smirnov, 1993). ROS oxidize protein and DNA and are responsible for membrane leakage, cellular damage and ultimately cell death (Neill et al., 2002). It has been reported that tolerant plants have high level of osmolytes and a cascade of chemical reactions caused by increased level of

defense enzymes. Superoxide dismutase (SOD), catalase (CAT), peroxidase (POX), ascorbate peroxidase (APX) and glutathione reductase (GR) are the main enzymes/constituents of the antioxidant defense system which help in scavenging the lethal and semilethal ROS. Due to the global warming a change in pattern of precipitation has been observed. Sometimes prolonged precipitation is followed by a dry period. In this situation plants experience water stress under allelopathic conditions. During the precipitation large amounts of leachates accumulate in the soil due to the donor plants. It is interesting to find out the role of allelochemicals in the modification of physiological processes under the influence of water stress (WS) and also to study whether the allelochemicals buttress or counter the effect of water stress.

The present interactive study was undertaken in order to investigate the degree of oxidative stress caused by the cumulative effect of these two factors. A study of water status, pigments and protein content and the activities of nitrate reductase and antioxidative enzymes in *Zea mays* as a test crop was done under water stress and crude mixture of allelochemicals leached out from *Nicotiana plumbaginifolia*, a weed rich in allelochemicals. It is a common weed of crop fields in North India. *N. plumbaginifolia* contains several compounds including phenol, polyphenols (rutin, chlorogenic acid, quinic acid, shikimic acid, quercetin, isoquerciterin, scopoletin and kaempferol) and tannin classes, mostly in the form of glycosides. It also contains alkaloids, such as nicotine and non-nicotine with the highest concentration found in the leaves (Anonymous, 1966). Our study will definitely help to sketch out the physiological alteration caused by allelochemicals together with water stress.

## MATERIALS AND METHODS

### Leachate preparation

Plants of *Nicotiana plumbaginifolia* were collected in September, 2007 from the wild population found in vegetative stage in the Roxburgh Botanical Garden, Department of Botany, University of Allahabad, Allahabad (24°47' and 50°47' N latitude; 81°91' and 82°21'E longitude; 78 m above sea level ). 250 plants (0.5 kg) were placed in a 50×50 cm tray. The rain was simulated through a water dispersion unit over the plant material, which was kept in thin layer so as to facilitate the free circulation of 5 l distilled water (DW) (1:10 w/v). This process continued 24 h. Thereafter, the collected leachate was kept in a refrigerator at 8°C to avoid biodegradation. It was considered to have 100% concentration. The leachates of 25, 50 and 75% concentrations were made by diluting the parent leachate with distilled water.

### Soil culture

Seeds of maize (*Zea mays* cv. Uttam) were obtained from a seed agency in Allahabad, Uttar Pradesh, India. Healthy seeds of maize were surface sterilized in 0.001 M HgCl<sub>2</sub> and washed with double distilled water thoroughly. 25 seeds of uniform weight were sown in each experimental tray (length 30 cm, width 30 cm, height 10 cm) in 5 equidistant rows filled with 6 kg homogeneous soil (clay:sand, 2:1). The experiment was conducted in a culture room at a temperature 28±2°C, photoperiod 18/6h, humidity 61±5% and photon flux density 240 μmol m<sup>-2</sup> s<sup>-1</sup>. Six replicates were done. The trays were arranged in two blocks. One block was irrigated with DW and the trays of other block were irrigated with 200 ml of graded

concentration of leachate up to 12 d. After 12 d the first block was arranged into two sets. One set received regular water supply (control) and the other was subjected to water stress by withholding the water supply for 6 d (WS). The leachate irrigated block was also subjected to water stress for 6 d simultaneously. Eighteen-d-old seedlings were harvested. The first fully expanded leaves and roots were sampled for analyses.

### Relative water content

For the measurement of relative water content (RWC) leaves were cut into discs of uniform size, weighed for a fresh weight (FW) and were immediately floated on distilled water at 25°C in the darkness. After 12 h the turgid weight (TW) was measured and then discs were dried in an oven at 80°C for 48 h for the dry weight (DW). The RWC was calculated by the modified method of Bars and Weatherly (1962).

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

### Measurement of pigments and protein content

Chlorophyll a, chlorophyll b and carotenoids from the first fully expanded leaves were extracted in 80% acetone and were quantified following Lichtenthaler (1987). Protein content was determined according to Lowry et al. (1951) with bovine serum albumin as a standard.

### Extraction and assay of enzymes

Nitrate reductase (EC 1.7.99.4) activity was assayed by the modified procedure of Jaworski (1971) based on the incubation of fresh tissue (0.25 g) in 4.5 ml medium containing 100 mM phosphate buffer (pH 7.5), 3% KNO<sub>3</sub> and 5% propanol. 0.4 ml aliquot was treated with 0.3 ml 3%

sulphanilamide in 3 N HCL and 0.3 ml 0.02% N-(1-naphthyl)-ethylene diamine dihydrochloride (NEDD). The absorbance was measured at 540 nm. NR activity was calculated with a standard curve prepared using  $\text{NaNO}_2$ .

Superoxide dismutase (EC 1.15.11) activity was determined by the nitroblue tetrazolium (NBT) photochemical assay method following Beyer and Fridovich (1987). 0.2 g fresh leaf tissue was homogenized in 1 % polyvinyl pyrrolidone (PVP) prepared in 50 mM potassium phosphate buffer (pH 7.0) and centrifuged at  $15,000\times g$  for 30 min at  $4^\circ\text{C}$ . The reaction mixture contained 0.5 ml clear supernatant, 2 ml 0.15 mM ethylenediaminetetra acetic acid (EDTA), 20 mM methionine, 0.12 mM NBT and 0.5 ml  $11.96\ \mu\text{M}$  riboflavin, 0.5 ml PVP. The optical density (OD) was determined colorimetrically against a blank at 560 nm. One unit of SOD activity was defined as the amount of enzyme required to cause 50% inhibition of the reduction of NBT.

Catalase (EC 1.11.1.6) was assayed according to the method of Sinha (1972). 0.2 g of fresh leaf tissue was homogenized in 100 mM potassium phosphate buffer (pH 7.0) and centrifuged at  $10,000\times g$  for 30 min at  $4^\circ\text{C}$ . The reaction mixture contained 0.5 ml enzyme extract, 1.25 ml 0.2 M  $\text{H}_2\text{O}_2$ , 3.2 ml potassium phosphate buffer. The reaction mixture was mixed with potassium dichromate acetic acid reagent for colorimetry at 570 nm and one unit of enzyme activity is defined as the amount which produced an increase of 0.1 OD per minute.

Peroxidase (EC 1.11.1.7) was assayed following McCune and Galston (1959). Fresh leaf tissue (0.2 g) was homogenized in 0.1 M potassium phosphate buffer (pH 6.0) and centrifuged at  $10,000\times g$

for 20 min at  $4^\circ\text{C}$ . The reaction mixture contained 2.0 ml enzyme extract, 2 ml potassium phosphate buffer, 1.0 ml 0.1 N pyrogallol and 0.2 ml 0.02%  $\text{H}_2\text{O}_2$  and OD was determined at 430 nm. One unit of enzyme activity is defined as the amount which produced an increase of 0.1 OD per minute.

### Estimation of free proline

Extraction and determination of proline were performed according to Bates et al. (1973). Leaf samples were extracted with 3% sulphosalicylic acid. An aliquot was treated with acid-ninhydrin and acetic acid, boiled for 1 h at  $100^\circ\text{C}$ . The reaction mixture was extracted with 4 ml of toluene. The absorbance of chromophore containing toluene was determined at 520 nm. Proline content was expressed as  $\mu\text{mol g}^{-1}\text{FW}$  using a standard curve.

### Statistical analysis

Data were statistically analyzed using analysis of variance (ANOVA) by using SPSS (Ver. 10; SPSS Inc., Chicago, IL, USA). The appropriate standard error of means ( $\pm\text{SE}$ ) was calculated for presentation in the tables and graphs. The means were analyzed by Duncan's multiple range test (DMRT) at  $P < 0.05$ .

## RESULTS

### Root length, shoot length and relative water content

Water stress (WS) and allelochemical stress (AS) caused significant alterations in the height of seedlings (Table 1, right;  $P < 0.05$ ). Allelochemicals caused a gradual decrease in root length (RL) with higher dosage of leachates. N-100 was recorded for minimum RL and shoot length (SL). Maximum SL and RL were observed

Table 1. Effect of water stress and *Nicotiana plumbaginifolia* leachate on growth of *Zea mays* seedlings.

Treatments	RWC [%]	Seedling height [cm]		
		Root length (RL)	Shoot length (SL)	RL/SL
C	94.45±0.21 <sup>a</sup>	6.02±0.03 <sup>a</sup>	9.50±0.01 <sup>a</sup>	0.63±0.00 <sup>b</sup>
WS	91.63±3.71 <sup>a</sup>	6.19±0.07 <sup>a</sup>	8.63±0.04 <sup>b</sup>	0.72±0.00 <sup>a</sup>
N 25	84.15±3.04 <sup>ab</sup>	5.78±0.09 <sup>b</sup>	8.05±0.06 <sup>c</sup>	0.72±0.01 <sup>a</sup>
N 50	77.01±2.84 <sup>bc</sup>	4.71±0.06 <sup>c</sup>	7.80±0.01 <sup>d</sup>	0.60±0.01 <sup>c</sup>
N 75	67.87±5.47 <sup>cd</sup>	3.85±0.06 <sup>d</sup>	7.17±0.07 <sup>e</sup>	0.54±0.00 <sup>d</sup>
N 100	64.28±7.95 <sup>c</sup>	3.07±0.06 <sup>e</sup>	6.02±0.02 <sup>f</sup>	0.51±0.01 <sup>e</sup>

Mean ± (SE) values followed by the same letters within each column are not significantly different at 0.05 (ANOVA and Duncan's multiple range test) n=3. C-control; WS-water stress; N-leachate+water stress; 25, 50, 75, 100 represent concentrations (%).

in control (C) and water stressed plants (WS), respectively. Both WS and WS+AS decreased the SL.

Water status of the seedlings was differently influenced by stress treatment. The highest RWC was recorded in leaves of control seedlings. The decrease in RWC under WS was not significant, but RWC was sensitive to leachate. A gradual significant decrease in RWC was recorded with increasing leachate concentration ( $P < 0.05$ ). WS together with allelopathins present in the leachate caused a significant decrease in RWC. Minimum RWC was recorded in N-100 seedlings (Table 1, left).

### Photosynthetic pigments and protein content

Water deficit significantly decreased pigment content in the seedlings with and without leachates (Table 2). Leachate also adversely affected pigment content. Chlorophyll a (chl a) decreased gradually with the increase in leachate concentration. The control seedlings exhibited maximum amount

of chl a followed by WS. The minimum amount of chl a was recorded in N-100. However, chl b exhibited different pattern at the moderate concentrations of leachate. N-50 and N-75 increased chl b under WS condition while lower (N-25) and higher (N-100) concentrations of leachate showed a decrease of the same. The maximum amount of chl b was recorded in N-50. Total chlorophyll also decreased under water stress. Control seedlings showed maximum amount of total chlorophyll followed by WS, while a gradual decrease was recorded with increasing leachate concentration. Carotenoids exhibited a similar trend of changes as chlorophyll.

Control seedlings exhibited a maximum amount of protein in the leaves. A decrease in protein content was recorded in seedlings under WS and WS+AS. Among stressed seedlings WS showed a higher amount of protein in comparison to seedlings which were pretreated with graded leachate concentrations. Protein content was inversely proportional to the concentration of leachate.



Table 2. Effect of water stress and *Nicotiana plumbaginifolia* leachate on pigments and protein content in the leaves of *Zea mays* seedlings.

Treatments	Pigment [mg g <sup>-1</sup> FW]				Protein [mg g <sup>-1</sup> FW]
	chlorophyll a	chlorophyll b	Total chlorophyll	carotenoids	
C	5.25±0.24 <sup>a</sup>	0.50±0.00 <sup>a</sup>	5.74±0.12 <sup>a</sup>	2.07±0.04 <sup>a</sup>	28.35±0.00 <sup>a</sup>
WS	3.78±0.24 <sup>b</sup>	0.45±0.18 <sup>a</sup>	4.23±0.42 <sup>b</sup>	1.08±0.09 <sup>b</sup>	25.53±4.26 <sup>b</sup>
N 25	3.54±1.18 <sup>bc</sup>	0.39±0.14 <sup>a</sup>	3.93±1.32 <sup>bc</sup>	1.00±0.28 <sup>bc</sup>	24.68±3.18 <sup>bc</sup>
N 50	2.82±0.72 <sup>bcd</sup>	0.51±0.38 <sup>a</sup>	3.33±1.10 <sup>bc</sup>	0.73±0.06 <sup>cd</sup>	23.10±2.56 <sup>c</sup>
N 75	2.64±0.34 <sup>cd</sup>	0.50±0.04 <sup>a</sup>	3.14±0.30 <sup>bc</sup>	0.72±0.22 <sup>cd</sup>	18.40±1.14 <sup>d</sup>
N 100	2.34±0.13 <sup>d</sup>	0.46±0.07 <sup>a</sup>	2.80±0.05 <sup>c</sup>	0.65±0.11 <sup>d</sup>	16.40±3.24 <sup>e</sup>

Mean ± (SE) values followed by the same letters within each column are not significantly different at 0.05 (ANOVA and Duncan's multiple range test) n=3. C – control; WS – water stress; N – leachate + water stress; 25, 50, 75, 100 represent concentrations (%).

### Nitrate reductase activity

Nitrate reductase activity (NRA) in the leaves was slightly affected by water deficit but it was highly sensitive to potential allelopathins present in the leachate (Fig. 1). The maximum NRA was recorded in control seedlings. The decrease

in NRA was not significant under WS and in seedlings treated with the lower leachate concentration (N-25). However, a drastic decrease in NRA was recorded in stressed seedlings with higher concentrations of leachate.

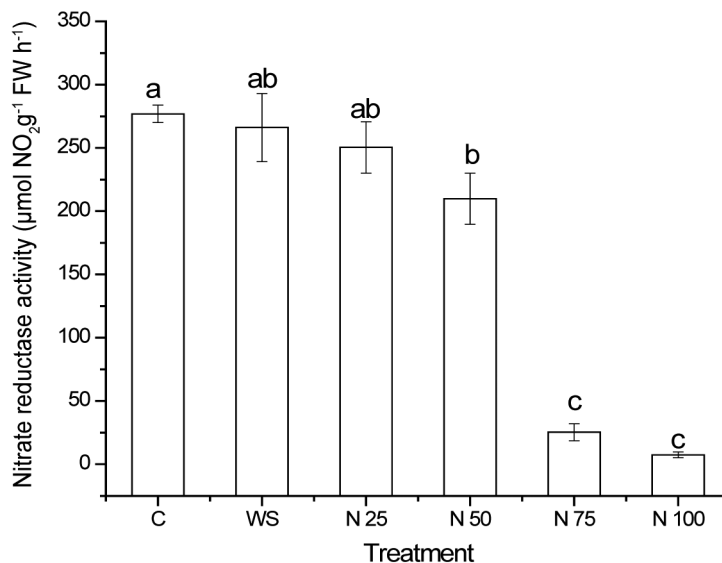


Fig. 1. Effect of water stress and *Nicotiana plumbaginifolia* leachate on nitrate reductase activity in leaves of *Zea mays* seedlings. Mean ± (SE) values followed by the same letters are not significantly different at 0.05 (ANOVA and Duncan's multiple range test) n=3. C – control; WS – water stress; N – leachate + water stress; 25, 50, 75, 100 represent concentrations (%).

### Antioxidant enzymes

SOD, CAT and POX, the important constituents of the antioxidative defense system were also analyzed. N-50 showed increased SOD activity under water deficit which was more than C and WS. However, higher concentrations (N-75 and N-100) decreased the activity of SOD in the leaves. The lowest SOD activity was recorded at N-100. CAT activity also increased in the seedlings under WS. WS exhibited maximum activity of CAT which decreased in stressed seedlings treated with leachate but was always higher than control except at N-100. The decrease was proportional to the leachate concentrations. The highest CAT activity in water stressed seedlings gradually decreased under the influence of leachate. An increase in POX activity in water stressed seedlings was not significant

but it was enhanced by allelochemicals. The maximum POX activity was recorded at N-25, which was gradually decreased with increasing leachate concentration (Fig. 2).

### Proline

WS significantly enhanced the accumulation of proline in the roots ( $P < 0.05$ ) of the stressed seedlings. Leaf proline remained unaffected in all treatments. The amount of root proline was higher than leaf proline. The higher amount of proline in the roots of WS seedlings gradually increased up to N-50 under the influence of leachate. A decrease in the amount of proline was recorded in the seedlings treated with higher concentrations of leachate but the amount remained still higher compared to control and WS (Fig. 3).

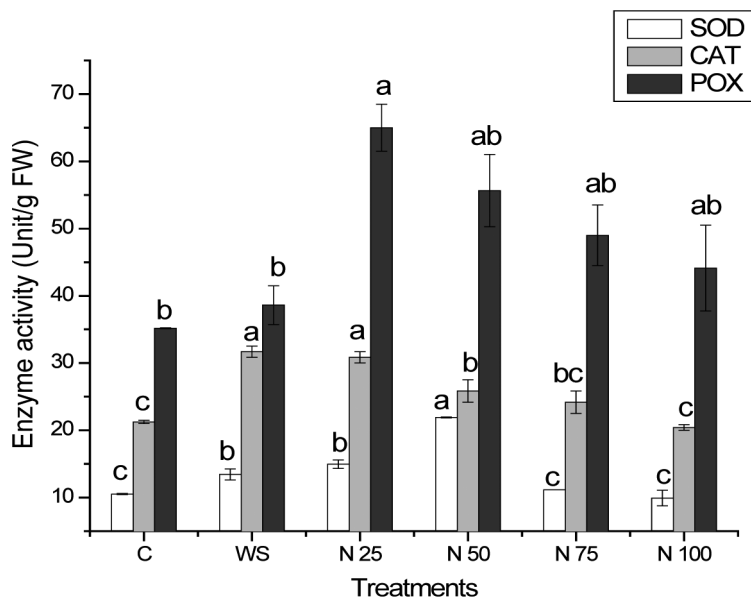


Fig. 2. Effect of water stress and *Nicotiana plumbaginifolia* leachate on antioxidant enzymes activity in leaves of *Zea mays* seedlings. Mean  $\pm$  (SE) values followed by the same letters are not significantly different at 0.05 (ANOVA and Duncan's multiple range test)  $n=3$ . C – control; WS – water stress; N – leachate + water stress; 25, 50, 75, 100 represent concentrations (%).

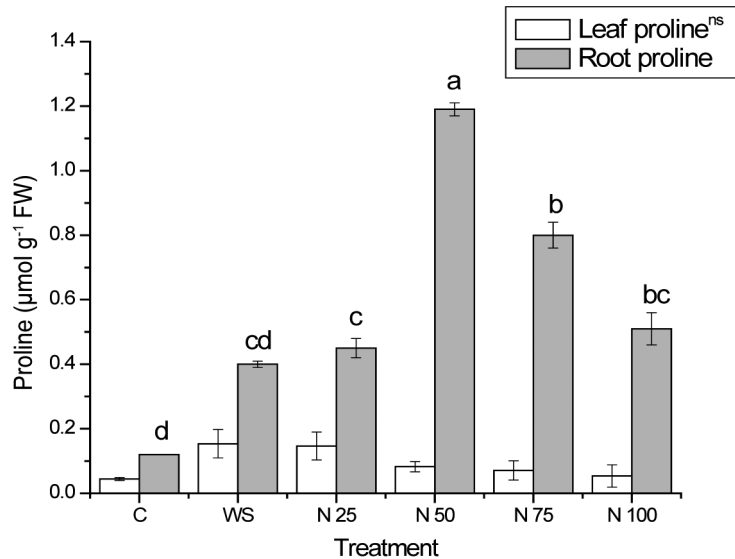


Fig. 3. Effect of water stress and *Nicotiana plumbaginifolia* leachate on proline accumulation in roots and leaves of *Zea mays* seedlings. Mean  $\pm$  (SE) values followed by the same letters are not significantly different at 0.05 (ANOVA and Duncan's multiple range test)  $n=3$ ; ns=not significant. C – control; WS – water stress; N – leachate + water stress; 25, 50, 75, 100 represent concentrations (%).

## DISCUSSION

Water stress resulted in decreased RWC (Jiang and Zhang, 2002). A further decrease in RWC of water stressed plants treated with leachate may be explained by the fact that allelochemicals caused physiological drought to plants (Barkosky and Einhellig, 2003). Allelochemicals intensified the water deficit in water stressed seedlings.

Water stress causes loss of turgor which affects rate of cell expansion and ultimately cell size and hence, reduces the shoot growth (Hale and Orcutt, 1987). A reduction of plant growth is one of the most prominent features of WS. It is part of drought avoidance mechanism (Ünyayar et al., 2005). Our results showed a slight induction in RL in water stressed plants, which is in agreement with other reports (Younis et al., 2000; Creelman et al., 1990), but a decrease in RL was recorded in seedlings treated with allelochemicals.

Allelochemicals inhibited the elongation, expansion and division of cells (Einhellig, 1995) which is a prerequisite for seedling growth. Allelochemicals also caused premature lignification of roots resulting in arrested growth. (dos Santosh et al., 2004).

Seedlings treated with various concentrations of leachate exhibited a severe decrease in photosynthetic pigments under WS. WS caused a reduction of chl a, chl b, total chlorophyll and carotenoids (Younis et al., 2000; Rahman et al., 2004). According to Smirnoff (1993) the decrease in photosynthetic pigments is an index of water deficit. The decrease in the amount of photosynthetic pigments was more pronounced in seedlings treated with various concentrations of leachate. Allelochemicals caused a decrease in photosynthetic pigments (Bagavathy and Xavier, 2007). Secondary metabolites inhibit the activity of protoporphyrinogen IX



and 4-hydroxyphenylpyruvate dioxygenase or phytyl desaturase, the key enzymes in chlorophyll and carotenoid biosynthesis, respectively (Romagni et al., 2004). The effect of allelochemicals is concentration dependent (Singh and Singh, 2003). In our experiment also the decrease in pigment content was expressed to a higher extent in seedlings treated with higher leachate concentrations. Both factors, (WS+AS), were highly unfavourable for photosynthetic pigments as well as for accessory pigments separately or in combination.

WS (Rahman et al., 2004) and allelopathins (Venkateshwarlu et al., 2001) caused a reduction of protein content. Both the WS and allelopathins are known to generate reactive oxygen species (ROS) (Zgallai et al., 2006; Lara Nunez et al., 2006) which caused oxidative modification/degradation of proteins (Pacifice and Davies, 1990). Inhibition of photosynthesis and other impaired metabolic activities resulted in decreased protein synthesis, which was evident from the correlation recorded in the decrease in photosynthetic pigments and protein contents. Another probable reason for the decrease in protein is its breakdown into amino acids which serve as osmolytes and defense enzymes (Stewart et al., 1977).

NRA was decreased in the water stressed seedlings. Several complementary factors and reactions are responsible for such a decline of NRA. As photosynthesis provides the energy, e<sup>-</sup> donors and carbon skeleton, NRA was found to be dependent on it (Kaiser et al., 1993). The reduced photosynthetic rate may be responsible for such a loss of NRA. Another reason for NRA reduction may be an inhibited synthesis or low induction of enzymes (Chen and Sung, 1983). The absorption of nitrate by the roots due to drying soil and

transport of nitrate from the roots to the leaves were affected by water deficit which consequently decreased the foliar nitrate (Foyer et al., 1998). Allelochemicals are known to inhibit NRA. Bagawathy and Xavier (2007) reported that NRA was inhibited in *Sorghum* plants treated with *Eucalyptus* leaf extract. Tripathi et al. (2000) also reported a decrease in NRA of *Vigna radiata* leaves by the leaf and root extracts of *Dalbergia sissoo*. Allelochemicals at higher concentrations were responsible for the drastic decrease in NRA in the water stressed seedlings.

Several enzymes of the defense system increased tremendously during oxidative stress in order to avoid the damage caused by ROS (Foyer and Noctor, 2003). These defense enzymes act as stress markers. Their assay is an essential aspect in assessing stress responses in plants. SOD is considered to be the first line of defense against ROS (Gomez et al., 2004). WS increased the activities of SOD. Plants adapted to water stress maintained a high level of antioxidants. Water stressed seedlings treated with N-25 and N-50 leachates showed increased activity of SOD. Allelochemical stress can generate ROS and cause oxidative stress (Lara-Nunez et al., 2006). Allelochemicals stimulated the activities of SOD and POX (Dobłinski et al., 2003). The seedlings treated with higher concentrations of leachate, when subjected to water stress, decreased the activity of SOD. This indicated failure of antioxidant defense system at higher concentrations of allelopathins, which may have adversely altered the plant-water relationship (Barkosky and Einhellig, 2003). The activity of SOD increased in water stressed seedlings and in the seedlings treated with lower concentration of leachate, but the same decreased in seedlings under

the influence of higher concentration of leachate. Under the combined effect of water stress and allelochemical stress (WS + AS) CAT activity decreased. Toxic  $H_2O_2$  might have accumulated in the tissues of target species which caused deleterious effects (Mehdy, 1994). However, an increase in CAT activity was recorded in seedlings under WS alone which was in agreement with other reports (Bailly et al., 2004; Jiang and Zhang, 2002). Leachate may cause a decrease in CAT activity. When maize seedlings were treated with leachate of *Callicarpa acuminata*, CAT activity decreased (Cruz-Ortega et al., 2002). This was evidence that in maize other enzymes of antioxidant defense system played an important role to cope up with WS + AS.

Proline accumulation is positively related to drought tolerance (Reddy et al., 2004). Proline acts as an osmoprotectant as well as a compatible solute (Ueda et al., 2008). In our experiment, proline accumulation was recorded in the root and the shoot of water stressed maize seedlings with and without allelochemicals. Proline accumulation was the result of a stress response induced by allelochemicals (Lata Kowska et al., 2008). WS tended to increase proline level, but together with allelopathins the accumulation of osmolyte was severely affected. The decrease in proline level by allelopathins suggested two possibilities (i) allelochemicals caused either relief from stress (ii) or allelochemicals affected the activity of the enzymes,  $\Delta^1$ -pyrroline-5-carboxylate synthetase (P5CS) and proline dehydrogenase (PDH), being two key enzymes in proline synthesis and degradation, respectively. Our results more or less favored the second possibility, because the degree of stress was increased as was evident from other physiological parameters.

In roots, proline level was increased at lower water potential due to proline transport. The relative expression level of proline transporters in vascular and cortical cells of roots is much higher than in leaves (Grallath et al., 2005). Our results were in agreement with the fact that mild increase in proline accumulation (2 to 3-fold) caused root elongation (Ueda et al., 2008) while overaccumulation suppressed the root growth (Maggio et al., 2002). The roots/root hairs, being the organ which absorbs water from the surrounding soil solution, have to increase the concentration of cell sap to absorb more water.

In conclusion, the present results showed a deleterious effect of WS in *Zea mays* seedlings which was further enhanced by the allelochemicals present in the aqueous leachate of *Nicotiana plumbaginifolia*.

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