Our Nature (2012) 10: 145-155

Photosynthetic, Biochemical and Enzymatic Investigation of *Azolla microphylla* in Response to an Insecticide-Hexachlorohexahydro-Methano-Benzodioxathiepine-Oxide

Waseem Raja*, Preeti Rathaur, Suchit A. John and P.W. Ramteke

Plant Physiology and Biochemistry, School of Basic Sciences, Sam Higginbottom Institute of Agriculture, Technology and Sciences, Allahabad, India
**E-mail: rajawaseem26@yahoo.in*

Abstract

A study on the heterocystous, nitrogen fixing Water fern, *Azolla microphylla* was carried out to investigate the effect of an organochlorine insecticide (hexachloro-hexahydro-methano benzodioxathiepine-oxide, called as endosulfan) at different concentrations of 0, 50, 100, 200, 400 and 600 ppm on fresh weight, dry weight, photosynthetic pigments, stress metabolites such as ascorbic acid, proteins, and nitrogen metabolism activity like nitrate reductase and nitrate uptake. The inhibition was found to be dose dependent. The insecticide endosulfan showed to be deleteriously affecting the activities in the *Azolla microphylla*. Endosulfan adversely depleted the cellular activities, leading to a marked increase in the Vitamin-C at lower concentration and gradually decreases at higher concentrations. Decrease in protein was clear and activities like nitrate reductase and nitrate uptake also increases up to certain concentration and at higher concentration slightly decreases. Despite of deleterious effects of endosulfan on the *Azolla microphylla*, a unique regenerating ability in presence of the insecticide was observed by the end of five days in the lower doses of insecticide. *Azolla* seems to help sustain the soil nitrogen supply by returning nitrogen to quantities roughly equal to those extracted from the soil by the rice plant.

Key words: *Azolla microphylla*, Endosulfan, Enzymes, Photosynthetic pigments, Stress metabolites, Nitrogen metabolism

Introduction

The increasing use of pesticides in agriculture demands investigation to examine the effect of pesticides on the non-target soil micro-organisms and plants including nitrogen fixing cyanobacteria and their symbionts. A pesticide (herbicides, fungicides and insecticides) adversely affects all aspects of primary and secondary metabolism in crops and animals when applied in agricultural fields. The important factor in using *Azolla* as a biofertilizer for rice crop is its quick decomposition in soil and efficient availability of its nitrogen to rice plant. The quick multiplication rate and rapid decomposing capacity of *Azolla* has become paramount important factor to use as green manure cum biofertilizer in rice field. The application of insecticide a group of pesticides, in crop fields for selective control of pests in the modern age has led to serious environmental contamination resulting in greater loss of crop productivity

and growth of many beneficial microorganisms, phytoplankton's etc. (Meghraj *et al*., 1988). Though the application of many insecticides are forbidden, the low cost, easy availability, lack of awareness and lax regularity implementation have contributed to the continuous use of the insecticides in tropical and subtropical regions. The removal of these insecticides from soil and aquatic systems has become a difficult problem and as a result of this, they persist in these ecosystems for a long period of time (Singh, 1988). Water bodies such as ponds, water reservoirs, aquaculture, shallow water and paddy fields are highly eutrophic and maintain large standing crops of phytoplankton's and floating plants and some aquatic fern like *Azolla* (Aida *et al*., 2006). An *Azolla- Anabaena* association is a favourite biofertilizer of crops, especially in rice paddy fields because of its ability to fix dinitrogen at high rates and low cost. In addition, *Azolla* is a suitable candidate as an animal feed, human food water purifier, medicine, hydrogen fuel, biogas producer, weed controller, suppresser of weeds and reduces ammonia volatilization after chemical nitrogen application and rightly called as green gold (Wagner, 1973). Though the considerable amount of work on abiotic stress induced inhibitory effect of growth, photosynthetic pigments content and nitrogen metabolism have been done in recent years (Masood *et al.,* 2008) Detrimental effect of pesticides on the growth of aquatic macrophytes are generally known (Seulthorpe, 1967), but still the knowledge of the indirect effect of applied pesticides like monocrotophos on soil micro and macro flora is fragmentally and partly outdated. The influence of pesticides on soil and aquatic algae including *Azolla* has been a growing concern. Plant exposed to stress shows an increased accumulation of proline (Chris *et al.,* 2006). Studies also showed that organophosphorus insecticides interfere with carbohydrate accumulation in paddy seeds (Gupta and Mishra, 1983). Quinalphos (O, O-diethyl-O-quinoxalin-2-yl phosphorothioate) due to its acaricidal and insecticidal properties is in large scale use in this country. From an annual consumption of 300 metric tons during 1977, the use has risen to 1000 metric tons. But insecticides particularly endosulfan induced effect on growth, enzyme assay and nitrogen metabolism in *Azolla* particularly *A. microphylla are* yet to be investigated. Considering the importance of *Azolla* in rice fields, and frequent use of pesticides against pests, the authors set forth the objective of investigating the Photosynthetic, biochemical and enzymatic investigation of *A. microphylla* in response to an insecticide – hexachlorohexahydro – methano – benzodioxathiepine - oxide.

Materials and methods

Plant material and growth conditions

Azolla microphylla, selected for present study, was procured and collected from National Centre for Conservation and Utilization of Blue Green Algae, IARI, New Delhi and cultured in Department of Biological sciences, SHIATS Allahabad. The plants were surface sterilized quickly with a solution of mercuric chloride (0.1% for 30s) followed by dipping the plants into a large volume of sterile distilled water. Washing of the Azolla microphylla with sterile distilled water was repeated several

times. Fronds were then transferred into plastic trays (32×25×6 cm^3) containing nitrogen free medium. The pH of the medium was adjusted to 7.2. Plastic trays were placed in the Culture Lab, Department of Biological Sciences, SHIATS Allahabad. During the experimental period, average minimum and maximum temperature ranged from 16.7 to 36.8°C, and relative humidity from 55 to 71%. Photosynthetic active radiation (PAR) ranged between 800 -1000 μmol photon $m^{-2}s^{-1}$.

Pesticide treatment

Widely used insecticide endosulfan (hexachloro hexahydro-methanobenzodiox-athiepine-Oxide) 35% EC was selected for the treatment which was manufactured by Hindustan Pulversing Mills, Industrial Growth Center Samba Jammu. Its various concentrations 0, 25, 50, 100, 200, 400 and 600 ppm in nutrient medium were prepared for screening experiment.

Growth estimation

Azolla microphylla plants were rinsed in an aerated iso-osmotic solution of sorbitol were blotted dry on filter paper and weighed to represent their fresh weight (FW). Dry weight (DW) was determined by drying the samples in a hot air oven at 60°C for 24 h to a constant weight.

Chlorophyll and Carotenoid estimation

Chlorophyll and carotenoids were extracted from fronds with 80% acetone. Chlorophyll and carotenoids were estimated spectrophotometrically according to the method of Lichtenthaler and Welburn (1983).

Protein estimation

Protein was estimated by method given by (Lowry *et al*., 1951). The protein content was determined by the standard curve prepared out of the Bovine serum albumin protein and absorbance was measured at 660 nm.

Ascorbic acid estimation

Ascorbic acid is an important chemical antioxidant which is responsible for the non-enzymatic scavenging of superoxide radical and hydrogen peroxide, its estimation is based on the formation of pink coloured complex due to the reduction of dinitrophenylhydrazine by ascorbic acid to phenyl hydrazene in acidic medium. It is estimated by the method given by (Mukherjee and Choudhary, 1983) and absorbance is recorded at 530 nm.

Nitrate uptake estimation

Nitrate uptake by the *Azolla microphylla* cells were estimated by measuring the depletion of nitrate from external medium spectrophotometrically by brucine-sulphuric acid method of (Nicholas and Nasen, 1957). Nitrate Calibration curve was prepared in the range of 1-100 μm using potassium nitrate as standard solution.

Nitrate reductase activity (EC 1.6.6.1) estimation

A known volume of *A. microphylla* was centrifuged (8000 rpm, 5 min) and washed 3-4 times and transferred into culture medium containing 5 mm KNO_3**.** Samples were withdrawn at regular intervals and formation of nitrite was measured at 540 nm by the diazo-coupling method of (Lowe and Evans, 1964). A calibration curve as

standard solution and the values were calculated accordingly.

Statistical analysis

All the data obtained of *Azolla microphylla* in terms of growth, chlorophyll, carotenoids, protein, ascorbic acid, nitrate reductase activity and ammonia uptake in response to different levels of endosulfan were statistically analysed for their significance. An analysis of variance (ANOVA) was performed using SPSS 10 program. The significance was tested at 0.05 (5%) level. Values presented in the text indicate mean values ± of five replicates.

Results

The present study shows the effect of different concentrations of endosulfan on growth, photosynthetic pigments and nitrogen metabolism of *A. microphylla.* The growth response of the tested *A. microphylla* to insecticide exposure was inhibitory and the effect varied with insecticide dose. All the concentrations of the insecticide reduced the growth parameters shown in table 1. Fresh weight of insecticide stressed plants were reduced by 7.15, 21.43, 28.58 and 47.15% at 50,100,200 and 400 ppm concentration respectively and in case of dry weight, the dry weight of the test organism reduced by 14.67, 23.17, 38.42 and 52.79% at 50, 100, 200 and 400 ppm respectively. Plants were highly damaged at the concentration of 600 ppm. Contents of total chlorophyll and carotenoid in test *A. microphylla* reduced considerably following insecticide exposure and decrease was dose dependent (Tab. 1). As a result of increased exposure total chlorophyll content decrease in *A. microphylla* was 3.85, 19.24, 30.77, 44.24 and 61.54% at 50, 100, 200, 400 and 600 ppm respectively and carotenoid content by 9.04, 28.67, 38.95, 56.39 and 68.54% at same concentrations. As compared to total chlorophyll, carotenoid content was severely affected. The test organism was highly damaged at 600 ppm. Thus summing up the results the effect of endosulfan on *A. microphylla* is detrimental and there is an inverse relation between concentration of insecticide on growth and photosynthetic pigments.

Being essential macromolecule of living cells protein play a paramount role in metabolic pathway to understand the effect of endosulfan on *A. microphylla*. Analysis of protein was done after five days of treatment of insecticide (Fig. 1).The concentration of of protein was maximum in control showed a considerable decrease with increasing concentration of endosulfan. Protein content reduced by 15, 26 and 48% at 50, 100, and 200 ppm, respectively. Further there was a decrease in protein as concentration increases. The highest decrease was shown at 600 ppm which was about 70%. Ascorbic acid (Vitamin-C) content increases as the concentration of endosulfan increases as depicted in (Fig. 2).The ascorbic acid content increases by 19, 30 and 42% at 50,100 and 200 ppm, respectively. Beyond 200ppm there was a gradual decrease. Thus our result reveals that Vitamin-C content increases up to certain limit and then starts decreasing.

An attempt was made to study the effects of various concentrations of endosulfan on nitrogen metabolism of test

Table 1. Effect of endosulfan on fresh weight, dry weight, total chlorophyll and carotenoid content of Azolla microphylla. Values in parenthesis are percent decrease (-) with reference to respective controls. Mean ±SE (n=3). Values are significantly (P<0.05) different from each other (Analysis of variance).

Endosulfan (ppm)	Fresh Weight (gm)	Dry Weight (gm)	Total Chlorophyll (mg/gm f. wt.)	Carotenoids (mg/gm f.wt.)
0	1.4±0.3	0.341±0.1	0.52±0.4	0.321±0.1
50	1.3±0.3 (-7.15)	0.291±0.1 (-14.67)	0.50±0.3 (-3.85)	0.292±0.2 (-9.04)
100	1.1±0.4 (-21.43)	0.262±0.1 (-23.17)	0.42±0.1 (-19.24)	0.229±0.1 (-28.67)
200	1.0±0.4 (-28.58)	0.211±0.1 (-38.42)	0.36±0.2 (-30.77)	0.196±0.3 (-38.95)
400	0.74±0.4 (-47.15)	0.161±0.0 (-52.79)	0.29±0.2 (-44.24)	0.140±0.0 (-56.39)
600	0.46±0.1 (-67.15)	0.109±0.1 (-68.04)	0.20±0.1 (-61.54)	0.101±0.0 (-68.54)

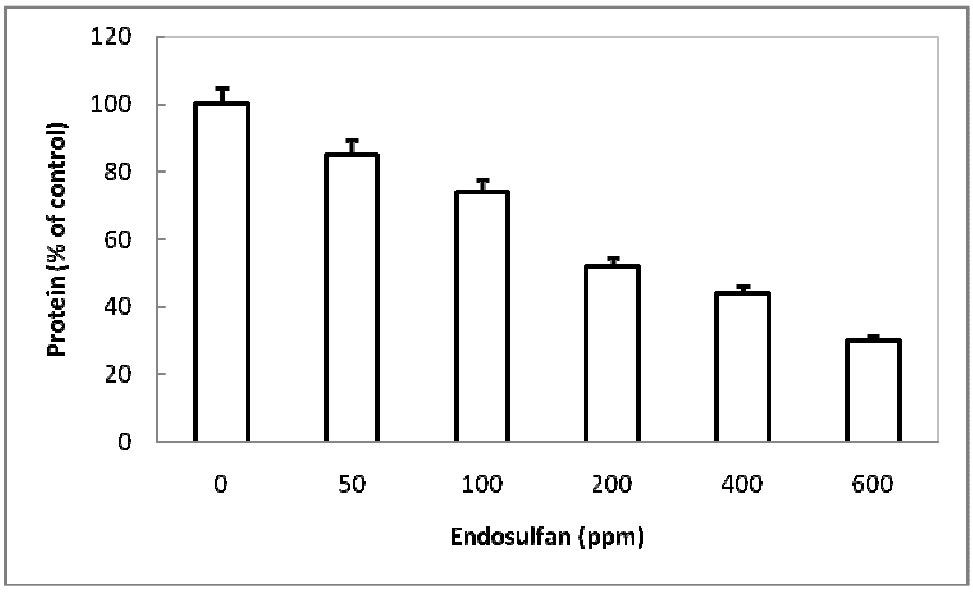

Figure 1. Effect of different concentrations of endosulfan on Protein content in *Azolla microphylla*. Protein content in untreated control was 0.998±0.4 mg/gm fresh weight. Mean± SE... All the values are significant at P<0.05.

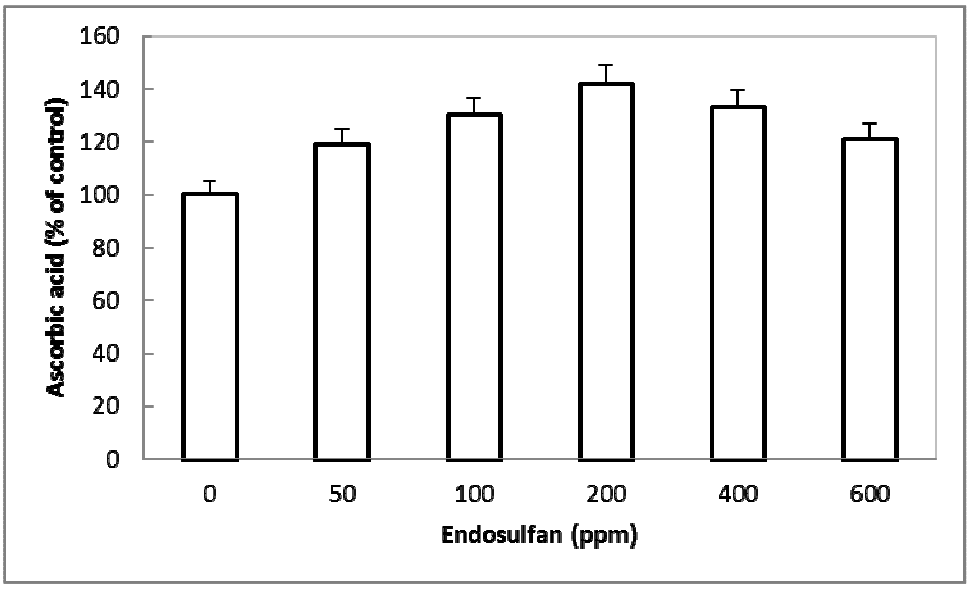

Figure 2. Effect of different concentrations of endosulfan on Ascorbic acid content in *Azolla microphylla*. Ascorbic acid content in untreated control was 24.7±1.3 µg gm^{-1} fresh weight. Mean± SE... All the values are significant at P<0.05

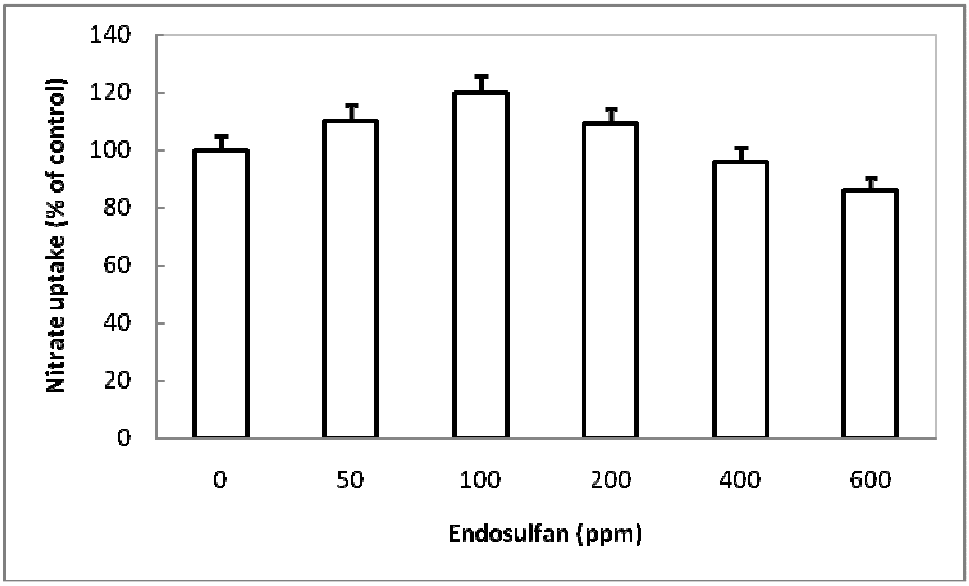

Figure 3. Effect of different concentrations of endosulfan on Nitrate uptake content in *Azolla microphylla*. Nitrate uptake content in untreated control was 9.01±1.7 µ mol mg^{-1} Protein h^{-1} Mean± SE.. All the values are significant at P<0.05.

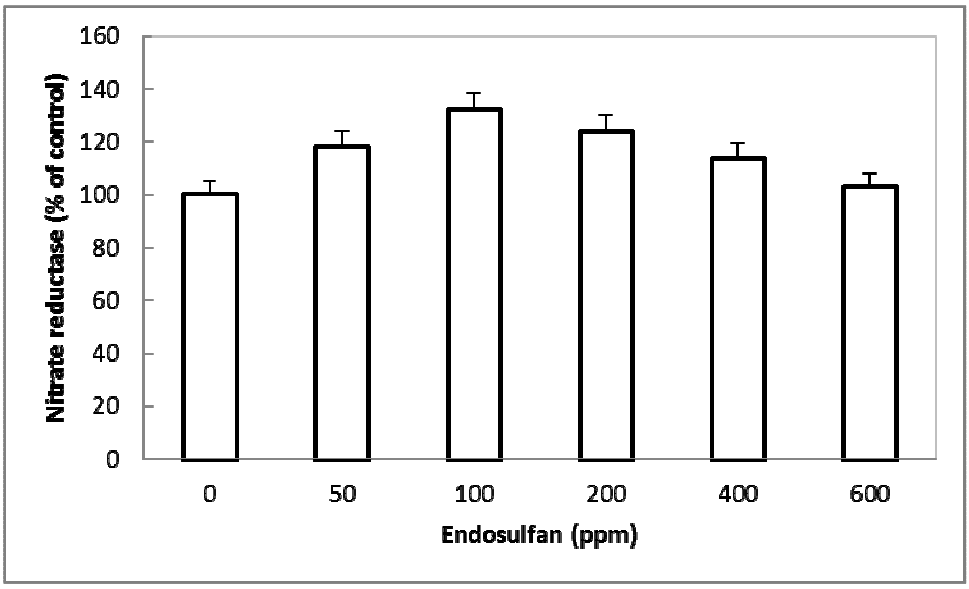

Figure 4. Effect of different concentrations of endosulfan on Nitrate reductase activity in *Azolla microphylla*. Nitrate reductase activity in untreated control was 13.6±2.0 µ mol $NO_2^{-1}mg^{-1}$Protein h^{-1}. Mean± SE. All the values are significant at P<0.05

organism (Figs. 3-4). Nitrate uptake by *A. microphylla* after five days of treatment was clear that as the concentration of endosulfan increases, the nitrate uptake also increases by 10, 20%, at 50 and 100 ppm. However concentration beyond 100 ppm there was a slight decrease in nitrate uptake. The highest decrease was shown at 600 ppm which was about 14%. Also a case of nitrate reductase activity the concentration of endosulfan increases the activity by 18, 32% at 50 and 100 ppm. But beyond there was also a slight decrease in nitrate reductase activity. Thus, summing up the results, at higher concentration the nitrate uptake declined. The accumulation increases at lower concentration of pesticides; this could be due to more demand of test organism for nitrogen. Thus, we put forth that present nitrogen and nitrogen yield were significantly affected by endosulfan but nitrate reduction activity was affected at higher concentration of endosulfan.

Discussion

Heavy use of pesticides reduces the growth of plants higher as well as lower plants. Several physiological and biochemical mechanisms are involved in response of *Azolla* to pesticide stress. Reduction in fresh weight and dry weight was clear after five days of incubation at different concentration in ppm of endosulfan .our results are comparable to the observations made by (Aida *et al.*, 2006) and recently done by (El-Shahate *et al.*, 2011). It is found that pesticide has an inhibitory effect on photosynthetic CO_2 assimilation and protein synthesis (Battah *et al.*, 2001) which could be due to disturbances in nitrogen

metabolism and photosynthetic activity or due to increase in protease activity (Kaushik and Venkataraman, 1983) such effects might exert much secondary effect on growth. (Kalita, 1997) has demonstrated that high concentration of melathion inhibit the growth of *Azolla pinnata*. The reduction in dry weight and fresh weight by endosulfan might be due to chemical which effects, the tissue binding process in *Azolla* at higher concentrations. This may also be caused by the disturbance with Hill reaction and electron transport system in photosynthesis as has been observed in spinach due to application of an insecticide methyl parathion (Moreland and Novitzky, 1984). The reduced growth in response to higher concentration of melathion may result from reduction in protein and DNA content (Sengupta *et al*., 1986).

The damaging effect of endosulfan on photosynthetic pigments of *A. microphylla* was noticed after five days of treatment. Deleterious effects were observed on both the photosynthetic pigments, chlorophyll was more affected than carotenoids. The pesticides are known to inhibit chlorophyll biosynthesis particularly by inhibiting δ-aminolevuliniceacid dehydrogenase and protochlorophyllide reductase (Dubey, 1997). Under stress conditions carotenoid pigments are less affected than chlorophyll resulting in a low chlorophyll/carotenoid ratio, and results are obtained in *Azolla* fronds treated with pesticides and are in consequences with earlier findings (Prasad *et al.*, 2005). Since carotenoids are less affected, it also act as an antioxidant metabolite (Chris *et al*., 2006; Dai *et al.*, 2006), it protects chlorophyll and photosynthetic membrane from oxidative damage, therefore decline in carotenoids could have serious consequences on chlorophyll as well as thylakoid membrane which may lead to reduction in photosynthetic capability of *A. microphylla*. Like chlorophyll, protein of the *A. microphylla* was also inhibited by enhanced doses of pesticides which are in agreement with results of earlier work of (Holst *et al*., 1982). Malaga and (Malliga and Subramanian, 1989) also reported inhibition in the protein content of *Azolla* fronds following different doses of pesticides and it could be co-related with reduced photosynthetic activity, nitrogen metabolism and nucleic acid damage under pesticide stress. (Reddy *et al.*, 2004). Recently (Prasad and Zeeshan, 2004) have shown reduction in growth (Protein) of cyanobacterium *Plectonema boryanum* under monocrotophos stress which is in agreement with our findings.

A significant increase in ascorbic acid (Vitamin C) content in fronds of *Azolla microphylla* was observed in response to pesticide stress. Ascorbic acid an important antioxidant, which react not only with H_2O_2 but also with O_2^-, OH^- and lipid hydro peroxidases (Reddy *et al*., 2004). Diazole-treatment increased the ascorbic acid content in tomato seedlings (Senaratna *et al*., 1988) Ascorbate can function as a terminal antioxidant because of the redox potential of ascorbic acid/ monodehydro ascorbate (MDA) pair (+280 nm) is lower than that of most of the bioradicals (Scandalios *et al*., 1997), however very little is known about the regulation of ascorbic acid biosynthesis in higher plants. A high level of endogenous ascorbate is essential to effectively maintain the antioxidant system

that protects plants from oxidative damage due to the biotic and abiotic stress (Shigeoka *et al*., 2002).

The nitrate reductase activity was increased significantly at 100 ppm of both the pesticide concentration. According to Singh and Mahapatra (1998), nitrate reductase activity was mostly determined by nitrate flux into metabolic pool, so it may be possible that there is an indirect effect of salinity on nitrate reductase activity through modification in nitrate uptake. Expression and activity of nitrate reductase was affected by nitrate under pesticide stress and nitrate is reported to have protective role for nitrate reductase enzyme against action of proteases and/inhibitors besides inducing the synthesis of nitrate reductase protein through nitrate reductase gene expression. 100 ppm concentration of both the pesticide stress increased the nitrate uptake and nitrate reductase activity possibly because of high demand of nitrogen during stress condition (Holst *et al*., 1982; Suseela, 2001).

Conclusion

The environmental hazards of pesticides would be intensified far greater than expected in the soils already contaminated with pesticide which in turn affect the productivity of *Azolla* plants under field conditions (Raja *et al*., 2012). In a review on Azolla calls Azolla “a green gold mine The results demonstrated differential response in terms of growth, chlorophyll, carotenoids, Proteins, Ascorbic acid content, and Nitrogen metabolism activities of *Azolla microphylla* in response to endosulfan. The strong inhibitory effect on the growth and photosynthetic pigments could be correlated with endosulfan induced inhibition. In contrast to this, Proteins, Ascorbic acid and nitrogen metabolism activities were enhanced. These observations serve as baseline data for the evaluation and quantification of *Azolla* genotypes towards increasing the usefulness of *Azolla-Anabaena* association as biofertilizer. Elucidation of physiological and biochemical response to endosulfan is important in this organism because the application of pesticide in crop fields is increasing and potential use of *Azolla* as a biofertilizer in such environment needs to be investigated in detail especially at molecular level .

Acknowledgments

We are grateful to University administration and Head, Department of Biological Sciences, SHIATS, India for providing necessary laboratory facilities. We like to acknowledge the Vice Chancellor, Dean P.G. studies and Director Research for encouragements.

References

Aida, M., I. Kozuyuki, I. Hiroaki, H. Naokuni, I. Yasuo and U. Kenji 2006. Susceptibility of aquatic ferns to paddy herbicides benzulfuron methyl. *Weed Boil. Manag.* ***4*****(*****3*****):** 127.

Battah, M.G, E.F. Shabana, I.A. Kobbia and H.M. Eladel 2001. Differential effects of thiobencarb toxicity on growth and photosynthesis of *Anabaena variabilis* with change in phosphate level. *Ecotox. Environ. safe* ***49*****(3):** 235-239.

Chris, A., M. Zeeshan, G. Abraham and S.M. Prasad 2006. Proline accumulation in *Cylindrospermum* sp. *Env. Exp. Bot.* ***57*****:** 154-159.

Dai, P., Z.T. Xiong, Y. Huang and M.J. Li 2006. Cadmium induced changes in pigments, total phenolics, and phenylalanine, Ammonia-lyase activity in fronds of *Azolla imbricate. Environ. Toxicol.* ***21*****:** 505-512.

Dubey, R.S. 1997. Photosynthesis in plants under stressful conditions. In *handbook of photosynthesis*, (Ed. M. Dekker), New York. pp.859-876.

EL-Shahate, R.M., M.M.I. EL-Araby, E.W. Eweda and M.N. El-Berashi 2011.Evaluation of the effect of three different pesticides on *Azolla pinnata* growth and NPK uptake. *J. American. Sci.* ***7(1):*** 1020-1031.

Gupta, A.K. and S.G. Misra 1983. Effect of organophosphorus insecticides on seed germination of moong (*Vigna radiate* L.) and paddy (*Oryza sativa* L.). *Pesticides* ***2*****:** 3-10.

Holst, R., H. John and G.Y. Kapusta 1982. Effect of several pesticides on the growth and nitrogen Assimilation of the *Azolla-Anabaena* symbiosis. *Weed. Sci.* ***30*****:** 54-58.

Kalita, M.C. 1997. Effect of melathion on growth, chlorophyll biosynthesis and total nitrogen accumulation of *Azolla,-Anabaena* symbionts. *J. Ecotoxicol. Environ. Monit.* ***7(1):*** 059-063.

Kaushik, B.D. and G.S. Venkataraman 1983. Response of cyanobacterial nitrogen fixation to insecticides. *Curr. Sci.* ***52*****:** 321-323.

Lichtenthaler, H.K. and A.R. Welburn 1983. Determination of total carotenoids and chlorophyll a and b of leaf extracts in different solvents. *Biochem. Soc. Transac.* ***11*****:** 591-592.

Lowe, R. and H. Evans 1964. Preparation of some properties of a soluble nitrate reductase from *Rhizobium japonicum*. *Biochem. Biophy. Act.* ***85*****:** 377-389.

Lowry, O.H., N.J. Rasebrough, A.L. Farr and R.J. Randoll 1951. Protein measurement with the folin phenol reagent. *J. Bio. Chem.* ***193*****:** 165-175.

Malliga, P. and G. Subramanian 1989. Effect of certain chemicals on the in vivo status of the endosymbiont *Anabaena-Azolla* in *Azolla pinnata* R. BR. *Curr. Sci.* ***58*****:** 634.

Masood, A., M. Zeeshan and G. Abraham 2008. Response of growth and antioxidant enzymes in *Azolla* plants (*Azolla pinnata* and *A. filiculoides*) exposed to UV-B. *J. Acta Biol. Hung.* ***59(2):*** 247-258.

Meghraj, M., K. Venkateshwarlu and S. Rao 1988. Effect of insecticides and phenolics on nitrogen fixation by *Nostoc*. *Bull. Envir. Contam. Toxicol.* ***41*****:** 277-281.

Moreland, D.E. and W.P. Novitzky 1984. Interaction of insecticides with chloroplast membranes. In *Advances in Photosynthetic Research*, Sybesma. Junk Publisher, The Netherlands. pp. 81-94.

Mukherjee, S.P. and M.A. Choudhuri 1983. Implications of water stress-induced changes in the leaves of indigenous ascorbic acid and hydrogen peroxide in *Vigna* seedlings. *Physiol. Plant.* ***58*****:** 166-170.

Nicholas, D.J.D. and A. Nason 1957. Determination of nitrate and nitrite. In *Methods Enzymology*, (Eds. S.P. Colowick and N.O. Kaplan). III Academic Press, New York. pp. 981-984.

Prasad, S.M. and M. Zeeshan 2004. Effects of UV-B and monocrotophos singly and in combination on photosynthetic activity and growth of non-heterocystous cyanobacterium *Plectonama boryanum*. *Environ. Exp. Bot.* ***52*****:** 175-185.

Prasad, S.M., D. Kumar and M. Zeeshan 2005. Growth, photosynthesis, active oxygen species and antioxidants responses of paddy field cyanobacterium *Plectonema boryanum* to endosulfan stress. *J. Gen. Appl. Microbiol.* ***51*****:** 115-123.

Raja, W., P. Rathaur, S.A. John, P.W. Ramteke and R.A. Dar 2012. Effect of monocrotophos on electrolytic leakage, proline content and nitrogen metabolism of floating pteridophyte A*zolla microphylla*. *The Holistic Approach to Environment* ***2(3):*** 111-120.

Reddy, A.R., K.V. Chaiyanya and M. Vivekanandan 2004. Drought induced responses of photosynthesis and antioxidant metabolism in higher plants. *J. Plant Physiol.* ***161*****:** 1189-1202.

Scandalios, J.G., L. Guan and A.N. Palidros 1997. *Catalases in plants: gene structure, properties, regulation and expression*. Gold Spring Harbour, New York. pp. 343-406.

Senaratna, T., C.E. Mackay, B.D. McKersie R.A. Fletcher 1988. Uniconazole induced chilling tolerance in tomato and its relationship to antioxidant content. *J. Plant. Physiol.* ***133*****:** 55-61.

Sengupta, P.K., A. Chakraborti and S.K. Banerjee 1986. Biochemical changes induced by toxic concentration of melathion in germinating wheat seeds. *Curr. Sci.* ***55*****:** 492-494.

Seulthorpe, C.D. 1967. *The biology of aquatic vascular plants*. Edward Arnold (Pub.) Ltd., London. 610 p.

Shigeoka, S., T. Ishikawa, M. Tamoi, Y. Miyagawa, T. Takeda, Y. Yabuta and K. Yoshimura 2002**.** Regulation and function of ascorbate peroxidase isoenzymes. *J. Exp. Bot.* ***53*****:** 1305-1319.

Singh, P.K. 1988. Biofertilezers-potentialities and problems. In *Plant Physiology Forum* (Eds. S.P. Sen and P. Patil), Calcutta. pp.109-114.

Singh, P.K. and J.K. Mahapatra 1998. Basic biology of *Azolla-Anabaena:* Association and free – living blue green algae. In *The changing scenario in plant Science* (Eds. V.S. Jaiswal, A.K. Rai, U. Jaiswal and J.S. Singh). Allied Publ. Ltd., New Delhi.

Suseela, M.R. 2001. Effect of butachlor on growth and nitrogen fixation by *Anabaena sphaerica*. *J. Environ. Biol.* ***22*****:** 201-203.

Valentine, J.P. and S.W. Bighan 1974. Influence of several algae on 2, 4-D residue in water. *Weed Sci.* ***22*****:** 358-363.

Wagner, G.M. 1973. *Azolla*: a review of its biology and utilization. *Bot. Rev.* pp. 1-26.