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Growth, photosynthetic electron transport, and antioxidant responses of young soybean seedlings to simultaneous exposure of nickel and UV-B stress

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Abstract

The effects of enhanced ultraviolet-B (UV-B, 0.4 W m^{-2}) irradiance and nickel (Ni, 0.01, 0.10 and 1.00 mM; $Ni_{0.01}$, $Ni_{0.10}$, $Ni_{1.00}$, respectively) treatment, singly and in combination, on growth, photosynthetic electron transport activity, the contents of reactive oxygen species (ROS), antioxidants, lipid peroxidation, and membrane leakage in soybean seedlings were evaluated. $Ni_{0.10}$ and $Ni_{1.00}$ and UV-B declined the growth and chlorophyll content, which were further reduced following combined exposure. Contrary to this, $Ni_{0.01}$ stimulated growth, however, the effect together with UV-B was inhibitory. Carotenoids showed varied response to both the stresses. Simultaneous exposure of UV-B and Ni as well as UV-B alone reduced the activities of photosystems 1 and 2 (PS1 and PS2) and whole chain activity significantly, while Ni individually, besides strongly inhibiting PS2 and whole chain activity, stimulated the PS1 activity. Both the stresses, alone and together, enhanced the contents of superoxide radical ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2), malondialdehyde (MDA), electrolyte leakage, and proline content, while ascorbate content declined over control. Individual treatments increased the activities of catalase (CAT), peroxidase, and superoxide dismutase (SOD), but $Ni_{1.00}$ declined SOD activity significantly. Combined exposure exhibited similar response, however, CAT activity declined even more than in control. Compared to individual effects of UV-B and Ni, the simultaneous exposure resulted in strong inhibition of photosynthetic electron transport and excessive accumulation of ROS, thereby causing severe damage to soybean seedlings.

Additional key words: *Glycine*; lipid peroxidation; oxidative stress; photosynthetic electron transport activity; photosynthetic pigments; reactive oxygen species.

Introduction

Depletion of stratospheric ozone layer due to anthropogenically produced CFC's is enhancing the solar ultraviolet-B irradiation (UV-B, 280–320 nm) at the Earth's surface (Madronich *et al*. 1998). UV-B irradiation affects plants from the molecular to the ecosystem level (Caldwell *et al*. 1998) and is posing threat to very survival of the biotic communities. It may damage nucleic acids, proteins, and lipids, and thus produce mutagenesis and depression of key physiological processes (Teramura and Sullivan 1994, Jansen *et al*. 1998). Although enhanced UV-B irradiation causes a multitude of deferring effects, changes in electron transport capability, chloroplast ultrastructure, stomatal aperture, and contents of both photosynthetic and protective pigments are important mechanisms through which UV-B exposure leads to inhibition of photosynthesis (Teramura and Sullivan 1994). Studies related to the effects of elevated UV-B irradiation on physiology, growth, and development of plants have shown that effectiveness of UV-B irradiation is modified by abiotic factors such as ozone (Rao *et al.* 1996), heavy metals (Dubé and Bornman 1992), *etc.* Heavy metals (Pb, Cd, Zn, Hg, Ni, *etc*.) are important components of aquatic and terrestrial ecosystems. Some of these metals are required in some plants but most of

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Abbreviations: ASC – ascorbate; CAT – catalase; Chl – chlorophyll; DCMU – 3-(3,4 dichlorophenyl)-1,1-dimethyl urea; DCPIP – 2,6-dichlorophenol indophenol; MDA – malondialdehyde; MV – methyl viologen; NBT – *p*-nitroblue tetrazolium chloride; p-BQ – *p*-benzoquinone; PPFD – photon flux density; POD – peroxidase; PS – photosystem; SOD – superoxide dismutase; UV-B – ultraviolet-B radiation (280–320 nm).
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them are toxic to plants and enter into the ecosystems through air, drainage water, and river canal systems carrying industrial effluents. Plant absorbs and accumulates metals in leaves at high rate which adversely affects various physiological activities (Carlson *et al.* 1975). Nickel, one of the major heavy metals in effluents, released from steel, iron, and electroplating industry inhibits the photosynthetic electron transport activity in isolated chloroplasts of barley seedlings (Mohanty *et al.* 1989) and alters chlorophyll (Chl) fluorescence characteristics, *e.g.* in young mung bean seedlings (Gopal *et al.* 2002). Under stress the plant stimulates the formation of reactive oxygen species (ROS) at various sites of respiratory and photosynthetic electron transport chain (Arora *et al.* 2002, Matysik *et al.* 2002) and thus creates oxidative stress in cellular systems (Hideg and Vass 1996). The ROS hydrogen peroxide (H_2O_2), superoxide radical ($O_2^{\cdot-}$), and hydroxyl radical ($OH^{\cdot}$) are highly reactive and induce lipid peroxidation, thereby affecting the structural integrity and permeability of cellular membranes (Dai *et al.* 1997). Plant metabolizes activated oxygen species by invoking an increased activity of enzymatic antioxidants such as superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), and glutathione reductase (Kondo and Kawashima 2000) and also by producing larger amounts of non-enzymatic antioxidants such as proline, flavonoids, ascorbate (ASC), and carotenoids (Car) (Arora *et al.* 2002, Matysik *et al.* 2002).

Changes in the incidence of UV-B irradiation and deposition of heavy metals in soil are important components of a changing climate, and together they may considerably affect the plants. Recently, the combined effect of UV-B and metal on growth, nutrient uptake, and photosynthesis in cyanobacterium *Anabaena doliolum* (Rai *et al.* 1998) and higher plants (Dubé and Bornman 1992) was studied. But detailed mechanism of action of UV-B and heavy metal (particularly Ni) in simultaneous exposure has not been investigated in plants. Therefore, we tested the impact of UV-B and Ni alone as well as in combination on growth, photosynthetic electron transport activities, state of ROS, antioxidant systems, lipid peroxidation, and membrane damage of soybean seedlings.

Materials and methods

Plants and growth conditions: Surface sterilized healthy seeds of soybean (*Glycine max* L. cv. Punjab 1) were sown in sterilized sand and 5-d-old seedlings of the same size were transferred to 0.2 % strength of Rorison nutrient medium (pH 7.5) containing 0.4 mM $Ca(NO_3)_2$, 0.2 mM $MgSO_4$, 0.2 mM KH_2PO_4, 0.1 μM $CuSO_4 \times 5\,H_2O$, 0.2 μM $ZnSO_4 \times 7H_2O$, 9.2 μM H_3BO_3, 1.8 μM $MnCl_2 \times 4\,H_2O$, 0.2 μM $NaMoO_4 \times 2\,H_2O$, and 10 μM Fe-EDTA. Seedlings were kept in the growth chamber maintained at 27±2 °C and irradiated for 13 h per day with photosynthetic photon flux density (PPFD) of 150 μmol $m^{-2}\,s^{-1}$ from fluorescent tubes.

Nickel and UV-B treatments: After acclimatizing in nutrient medium for two days, the seedlings were transferred to the fresh nutrient medium with various Ni concentrations (0.01, 0.10, and 1.00 mM; further $Ni_{0.01}$, $Ni_{0.10}$, and $Ni_{1.00}$, respectively) for next two days, and thereafter exposed to UV-B irradiation (0.4 W m^{-2}, simulating 15 % ozone depletion at Varanasi, adjoining to Allahabad) for 45 min (which corresponds to 1.08 kJ m^{-2}) together with PPFD of 50 μmol $m^{-2}\,s^{-1}$. UV-B irradiation was provided by fluorescent UV-tube (*Philips*, *TL 40 W/12*, The Netherlands) with its main output at 312 nm. Required irradiance was obtained by adjusting the distance between the source and seedlings. The radiation was filtered through 0.127 mm cellulose di-acetate (*Johnston Industrial Plastics*, Toronto, Canada) to remove all incident UV-C (<280 nm). The irradiance was measured with a power meter model *407, A-2* (*Spectra Physics*, USA).

Ni accumulation was estimated according to the method of Gabrielli *et al.* (1991) after 48 and 96 h of treatment. In another set, 48 h Ni treated seedlings were exposed to UV-B, and Ni accumulation was recorded after next 48 h. Leaves were rinsed in distilled water, dried at 80 °C for 24 h, and wet ashed in nitric and perchloric acid mixture (3/1, v/v) on an electric thermostatic plate (300 °C). Nickel content was determined by atomic absorption spectrophotometry (model *2380*, *Perkin Elmer*).

Growth and pigment analysis: 11-d-old seedlings were harvested. Fresh leaves were extracted with 80 % acetone for determining Chl and Car contents. The amount of Chl was quantified by using the formula of Arnon (1949) and the correction coefficient given by Porra (2002). Car content was estimated by the method of Goodwin (1954).

Photosynthetic electron transport activities: Chloroplasts were isolated from leaves of soybean seedlings according to the method of Mohanty *et al.* (1989). Chloroplasts were pre-treated with various concentrations of Ni for 15 min in darkness and exposed to UV-B for 30 min. Photosystem 1 (PS1) activity in treated and untreated chloroplasts was measured in terms of O_2 consumption in reaction mixture containing DCMU (10 μM), methyl viologen, MV (0.5 mM), DCPIP (0.05 mM), sodium ASC (1.0 mM), and sodium azide (NaN_3, 1.0 mM), whereas whole chain electron transport activity was measured by using 0.5 mM MV and 1.0 mM sodium azide. Photosystem 2 (PS2) activity was determined polarographically by Clark type oxygen electrode (*Rank Brothers*, U.K.) as O_2 evolution using 1.0 mM p-BQ. In each case chloroplasts equivalent to 6 μg(Chl) cm^{-3} were suspended in reaction medium containing 50 mM

HEPES–NaOH buffer (pH 7.5), 100 mM sucrose, 10 mM NaCl, and 2 mM $MgCl_2$.

Determination of superoxide radical and hydrogen peroxide contents: Superoxide radical was measured by the method of Elstner and Heupel (1976) by monitoring the nitrite formation from hydroxylamine in the presence of $O_2^{\cdot-}$ in supernatant obtained from leaf homogenate of UV-B and Ni treated and untreated seedlings. Similarly, leaves were also homogenized in 3.5 cm^3 of 5 % trichloroacetic acid, centrifuged at 10 000×*g* for 15 min, and the total peroxide amount in the supernatant was estimated by following ferrithiocyanate method as described by Sagisaka (1976).

Assay of enzymes: POD (EC 1.11.1.7) and CAT (EC 1.11.1.6) were extracted by homogenizing 50 mg fresh leaves at 4 °C in 100 mM potassium phosphate buffer (pH 7.0), while SOD (EC 1.15.1.1) was isolated in 100 mM EDTA-phosphate buffer (pH 7.8). The homogenate was filtered and centrifuged at 10 000×*g* for 15 min and the supernatant obtained was used for enzyme assay. CAT activity was determined as dissociation of H_2O_2 by measuring O_2 release in darkness for 1 min by Clark type oxygen electrode (*Rank Brothers*, U.K) as described by Egashira *et al*. (1989). Oxygen produced by enzymatic reaction was calculated after correction for auto-production of oxygen from H_2O_2. Temperature around the vessel was maintained at 25 °C. POD activity was determined spectrophotometrically according to the method of Gahagen *et al.* (1968). The activity was assayed in a reaction mixture (3 cm^3) containing 16 mM H_2O_2, 10 mM pyrogallol, and crude extract (150 µg protein). SOD activity was assayed at 25 °C according to the method of Giannopolitis and Ries (1977) using a reaction mixture (3 cm^3) containing 1.3 µM riboflavin, 13 mM methionine, 63 µM NBT, 0.05 M sodium carbonate (pH 10.2), and crude extract (100 µg protein).

Proline and ASC estimation: Proline concentration in leaves of treated and untreated seedlings was determined spectrophotometrically by the method of Bates *et al.* (1973). ASC from leaves was extracted in 5 % (m/v) sulfosalicylic acid and the amount of ASC was determined in supernatant using the method of Oser (1979).

Measurement of lipid peroxidation and membrane leakage: Lipid peroxidation in each sample was determined by 2-thiobarbituric acid-malondialdehyde (TBA-MDA) adduct formation as described by Heath and Packer (1968). Intactness of plasma membrane in leaves was measured as the leakage percentage of electrolytes, as described by Gong *et al.* (1998).

Results

Growth and pigment contents: Seedlings treated with $Ni_{0.10}$ and $Ni_{1.00}$ and UV-B alone showed a significant decrease in height, leaf area, and fresh and dry mass. Combined treatment reduced growth more than additive values suggest (Fig. 1). $Ni_{0.01}$ stimulated growth of soybean seedlings, however, together with UV-B, the growth parameters were inhibited significantly. Fig. 2 reveals that Ni accumulation in leaves exhibited increasing trend with rising concentration of Ni in nutrient medium, however, the accumulation decreased considerably following UV-B exposure.

Similar to growth, contents of Chl *a*, Chl *b*, and Car decreased significantly following $Ni_{0.10}$ and $Ni_{1.00}$ treatments, while a marked increase in pigment contents of $Ni_{0.01}$ seedlings was found (Table 1). UV-B alone declined Chl *a* and Chl *b* contents by 32 and 39 %, respectively, but enhanced the Car content by 13 %. On combining with UV-B, a further decrease in Chl *a* and Chl *b* contents was observed in $Ni_{0.10}$ and $Ni_{1.00}$ treatments. Even with the stimulatory dose of $Ni_{0.01}$, UV-B caused a significant reduction in Chl *a* and *b* contents, while the Car content showed still a 17 % higher value over untreated control.

Photosynthetic characteristics: PS1 activity in $Ni_{0.01}$ and $Ni_{1.00}$ treated chloroplasts increased by 15 and 6 %, respectively, over the control value, while similar Ni treatments declined PS2 activity by 8 and 35 % and whole chain activity by 10 and 40 %, respectively (Table 2). Unlike Ni induced stimulation of PS1 activity, UV-B alone decreased the PS1 activity by 6 %, while PS2 and whole chain showed similar trend by diminishing the activity by 30 and 33 %, respectively. PS1 activity, which was stimulated at tested dose of Ni alone, was inhibited significantly following combined treatments of Ni and UV-B as $Ni_{0.01}$+UV-B and $Ni_{1.00}$+UV-B caused 11 and 15 % of reduction over the value of control, respectively. PS2 and whole chain activities declined further, when the chloroplasts were exposed simultaneously to Ni and UV-B ($Ni_{0.01}$+UV-B and $Ni_{1.00}$+UV-B) and the inhibitory effects were even greater than suggested by their additive values.

ROS and antioxidants: $O_2^{\cdot-}$ and H_2O_2 contents in leaves increased with UV-B exposure and followed the increasing trend with rising concentrations of Ni. The effects were further enhanced when both stresses were combined (Fig. 3*C*,*D*). SOD, CAT, and POD activities were stimulated following Ni treatments, but the activity of SOD declined by 28 % in $Ni_{1.00}$ treated leaves (Fig. 4*A*,*B*,*C*). The seedlings exposed to UV-B alone exhibited an increase of 4, 188, and 85 % in the activities of CAT, POD, and SOD, respectively. Both the stresses together caused a significant decrease in the CAT

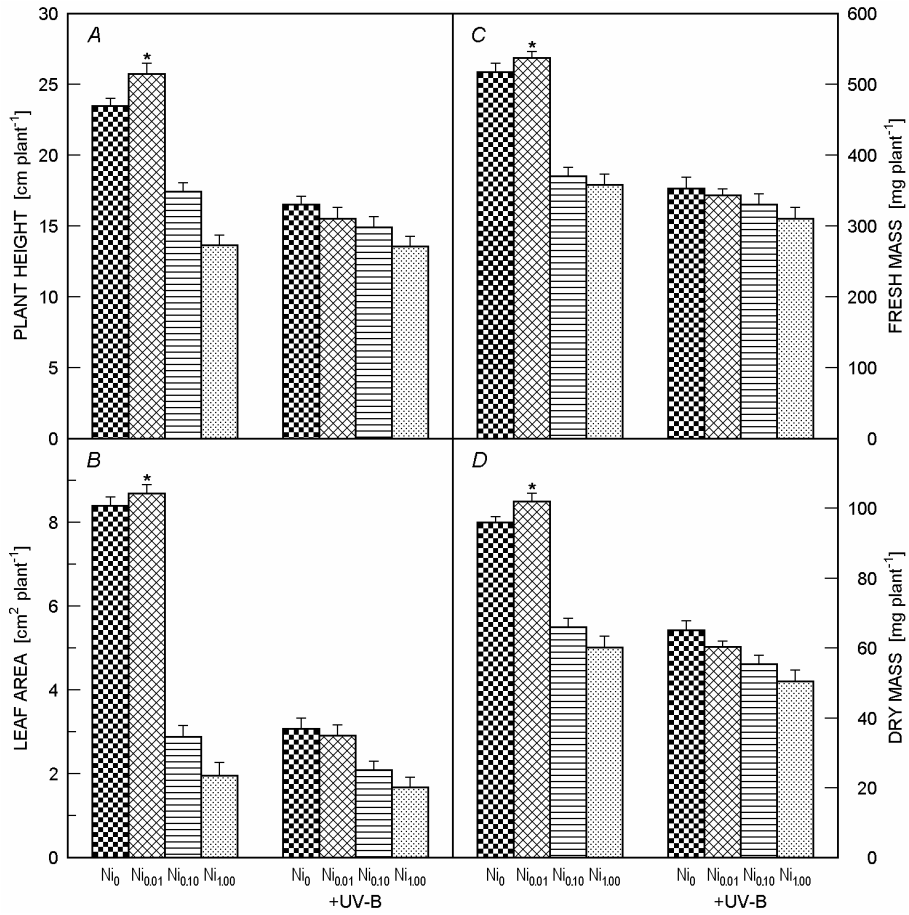

Fig. 1. Effects of enhanced UV-B radiation and nickel, singly and in combination, on plant height (*A*), leaf area (*B*), and fresh (*C*) and dry (*D*) masses of soybean. Means±SE. *Significantly different from control (p<0.05).

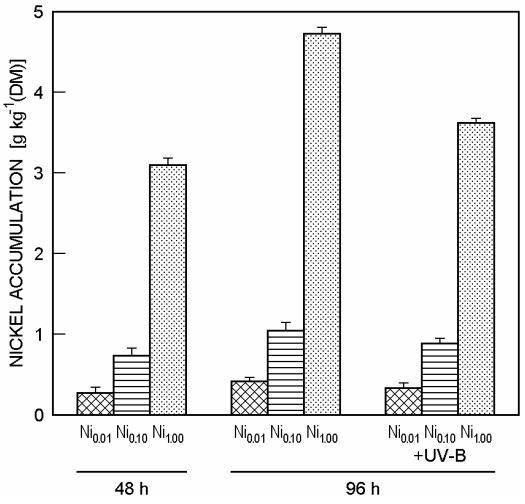

Fig. 2. Effect of UV-B on Ni accumulation in leaves of soybean seedlings grown in presence of different concentrations of Ni. Means±SE. All values are significantly different from control (p<0.01).

activity, while the activities of POD and SOD were still higher than those of control except the activity of SOD in $Ni_{1.00}$+UV-B treated seedlings.

Following $Ni_{0.10}$ and $Ni_{1.00}$ treatments, the content of proline in leaves was enhanced by 18 and 38 %, respectively (Fig. 5*A*). UV-B alone also raised the content of proline in leaves by 11 % and the simultaneous exposure caused a further rise in cellular contents of proline. In contrast to this, ASC content decreased following individual and combined stress treatments (Fig. 5*B*).

Lipid peroxidation and electrolyte leakage: Compared to control the content of MDA in leaves increased considerably following Ni and UV-B treatments and the increasing trend continued with rising doses of Ni (Fig. 3*A*). Combined doses of UV-B and Ni further enhanced the content of MDA. A similar trend was noticed, when Ni and UV-B exposed leaves were analysed for membrane damage as both stresses, individually and in combination, enhanced the electrolyte leakage (Fig. 3*B*).

Discussion

High Ni concentrations ($Ni_{0.10}$ and $Ni_{1.00}$) reduced the growth of soybean seedlings significantly, which could be due to an arrest of key physiological and biochemical processes. This inhibition was caused by accumulation of Ni in the leaves of soybean seedlings which are the major sites of physiological activities (Fig. 2). A similar result was observed in mung bean seedlings exposed to $Ni_{0.10}$ and $Ni_{1.00}$ (Gopal *et al.* 2002). In contrast to this, at low concentration ($Ni_{0.01}$), the stimulatory effect on growth performance of seedlings revealed that Ni is a micronutrient as reported by Brown *et al.* (1987). Considerable reduction in growth following UV-B exposure, alone and in combination with Ni, may also be correlated with strong inhibitory effect on photosynthetic electron transport activity (Table 2) and cellular degradation (Fig. 3*A*,*B*). Visual symptoms such as dwarfing of plants, distortion of shoots, and reduction in leaf dimensions in soybean seedlings exposed with UV-B alone and together with Ni were also observed. Previous studies showed that UV-B radiation alone significantly reduced the growth and biomass accumulation in soybean plants (Sullivan and Teramura 1990). Such reduction in growth has been correlated with reduced photosynthetic activities, destruction of growth promoting hormone indolylacetic acid (Kulandaivelu *et al.* 1989), and generation of free radicals (Kondo and Kawashima 2000). Tevini and Teramura (1989) suggest that UV-B induced stunting and dwarfing of plants may be associated with changes in cell division or cell elongation.

Table 1. Effects of UV-B and nickel, alone and together, on contents of chlorophyll (Chl) and carotenoids (Car) [g kg^{-1}(FM)] of soybean seedlings after 11 d of growth. Means ±SE. All treatments are significantly different ($p<0.01$ and $^{*}p<0.05$) from control (Student's *t*-test).

		Chl *a*	Chl *b*	Chl *a*+*b*	Car
Ni_0	control	0.37±0.02	0.28±0.01	0.65±0.04	0.24±0.01
$Ni_{0.01}$		0.46±0.02 (+24)	0.33±0.02* (+18)	0.79±0.05* (+22)	0.26±0.05* (+8)
$Ni_{0.10}$		0.25±0.01 (–32)	0.17±0.02 (–39)	0.41±0.02 (–37)	0.19±0.01 (–21)
$Ni_{1.00}$		0.16±0.01 (–57)	0.11±0.01 (–61)	0.27±0.03 (–58)	0.12±0.02 (–50)
Ni_0	+UV-B	0.25±0.02 (–32)	0.17±0.01 (–39)	0.42±0.02 (–35)	0.27±0.05 (+13)
$Ni_{0.01}$		0.23±0.02 (–38)	0.15±0.01 (–46)	0.38±0.04 (–42)	0.28±0.01 (+17)
$Ni_{0.10}$		0.14±0.01 (–62)	0.10±0.01 (–64)	0.24±0.02 (–63)	0.16±0.02 (–33)
$Ni_{1.00}$		0.11±0.01 (–70)	0.06±0.01 (–79)	0.17±0.02 (–74)	0.10±0.01 (–58)

Table 2. Effects of UV-B and nickel, singly and in combination, on photosynthetic electron transport activity [µmol(O_2) kg^{-1}(Chl) s^{-1}] in chloroplasts isolated from soybean seedlings. Means ±SE. All treatments are significantly different ($p<0.01$) from control (Student's *t*-test). PS = photosystem.

		PS1	PS2	Whole chain
Ni_0	control	68.3±0.6	28.3±0.3	24.4±0.3
$Ni_{0.01}$		78.3±0.8 (+15)	26.1±0.3 (–8)	21.9±0.3 (–10)
$Ni_{1.00}$		72.8±0.6 (+6)	18.3±0.3 (–35)	14.7±0.2 (–40)
Ni_0	+UV-B	64.2±0.8 (–6)	19.7±0.2 (–30)	16.4±0.3 (–33)
$Ni_{0.01}$		60.6±1.1 (–11)	13.3±0.1 (–53)	7.8±0.2 (–68)
$Ni_{1.00}$		58.1±0.6 (–15)	8.1±0.1 (–72)	4.4±0.1 (–82)

Nickel at high concentration and UV-B, alone and together, caused significant decrease in Chl content, which could be due to inhibition of Chl biosynthesis by inhibiting δ-aminolevulinic acid dehydrogenase and protochlorophyllide reductase activities, and breakdown of pigments or their precursors (Teramura and Sullivan 1994, Ouzounidou 1995). UV-B may also decline photostability of Chl as observed in other plants (Strid and Porra 1992). Jordan *et al.* (1991) suggested that UV-B alters m-RNA turnover of the Chl *a*/*b* binding proteins resulting in reduced Chl content. Nickel similar to other heavy metals might have replaced the central Mg from Chl molecules and thereby decline the photosynthetic light-harvesting ability of plants (Kupper *et al.* 1996). $Ni_{0.01}$ increased the Chl content over control, which was probably due to enhanced biosynthesis or stabilization of Chl in thylakoid membrane. Under stress, Car pigments are less affected than Chl, resulting in a low Chl/Car ratio, and our results obtained in soybean seedlings exposed to both stresses are in consonance with earlier findings (Krupa *et al.* 1987, Strid *et al.* 1990). UV-B alone increased the Car content which could be correlated with adaptive mechanism (Lingakumar and Kulandaivelu 1993). Car pigments protect Chl from photo-oxidative

destruction and therefore significant reduction in Car contents following simultaneous exposure of seedlings with UV-B and Ni could result in serious consequences on Chls leading to reduced photosynthetic efficiency of the plants.

Photosynthetic electron transport activity is sensitive to heavy metal ions (Mohanty *et al*. 1989) as well as to UV-B irradiation (Renger *et al*. 1989, Strid *et al*. 1990). We found that the whole chain as well as the PS2 driven electron transport was markedly suppressed in chloro-

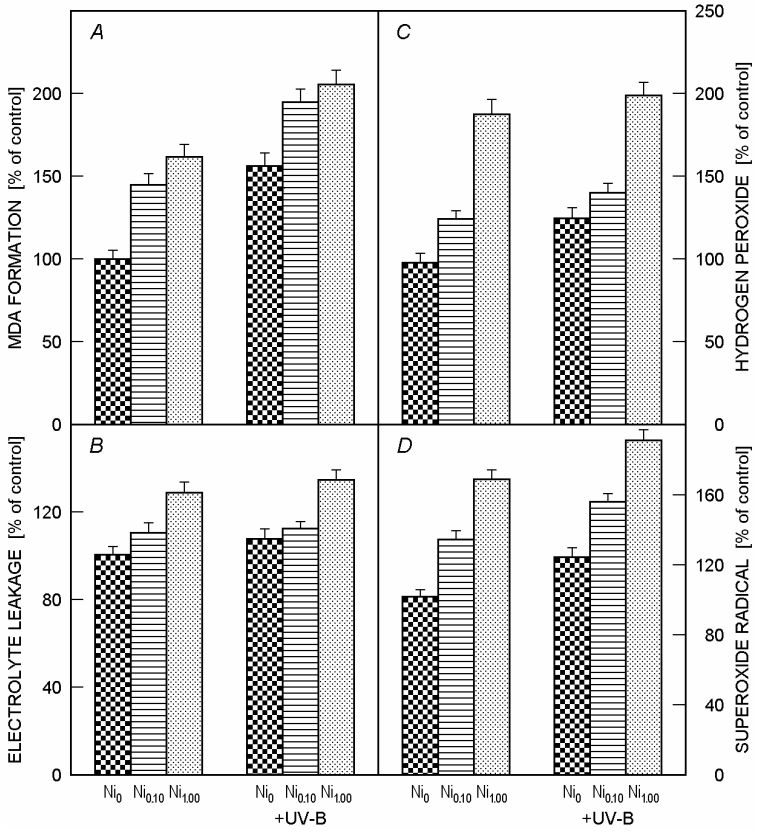

Fig. 3. Effects of enhanced UV-B radiation and Ni, singly and in combination, on lipid peroxidation, electrolyte leakage, and superoxide radical and hydrogen peroxide contents. The MDA (*A*), superoxide radical (*D*), and hydrogen peroxide contents (*C*) in untreated control were 5.6±0.1 mmol kg^{-1}(FM), 1.08±0.01 μmol kg^{-1}(FM), and 85±1 mmol kg^{-1}(FM), respectively. Means±SE. All values are significantly different from control (p<0.01).

plasts treated with Ni and UV-B, singly as well as in combination. The growth stimulating dose of $Ni_{0.01}$ retarded the PS2 and whole chain activities significantly, which could be due to direct exposure of chloroplasts with metal. The greater sensitivity of PS2 towards Ni and UV-B, singly as well as in combination, might have resulted in interaction of these stresses with the oxygen evolving complex, carriers of oxidizing as well as reducing side of PS2, and reaction centre itself (Mohanty *et al*. 1989, Renger *et al*. 1989). PS2 activity in the cyanobacterium *A. doliolum* was synergistically inhibited, when Cu and UV-B were applied simultaneously (Rai *et al*. 1995). In contrast to PS2, PS1 activity following Ni treatment was stimulated significantly which supports the view that PS1 is highly conserved and most resistant to stress (Almog *et al*. 1991). However, PS1 activity declined by UV-B, alone and together with Ni, could be due to direct effect on PS1 reaction centre.

Excessive accumulation of reactive oxygen species (H_2O_2 and $O_2^{\cdot-}$) in leaves following Ni and UV-B treatments, alone and together, was probably because of strong inhibition of photosynthetic electron transport activity as reported by Dai *et al.* (1997). Thus enhanced lipid peroxidation led to increased electrolyte leakage due to cell membrane damage (Pandolfini *et al*. 1992, Rai *et al.* 1998).

ROS, particularly H_2O_2 and $O_2^{\cdot-}$, are important in cellular signalling as second messenger and thus induce various genes and enzymes (Mahalingam and Fedoroff 2003). Therefore, the increased contents of $O_2^{\cdot-}$ and H_2O_2 in Ni and UV-B treated leaves triggered the activity of several anti-oxidative enzymes such as SOD, CAT, and POD in soybean seedlings. However, $Ni_{1.00}$ alone and together with UV-B might have directly inhibited the SOD activity resulting in greater accumulation of $O_2^{\cdot-}$. Nickel at the tested doses stimulated the CAT activity, but together with UV-B the significant decrease in CAT activity could be due to interactive effect of UV-B and Ni,

thus the level of H_2O_2 increased considerably. Similar increase in SOD, CAT, and POD activities was also observed in metal or UV-B exposed leaves (Srivastava and Tel 1993, Kondo and Kawashima 2000).

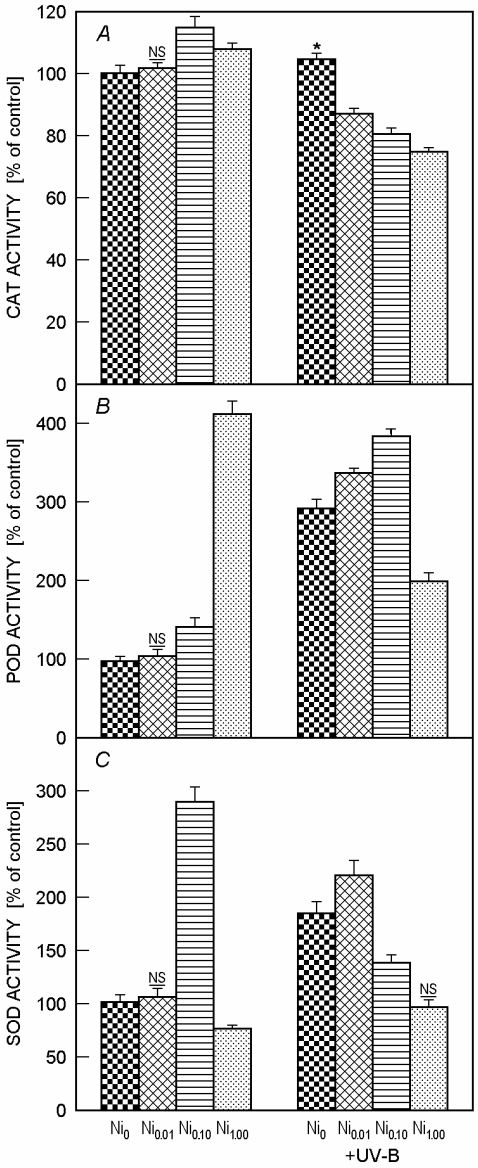

Fig. 4. Effects of enhanced UV-B radiation and nickel, singly and in combination, on catalase (*A*), peroxidase (*B*), and superoxide dismutase (*C*) activities (values in untreated control were 443.4±13.3 mmol(O_2 released) kg^{-1}(protein) s^{-1}, 19 667± 333 change in OD_{430} kg^{-1}(protein) s^{-1}, and 433 333±11 667 unit kg^{-1}(protein) s^{-1}, respectively). Means±SE. *Significantly different from control (p<0.05); NS, not significant.

Proline is regarded as an osmoprotectant, however, several authors implicated a role for proline in the detoxification of ROS (Pardha Saradhi *et al.* 1995, Matysik *et al.* 2002). Enhanced accumulation of proline in soybean leaves could be linked with detoxification against Ni and UV-B induced oxidative stress. In contrast to this, decreased accumulation of ASC following these treatments may be explained on the basis of less availability of NADPH, needed for reductive enzyme activities for ASC regeneration (Devine *et al.* 1993), due to strong depression of photosynthetic electron transport in chloroplasts by Ni and UV-B, alone and together.

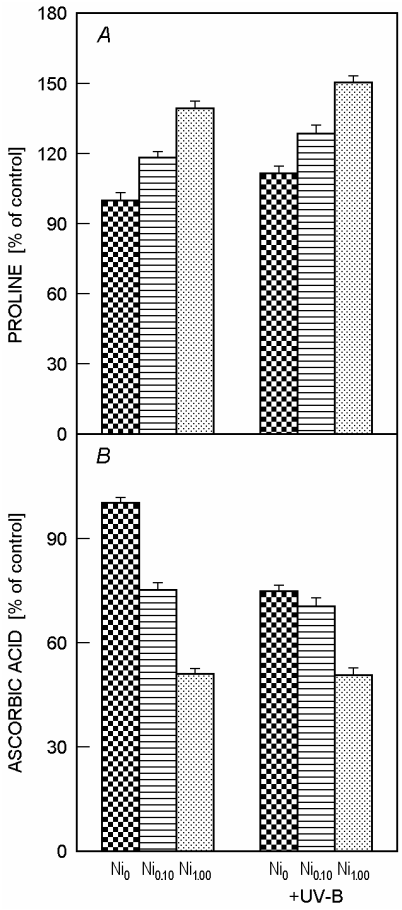

Fig. 5. Effects of enhanced UV-B radiation and nickel, singly and in combination, on proline (*A*) and ascorbic acid (*B*) contents. The proline and ascorbate contents in untreated control were 12.0±0.2 mg kg^{-1}(FM) and 1.16±0.02 mmol kg^{-1}(FM), respectively. Means±SE. All values are significantly different from control (p<0.01).

Thus enhanced UV-B irradiation and growth inhibiting dose of Ni alone caused significant reduction in physiological characteristics and biomass production of soybean seedlings. The combined effect of both the stresses became more expressed but was less than additive. Besides stress induced inhibited photosynthetic electron transport activities, the heavy accumulation of ROS due to declined CAT activity as well as ASC content, following combined treatments, led to an increased lipid peroxidation and cellular membrane damage, thereby the growth of soybean seedlings was reduced significantly.

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