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Differential responses of growth, antioxidant enzymes and oxidative stress in two species of *Azolla* (*Azolla microphylla* and *Azolla pinnata*) exposed to pretilachlor and enhanced UV-B radiation

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ABSTRACT

The present study examines the responses of growth, oxidative stress and antioxidant enzymes in two Azolla species (Azolla pinnata and Azolla microphylla) exposed to pretilachlor, a herbicide commonly used in rice field and enhanced ultraviolet-B (UV-B) radiation. Both species of Azolla were exposed to different concentration of pretilachlor (5, 10 and 20 µg ml^{-1}) and enhanced UV-B (UV-B_1: ambient + 2.2 kJ m^{-2} day^{-1} and UV-B_2: ambient + 4.4 kJ m^{-2} day^{-1}) alone and in combination. The dry mass, superoxide radical formation, lipid peroxidation, enzymatic antioxidants i.e. superoxide dismutase (SOD), catalase (CAT) and guaiacol peroxidase (POD), and non-enzymatic antioxidant proline were investigated under pretilachlor and UV-B stress. Pretilachlor and UV-B stress caused negative impact on growth of both the species in dose dependent manner. The damaging effect was further enhanced by combination of both the stresses that was due to generation of superoxide radical and lipid peroxidation (MDA). Both the stresses alone and together also caused the increase in activity of SOD, CAT and POD, and proline accumulation. Compared to individual effect of pretilachlor and UV-B, the simultaneous exposure resulted in a strong inhibition of growth and excessive accumulation of ROS, thereby causing severe oxidative damage as evidenced by increased content of MDA, despite increased level of antioxidants. The overall results showed that pretilachlor and enhanced UV-B adversely affected growth in both species and their combined doses further deteriorated the growth. Results also showed that A. microphylla is comparatively less affected by pretilachlor and enhanced UV-B radiation than A. pinnata. The study also suggested that A. microphylla may used to grow for mass cultivation for medicine production and also as biofertilizer.

Keywords: Pretilachlor, UV-B, MDA, SOD, CAT, POD.

INTRODUCTION

Recent researches have shown that global stratospheric Ozone has significantly decreased during the last decade. Man-made chemicals such as chlorofluorocarbons, invented in the 1930s, are the main culprits for the depletion of stratospheric ozone layer [1]. This leads to increase in UV-B radiation (280–315 nm) in the biosphere. The electromagnetic radiation emitted from the sun in the ultraviolet (UV) range (200–400 nm) constitutes about 7% of the total radiation. As it passes through the atmosphere, the total flux transmitted is greatly reduced and the composition of UV radiation is modified [2]. Owing to its high energy, the impact of UV-B on metabolic processes of plants can be very harmful [3,4,5]. UV-B can induce damage to DNA, proteins, membrane, and photosynthetic apparatus [6]. To keep this damage to a minimum, plants induce enzymatic and non-enzymatic antioxidative defense systems along with other morphological and anatomical changes.

The increasing use of pesticides in agriculture demands investigation to examine the effect of pesticides on the nontarget soil micro-organisms and plants including nitrogen fixing cyanobacteria and their symbionts. Pesticides (herbicides, fungicides and insecticides) adversely affect all aspects of primary and secondary metabolism in crops and animals when applied in agricultural field [7]. There have been several studies which show worst effect of the pesticides on growth and photosynthesis [5,8]. Besides this, pesticides also enhanced the generation of active oxygen species [9]. However, pesticides induced generation of active oxygen species was found in association with simultaneous induction of antioxidant system in plants [9], bacteria [10] and animals [11]. The effect of pesticides may be aggravated when they combined with other stress like UV-B [12].

UV-B and pesticide are photosynthetic inhibitors [13,5] interfere with photosynthetic electron transport and produce active oxygen species [12]. Active oxygen species includes $O_2^{\bullet -}$, H_2O_2 and $^{\bullet}OH$ etc. These radicals are produced at various sites of electron transport of chloroplasts and mitochondria and in various cellular organelles like peroxisomes, endoplasmic reticulum and glyoxysomes [14], which can react with lipids, proteins, pigments, and nucleic acids; causing membrane damage, and inactivation of enzymes and alteration of DNA activity, thus affecting cell viability [15,16]. To cope with such damage cells have been naturally equipped with an efficient antioxidant system which consists of enzymatic and non-enzymatic antioxidants [17]. The antioxidant system of plants comprises several enzymes: superoxide dismutase (SOD), catalase (CAT), guaiacol peroxidase (POD), glutathione reductase (GR), glutathione-S-transferase (GST) and ascorbate peroxidase (APX) [18,9,19] and play a crucial role to protect plant from oxidative damage, and it is believed that their activities and amount determine the degree of tolerance in plant.

Under natural environmental condition multiple stressor are likely to exist and one stress can modify the effect other stress, some time they may cause more severe damage to plants and other non-selective organisms. In recent years, studies have indicated that stresses such as temperature, pesticides, metals, trophospheric O_3 and CO_2 have altered the growth and development of microorganisms and higher plants up to certain extent [5,18].

Azolla is a heterosporus, free floating, fast growing nitrogen fixing aquatic fern and is widespread in fresh water habitat of India, Sri Lanka, Japan, China and Philippines. *Azolla* is a suitably called as green gold [20] because it is economically important as an animal feed, medicine [21], hydrogen fuel, biogas producer, weed Controller as well as a biofertilizer. Although, numbers of studies have been carried out regarding physiological and biochemical effects of UV-B and pesticide individually on cyanobacteria, algae and higher plants and animals, however, their interactive effects are still poorly known. The major contribution of *Azolla* is in nitrogen economy in paddy field, and its sensitivity towards pesticide and UV-B has created interest to study the response of *Azolla* under these stresses. As far as our knowledge goes, no report is available regarding the changes in growth and antioxidant metabolism of *Azolla* when the two stresses are simultaneously imposed. Therefore, an attempt was made to investigate the effect of pretilachlor and enhanced UV-B radiation, singly and in combination on growth and antioxidant metabolism of *Azolla pinnata and Azolla microphylla*.

EXPERIMENTAL SECTION

Plant material and Growth conditions

Two species of *Azolla*, *viz*. *Azolla pinnata* var. imbricata and *Azolla microphylla* have been selected for present study. *A. pinnata* was procured from the pond of the Botany Department of university of Allahabad, and the *A. microphylla* was collected from National Centre for Conservation and Utilization of Blue Green Algae, IARI, New Delhi and cultured in Roxburg garden, Department of Botany, University of Allahabad. The plants were surface sterilized quickly with a solution of mercuric chloride (0.1% for 30 s) followed by dipping the plants into a large volume of sterile distilled water. Washing of the *Azolla* with sterile distilled water was repeated several times. Fronds were then transferred into plastic trays ($32\times25\times6$ cm^3) containing combined-N free Espianase and Watanabe medium [22]. The pH of the medium was adjusted to 7.2. Plastic trays were placed in the Roxburg garden, Department of Botany, University of Allahabad, during the experimental period, average minimum and maximum temperature ranged from 16.7 to 36.8 ^oC, and relative humidity 55 to 71%. Photosynthetic active radiation (PAR) ranged between 800 -1000 µmol photon m^{-2} s^{-1}.

Pesticide and UV-B treatments

Pesticide, pretilachlor [2-chloro-2′, 6′-diethyl-*N*-(2-propoxyethyl) acetanilide] 50% EC was selected for the treatments. This is widely used herbicide to control grasses, sedges, broad leaved weeds like *Echinochloa* spp. *Cyperus iria*, *Cyperus difformis*, *Fimbristylis milliaceae*, *Ludwigia parviflora*, *Pannicum repens* etc. in rice fields. Its various concentrations 5, 10, 20, 25, 30, 35, 40, 50 and 100 µg ml^{-1} in nutrient medium was prepared for screening experiment and finally 5, 10 and 20 µg ml^{-1} of pretilachlor were selected. Similarly, out of various doses of UV-B enhanced UV-B radiation was provided artificially by UV-B lamps (Q-Panel Co, UV-B-313 flourescent lamps, OH, USA), hanging above and perpendicular to the pot rows on an adjustable frame. Enhanced UV-B was provided daily from 9:30 (3.5 h after the beginning of the photoperiod) to 15:30 hr. The radiation was filtered through 0.127 mm cellulose acetate (Johnston Industrial Plastics, Toronto, Canada) to remove all incident UV-C (< 280 nm). The UV-B irradiance at the top of the pots under the lamps was measured with the help of power meter (Spectra physics, USA Model 407, A-2). Each sample was also receiving ambient level of UV-B (8.6 kJ m^{-2} day^{-1}). For treatment fronds were exposed to two levels of enhanced UV-B

radiation, the low (UV-B_1: ambient + 2.2 kJ m^{-2} day^{-1}) and high (UV-B_2: ambient + 4.4 kJ m^{-2} day^{-1}) biologically effective UV-B (UV-B_{BE}), simulating 6 and 12% depletion, respectively in stratospheric ozone at Allahabad (25° 28' N latitude, 81° 54' E longitude). There were negligible differences between temperature, relative humidity and PAR under enhanced UV-B exposed plants and other treatments.

Estimation of dry mass

The total plant dry mass from each set of treated and untreated samples was recorded. Dry mass was estimated by single pan electronic balance (Contech- CA 223, India) after oven drying of the plant material for 48 h at 90 ^oC.

Determination of superoxide radical

Superoxide radical (SOR; $O_2^{\bullet -}$) was measured by the method of Elstner and Heupel [23] with some modification as described by Jiang and Zhang [24], monitoring nitrite formation from hydroxylamine in the presence of $O_2^{\bullet -}$, in supernatant obtained from treated and untreated fronds. The required amount (200 mg) of *Azolla* fronds was homogenized with 2 ml of 65 mM potassium phosphate buffer (pH 7.8) and centrifuged in CPR-30 (Remi, India) at 10,000 g at 4 ^oC for 10 min. The reaction mixture containing 65 mM potassium phosphate buffer (pH 7.8), 10 mM hydroxylamine hydrochloride and supernatant was incubated at 25 ^oC for 20 min. After incubation for required time, 17 mM sulfanilamide and 7 mM α–naphthylethylene diamine dihydrochloride were added to the incubation mixture. The components were mixed and separated into two layers using same volume of diethyl ether to eliminate the interference caused by the pigments. The absorbance of pink colored solution was recorded at 530 nm.

Measurement of lipid peroxidation

Oxidative damage to lipids was estimated by measuring the content of malondialdehyde (MDA) in fronds of each test sample prepared in 10 % (w/v) trichloroacetic acid containing 0.65 % (w/v) 2-thiobarbituric acid (TBA) and heated at 95 ^oC for 25 min as described by Heath and Packer [25]. MDA content was calculated by correcting for compounds other than MDA which absorb at 532 nm by subtracting the absorbance at 600 nm of a reaction mixture incubated without TBA from an identical solution containing TBA. The amount of MDA was calculated by using extinction coefficient 155 mM^{-1} cm^{-1}.

Estimation of Superoxide dismutase activity

Superoxide dismutase (SOD; EC 1.15.1.1) activity was assayed by monitoring the inhibition of photochemical reduction of nitro blue tetrazolium (NBT) according to the method of Giannopolitis and Ries [26]. For extraction of SOD, fresh *Azolla* fronds (100 mg) were homogenized under ice cold condition with 100 mM EDTA-phosphate buffer (pH 7.8). The homogenate was centrifuged at 10,000 g for 15 min at 4 ^oC and the supernatant obtained was used for enzyme assay. The reaction was performed in a total volume of 3 ml containing 1.3 µM riboflavin, 13 mM L-methionine, 0.05 M Na_2CO_3 (pH 10.2), 63 µM NBT and 0.1 ml of enzyme extract. The reaction mixture in similar test tubes was irradiated with visible light (250 µmol photons m^{-2} s^{-1}) for 15 min at 25 ºC. The initial rate of reaction as measured by the difference in absorbance at 560 nm, in the presence and absence of extract was proportional to the amount of enzyme. One unit of the enzyme activity was defined as the amount of enzyme which is required to 50 % inhibition in the reduction of NBT.

Determination of catalase activity

Catalase (CAT; EC 1.11.1.6) activity was determined in terms of decrease in absorbance due to decomposition of H_2O_2 at 240 nm using an extinction coefficient of 39.4 mM^{-1} cm^{-1} [27]. Two ml reaction mixture contained 50 mM potassium phosphate buffer (pH 7.0) containing 1 mM EDTA, 10 mM H_2O_2 and enzyme extract. One unit of enzyme activity is defined as 1 nmol H_2O_2 decomposed min^{-1}.

Estimation of Guaiacol peroxidase activity

Guaiacol peroxidase (POD, EC 1.11.1.9) activity in treated and untreated fronds of both species was determined according to Zhang [28]. *Azolla* fronds (200 mg) of both species were homogenized in 2 ml 50 mM phosphate buffer (pH 6.1) The homogenate was centrifuged at 10,000 g for 10 min at 4 ^oC and the supernatant was used as the crude enzyme extract. Guaiacol peroxidase activity was measured with guaiacol as the substrate in a total volume of 3 ml. The reaction mixture consisted of 50 mM potassium phosphate buffer (pH 6.1), 1 % guaiacol, 0.4 % H_2O_2 and enzyme extract. Increase in the absorbance due to oxidation of guaiacol (E = 25.5 mM^{-1} cm^{-1}) was measured at 470 nm. Enzyme activity was calculated in terms of Unit g^{-1} fresh mass. One unit of enzyme activity is the amount of 1 nmol guaiacol oxidized in one min.

Estimation of Proline

Proline content in treated and untreated fronds was estimated according to the method of Bates et al [29]. Fresh *Azolla* fronds (100 mg) were crushed in 3 % (w/v) aqueous sulfosalicylic acid, centrifuged at 10,000 g for 10 min and then mixed with 3 % (w/v) glacial acetic acid and acid ninhydrin. Samples were heated for 1 h in a water bath at 95 ºC, cooled and extracted with 4 ml toluene by vortexing for 1 min with a test tube mixer. The toluene layer was then separated with the help of a pipette and the absorbance was read at 520 nm using toluene as blank. The amount of proline in sample was obtained by comparing with standard curve.

Statistical analysis

Duncan's new multiple range test ($P<0.05$) was used for data statistics of each treatment and lower case letters a, b, c, d, and e indicated statistical significance. The results presented are the means of three independent experiments.

RESULTS AND DISCUSSION

Present study deals with the effect of two stresses; pretilachlor (5, 10 and 20 µg ml^{-1}) and UV-B ($UV\text{-}B_1$ and $UV\text{-}B_2$) alone and in combination, on growth, oxidative stress and antioxidants in two *Azolla* species (*Azolla microphylla* and *Azolla pinnata*). Dry mass of both *Azolla* species was observed after 96 h of experiment. Dry mass decreased in dose dependent manner of pretilachlor and UV-B exposure (Fig.1). In *A. microphylla* pretilachlor at 5, 10 and 20 µg ml^{-1} decreased dry mass by 10, 14 and 20%, while in case of *A. pinnata* it declined by 17, 24 and 33%, respectively over the values of respective controls (Fig.1). Reduction in growth may be attributed to inhibition in normal cell division, as reported in barley plant under pretilachlor treatment [30]. However, toxic effect was considerably higher in *A. pinnata* than *A. microphylla.* The exposure of $UV\text{-}B_1$ and $UV\text{-}B_2$ alone caused a reduction in dry mass of 8.8% and 13.2% in *A. microphylla*, whereas 12.9% and 22.5% in *A. pinnata*, respectively. Pretilachlor and UV-B in combination, declined growth more intensively than their individual treatments indicating an interaction of

UV-B and pretilachlor. The overall growth reduction could be due to arrest of key physiological and biochemical processes such as photosynthesis (data not shown). For survival and protection, plants have evolved complex mechanism to adapt against stress conditions. These mechanisms may be developmental, morphological, physiological and biochemical. The differential response of growth of both species towards pretilachlor and UV-B could be due to genotypic differences. The reduction in growth due to genotypic differences to pesticide, salinity and UV-B has been reported in earlier studies [31, 32]. Further, decline in growth of both species by the combined treatment of UV-B_1 and UV-B_2 with pretilachlor was probably due to more severe effects of these stresses on cell membrane, photosynthetic membrane and enzymes involved in various metabolic processes. The results clearly show that *A. microphylla* was less affected than *A. pinnata*.

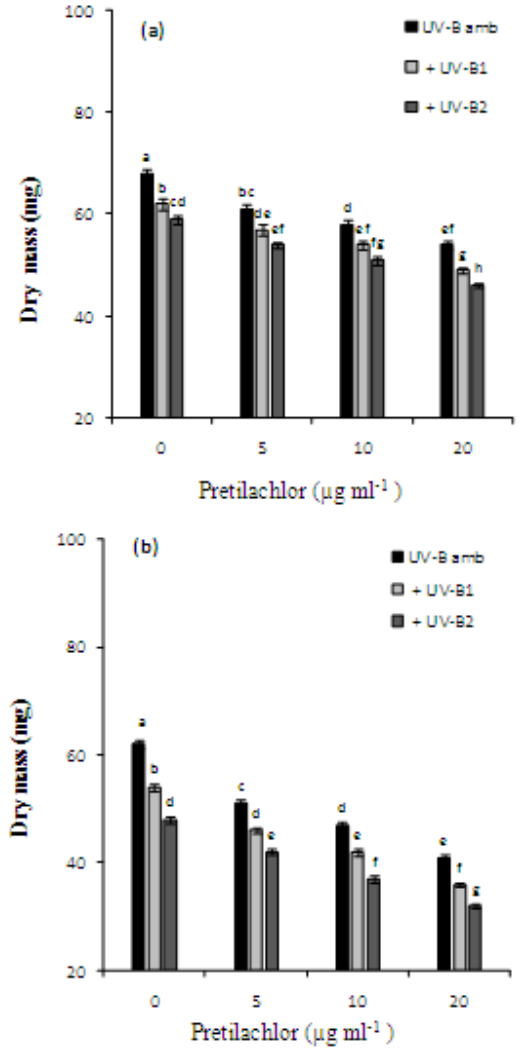

Fig. 1 Effect of pretilachlor and enhanced levels of UV-B radiation, singly and in combination, on dry mass of *Azolla microphylla* (a) and *Azolla pinnata* (b). Data are means ± standard error of three independent experiments.
Bars followed by different letters show significant difference at P<0.05 significance level according to Duncan's multiple range test.

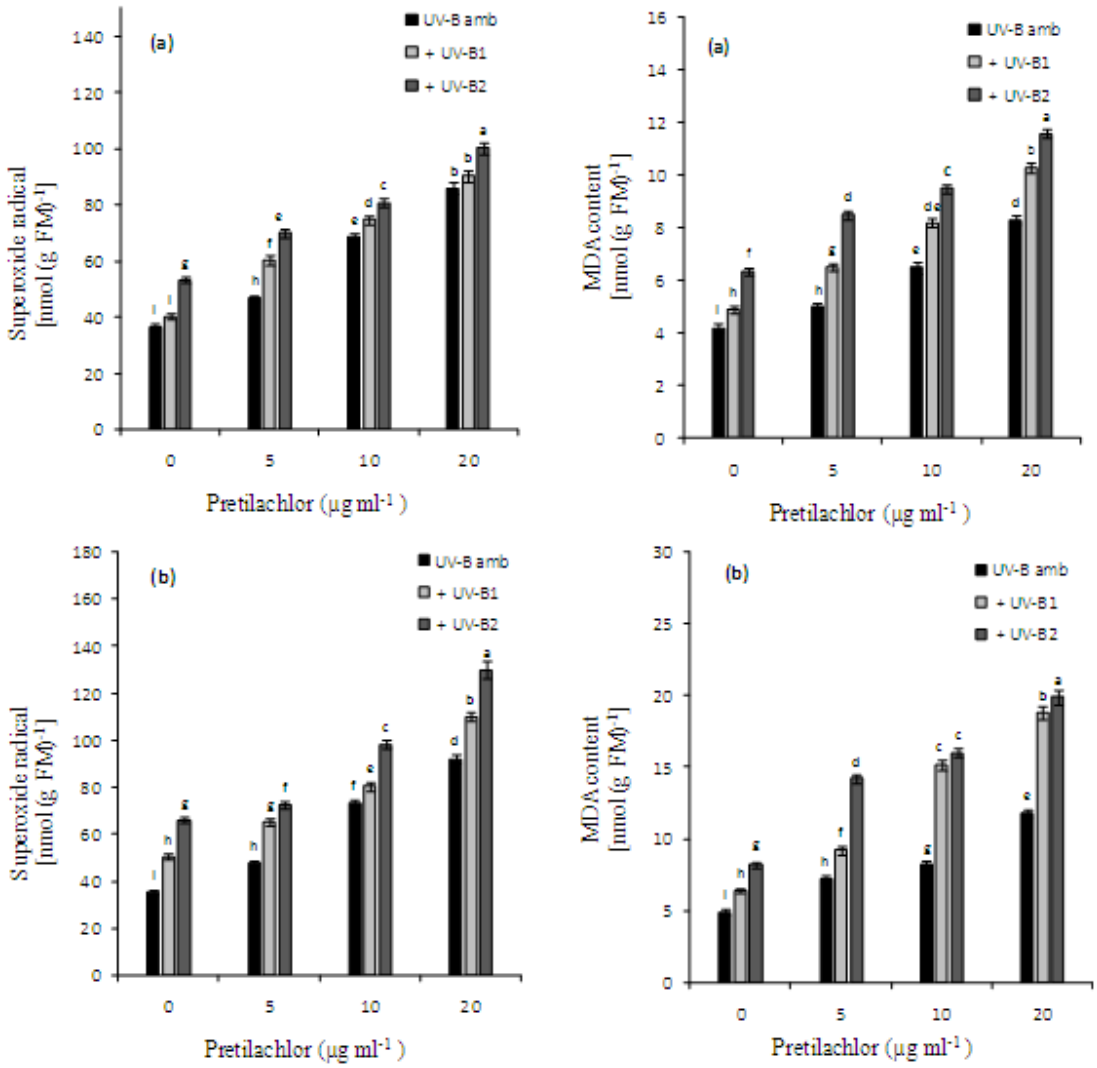

Fig. 2 Effect of pretilachlor and enhanced levels of UV-B radiation, singly and in combination, on level of MDA content and superoxide radical of *Azolla microphylla* (a) and *Azolla pinnata* (b). Data are means ± standard error of three independent experiments.

Bars followed by different letters show significant difference at P<0.05 significance level according to Duncan's multiple range test.

Further, deleterious effect of pretilachlor and UV-B on *Azolla* species were also correlated with oxidative stress. Level of SOR ($O_2^{\bullet -}$) increased following pretilachlor treatment, which was further increased in combination of UV-B stress. The increase in SOR content was an indicative of oxidative stress. Results depicted in Fig. 2, demonstrated the increased SOR content in both species of *Azolla* due to both stresses. The level of SOR increased by 27, 87 and 134% at 5, 10 and 20 µg ml^{-1} pretilachlor treatment in *A. microphylla*, while similar concentrations of pretilachlor increased SOR by 36, 108 and 162% in *A. pinnata*, respectively over the values of control samples. Further stimulation in SOR level was noticed, when pretilachlor concentrations were combined with UV-B_1 and UV-B_2. Similar trend was noticed in *A. pinnata* but SOR content was appreciably higher than *A. microphylla*. Production and accumulation of SOR in fronds exposed to pesticide and UV-B, alone and in combination was probably because of strong

inhibition of photosynthetic electron transport activities (data not shown) and also reported by Dai et al. [33].

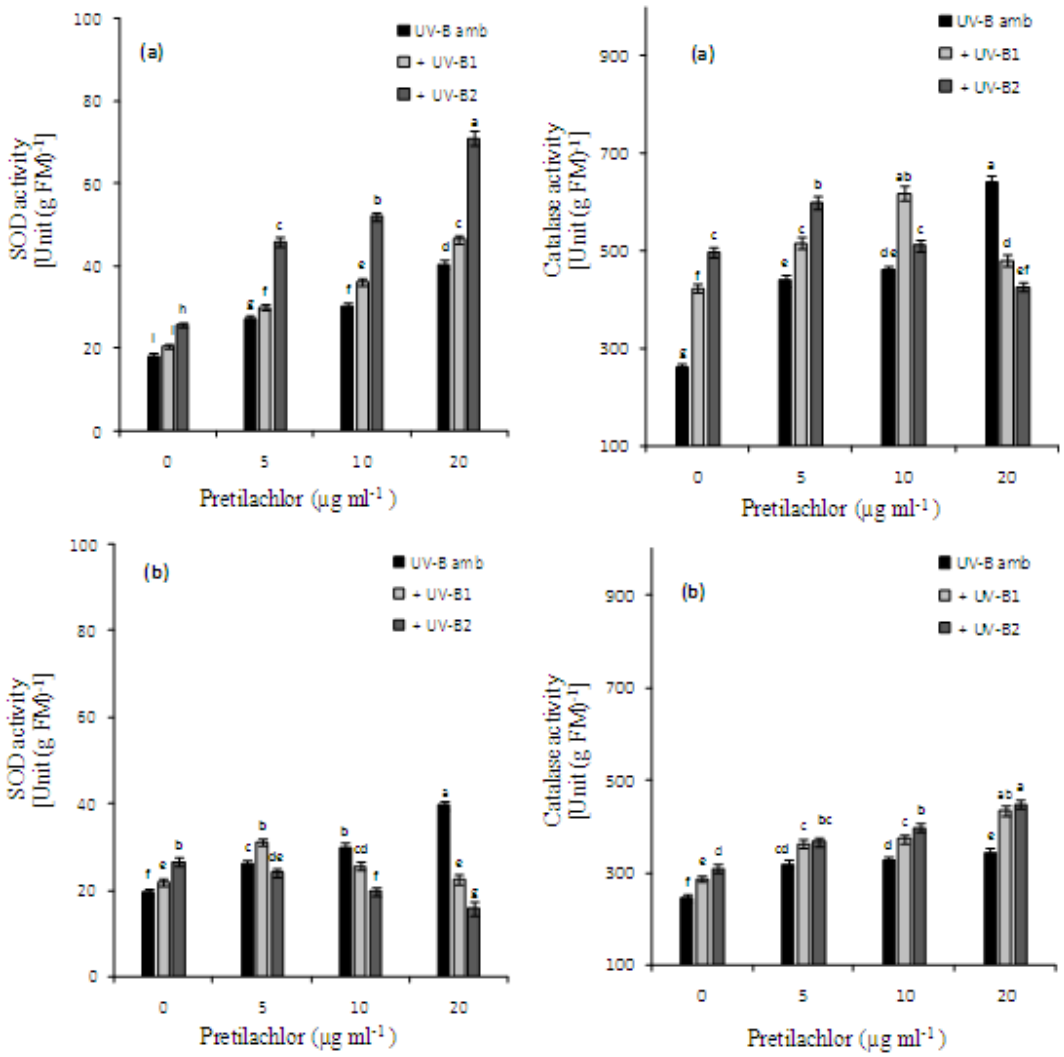

Fig. 3 Effect of pretilachlor and enhanced levels of UV-B radiation, singly and in combination, on superoxide dismutase and catalase activities of *Azolla microphylla* (a) and *Azolla pinnata* (b). Data are means ± standard error of three independent experiments.

Bars followed by different letters show significant difference at P<0.05 significance level according to Duncan's multiple range test.

The stimulation of active oxygen species production caused increased MDA content (malondialdehyde) in plant tissue. MDA is a decomposition product of polyunsaturated fatty acids, has been utilized very often as a suitable biomarker for lipid peroxidation, which is an effect of oxidative damage [34]. During the stress conditions, the polyunsaturated fatty acids of the membrane are peroxidized due to the formation of active oxygen species [35]. Membrane lipid peroxidation of two *Azolla* species was assessed by measuring MDA content (nmol g FM^{-1}) in fronds. Fig. 2 shows that the MDA content was appreciably higher in *A. pinnata* that indicated a higher degree of lipid peroxidation under pretilachlor and UV-B stress. Pretilachlor and UV-B, singly and in combination increased the MDA content in fronds of both species of *Azolla*, thereby indicating enhanced lipid peroxidation.

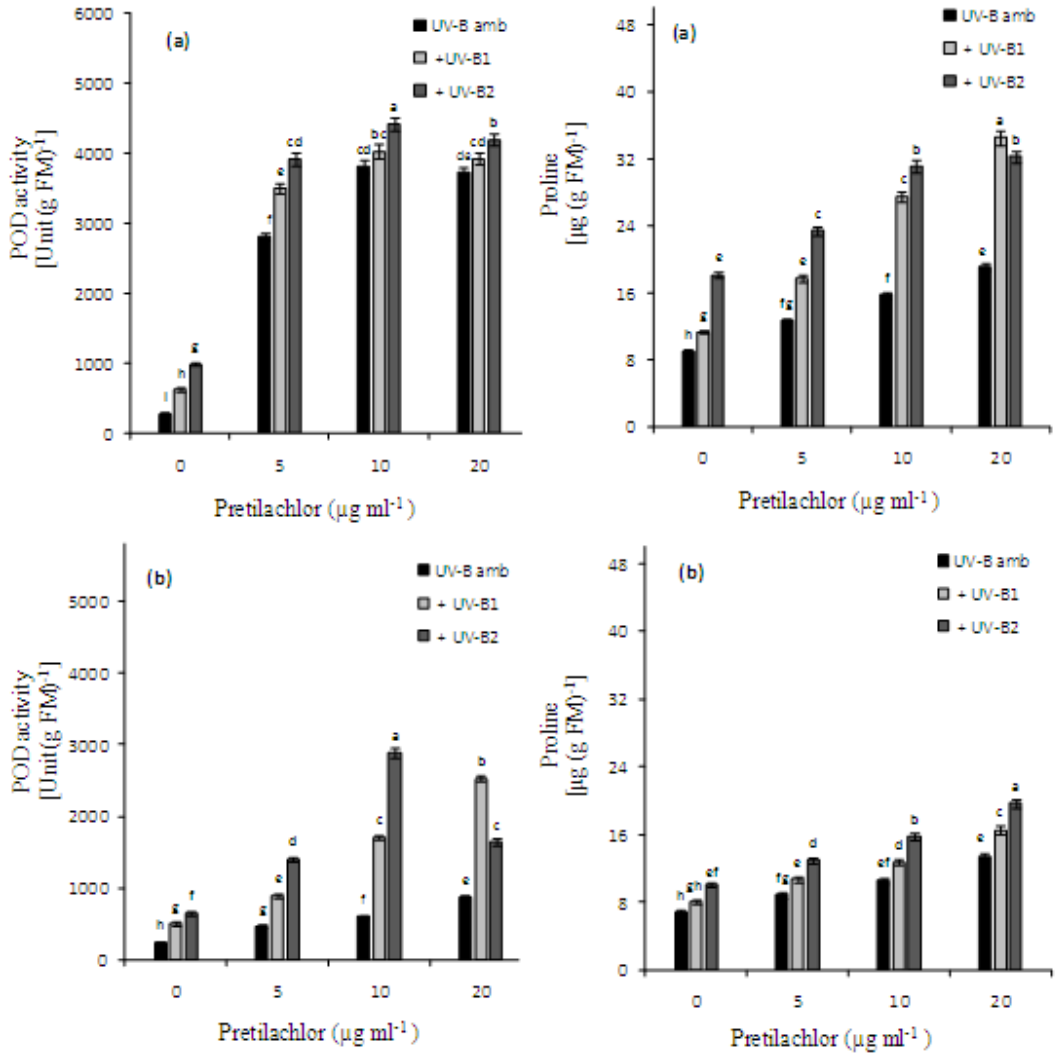

Fig. 4 Effect of pretilachlor and enhanced levels of UV-B radiation, singly and in combination, on guaiacol peroxidase activity and proline content of *Azolla microphylla* (a) and *Azolla pinnata* (b). Data are means ± standard error of three independent experiments.

Bars followed by different letters show significant difference at P<0.05 significance level according to Duncan's multiple range test.

The MDA content was increased by about 47, 67 and 140% in *A. pinnata* and 19, 55 and 98% in *A. microphylla* over the control of both species at 5, 10 and 20 µg ml^{-1} pretilachlor, respectively. After exposure to UV-B_2 with 5, 10 and 20 µg ml^{-1} of pretilachlor the content of MDA was increased by 189, 226 and 306% in *A. pinnata* whereas it was increased by 102, 126 and 176% in *A. microphylla*, respectively. Low level of MDA content may be one of the reasons for the observed tolerance of *A. microphylla* exposed to pretilachlor concentrations and UV-B radiation. Increased level of MDA content due to pesticide (dimethoate), singly and in combination with UV-B has been reported by Mishra et al. [5]. When the level of AOS formed exceeds the ability of the antioxidant system which is equipped in plants to cope with their damage to cellular components, the oxidative stress occurs. The changes of three antioxidant enzymes SOD, CAT and POD activity in pretilachlor and UV-B treated fronds are shown in Figs. 3 and 4. In organisms and higher plants, an oxidative stress is known to increase SOD activity [36]. SOD is

one of the most important antioxidant enzymes and present in all oxygen-metabolizing cells [37]. In our study, SOD activity in both species was found to be stimulated by pretilachlor and UV-B stress, causing dismutation of $O_2^{\bullet-}$ in to H_2O_2 [38]. After 96 h of experiment both species exhibited an increase in SOD activity, however, it was more marked in *A. microphylla*. In *A. pinnata*, SOD activity was increased in concentration dependent manner of pretilachlor, but decreased significantly at 10 and 20 µg ml^{-1}, when combined with UV-B_1 and 5, 10 and 20 µg ml^{-1} pretilachlor with UV-B_2. In *A. microphylla*, significant increase in this enzyme activity was found when fronds subjected to combined doses of UV-B_1 and UV-B_2 with pretilachlor. SOD activity increased quickly in *A. microphylla* at 20 µg ml^{-1} of pretilachlor + UV-B_2 treatment and reached the maximum 71.12 U (g FM)$^{-1}$ after 96 h of experiment.

A similar increasing trend in CAT activity was also found in both species. CAT enzyme is also a scavenger of H_2O_2 produced in the cellular system and its activity increased in both the species due to pretilachlor and UV-B stress. Stimulation in CAT activity has been reported in plants in earlier study [39]. But a decreasing trend in CAT activity but still higher than the values of control was observed in *A. microphylla*, when pretilachlor at 20 µg ml^{-1}, was combined with UV-B_1 and UV-B_2 doses. Foyer et al [40] reported that CAT is not a stable enzyme and it is susceptible to photoinhibition and degradation, so decrease in its activity at higher concentration of stress was observed. CAT activity increased markedly in *A. microphylla* and reached the maximum 640 U (g FM)$^{-1}$ at 20 µg ml^{-1} of pretilachlor, however, in *A. pinnata*, it was maximum at 20 µg ml^{-1} + UV-B_2 i.e. 446 U (g FM)$^{-1}$ (Fig. 3). The changing trend of POD activity was identical basically with SOD activity (Fig. 4). Activation of antioxidant enzymes SOD, CAT and POD by UV-B has been also observed in *Arabidopsis thaliana* by Rao et al. [41]. There were significant differences in enzymatic activities (SOD, CAT and POD) in both species of *Azolla* under stress condition. It has been earlier reported that the variations in antioxidant levels can serve as a signal for the modulation of AOS scavenging mechanisms and AOS signal transduction [42].

Both species of *Azolla* showed significant increase in proline content in response to pretilachlor and UV-B treatment as shown in Fig. 4. In *A. microphylla* fronds exposed to 5, 10 and 20 µg ml^{-1} of pretilachlor the content of proline was raised from 9.09 µg (g FM)$^{-1}$ up to 12.7, 15.8 and 19.9 µg (g FM)$^{-1}$ respectively, whereas in *A. pinnata* it increased from 6.8 µg (g FM)$^{-1}$ up to 8.86, 10.6 and 13.4 µg (g FM)$^{-1}$ respectively. Exposure of UV-B_1 and UV-B_2 alone also increased proline content as it was raised up to 11.4, 18.1 µg (g FM)$^{-1}$ and 8.1, 10.1 µg (g FM)$^{-1}$ in *A. microphylla* and *A. pinnata*, respectively. Proline content was further increased on combined treatment of pretilachlor and UV-B. Data presented in Fig. 4 showed that accumulation of proline content was more in *A. microphylla* than *A. pinnata* under control as well as treated condition. Similarly, increase in proline accumulation under UV-B stress has also been reported in *Azolla pinnata* and *Azolla filiculoides* by Masood et al [35], although the mechanism of accumulation of proline in plant or plant parts exposed to stress is still unknown. It is suspected that due to decrease in the activity of the respiratory electron transport system [43] leads to the accumulation of NADH and H^+. Proline accumulation might be an adaptive mechanism for reducing the level of accumulated NADH, and the acidity is used for synthesizing each molecule of proline from glutamic acid. Enhanced level of proline may have also conferred the capacity to detoxify active oxygen species efficiently in *A. microphylla* as compared to *A. pinnata*. Hyperaccumulation of proline in plants is linked with detoxification against stress induced

oxidative stress [44]. These results pointed out that the difference in the degree of oxidative stress (levels of MDA and SOR) in *Azolla* under stress condition was regulated by the capacity of antioxidant system and thus it may be the real cause of the differential sensitivity of both species towards stress. When we compared the growth, oxidative stress and antioxidant level between *A. microphylla* and *A. pinnata*, the more antioxidant activities were observed in addition to the less MDA formation in A. *microphylla*, which might be the probable reason for increasing stress tolerance as reported by earlier workers [31,35].

CONCLUSION

We conclude that although *A. microphylla* showed reduction in growth but quite good in resisting the stress caused by pretilachlor and UV-B. In *A. microphylla*, the levels of proline, and SOD, CAT and POD activities greatly enhanced the resistance capability towards pretilachlor and enhanced UV-B stress. On the basis of these results *A. microphylla* has been ranked as the more tolerant species than *A. pinnata*, so it can be used as biofertilizer and also for medicine purpose even under unavoidable stress conditions.

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