JJBS

Volume 5, Number 4, December.2012
ISSN 1995-6673
Pages 331 - 336

Jordan Journal of Biological Sciences **Short Communication**

Detrimental Upshot of Different Concentrations of Endosulfan on Growth and Lipid Peroxidation of Aquatic Pteridophyte *Azolla microphylla*

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Received 9th April, 2012; Accepted 11th July, 2012

Abstract

In the last decades, the use of insecticides in agriculture for selective control of pests has become crucial. Researches have produced a diverse range of products with novel modes of action. However, the extensive use of these compounds in the agriculture system raises public concern, because of the harmful potential of such substances in the environment and human health.When Endosulfan, an organochlorine insecticide, (0-400ppm), was added to an exponentially growing medium and the effects on *Azolla microphylla* were followed for 6 days, the following observations were made: Dry weight, Root number, root length, total chlorophyll and Carotenoid content decreased as compared to the control. Carotenoid content decreased more than chlorophyll content. These changes paralleled the inhibition of the rate of net photosynthesis, suggesting that the photosynthetic apparatus is one of the primary targets of the insecticide action. Insecticide exposure resulted in the reduction of relative growth rate at all the applied dose concentrations. An increase in the doubling time was also noticed which is an indication of delayed growth. Furthermore, Endosulfan induced the formation of MDA indicating enhanced Lipid peroxidation. Overall the effect of endosulfan was dose dependent. Since plants are sessile organisms and have limited mechanisms for insecticide application avoidance they need flexible means for acclimatization to changing environmental conditions. Hence in order to improve a plant protection, this kind of researches may be fruitful in understanding of mechanisms contributing to effect of endosulfan on plant system.

Keywords: Endosulfan, MDA, chlorophyll, carotenoid, doubling Time, relative growth rate.

1. Introduction

In modern agricultural production, herbicide application is a regular practice. While in developed countries, weeds and pests reduce yields of agricultural crops from 15 to 20%, reductions soar to 50% in undeveloped regions (Dobrovoljskiy and Grishina, 1985). The problems caused by the increased application of insecticides call for multidisciplinary approach. Incorrect and indiscriminate application of insecticides affects negatively the health of humans, plants and animals. In natural conditions, plants are exposed to multiple environmental stresses such as temperature, drought, cold, salinity, heavy metals, UV-B, pesticide etc. Pesticides are fetching major threat for the environment due to their increasing agricultural application and industrial production. The application of pesticide in paddy fields has also been endorsed to cause damaging effects on azolla plant which is a biofertilizer as well as a green manure to paddy fields (Raja *et al*., 2012a). The concept of using aquatic plant for different purposes is receiving special attention nowadays. Because of its growth habitat, high multiplication rate, excellent source of protein for mono-gastric animals, high biomass production and increasing demand as organic food (Raja *et al.*, 2012b). *Azolla microphylla* is a cosmopolitan free-floating water fern (Peters and Meeks, 1989). It has two features of particular interest: it is a fern despite its appearance, and the fronds of the six extent species have leaf cavities containing symbiotic, heterocyst-forming, N2-fixing cyanobacterium known as *Anabaena-azollae -strasb.*, a bacterial population (Peters and Meeks., 1989; Forni *et al*., 1989). Because of its habitat and nitrogen-fixing capability, the symbiotic association has been used for several decades as green manure in rice fields (Peters and Meeks., 1989). The *Azolla-Anabaena* association is important agronomically owing to its capacity to fix atmospheric nitrogen at cheaper and faster rates and making it available to crop plants (Raja *et al.*, 2012c). Azolla harvest light energy during photosynthesis and assimilate it into carbon compounds which provides cellular energy. This energy is utilized by azolla for fixation of nitrogen and other metabolic processes.

Detrimental effect of pesticides on the growth of aquatic macrophytes are generally known (Seulthorpe,

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1967), the influence of pesticides on soil and aquatic algae including *Azolla* has been a growing concern (Valantine and Binghan, 1974). Endosulfan due to its acaricidal and insecticidal properties is in large scale use in India and some other countries of world. From an annual consumption of 300 metric tons during 1977, the use has risen to 1000 metric tons in India (Anonymous, 1979) Endosulfan is an organochlorine insecticide which is systematic in action, penetrates plant tissue rapidly. Since *Azolla microphylla* is an important plant due to its ability to fix atmospheric nitrogen and pesticides like Endosulfan are also used to check the pest of paddy like leaf hoppers, white flies spider mites etc. so definitely this insecticide influence the growth and biomass property of *Azolla microphylla* and its other species. Azolla may be used for the production of hydrogen fuel and biogas, control of weeds and mosquitoes, and the reduction of ammonia volatilization that accompanies the application of chemical nitrogen fertilizer (Raja *et al.*, 2012a). Thus, azolla occupy an important position in food web and loss of azolla biomass may seriously affect soil fertility through nitrogen and carbon fixation. Therefore it becomes much imperative to study **"**detrimental upshot of different concentrations of Endosulfan on growth and lipid peroxidation of aquatic pteridophyte *Azolla microphylla*".

2. Materials and Methods

2.1. 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 30 s) 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 ^oC, and relative humidity from 55 to 71%. Photosynthetic active radiation (PAR) ranged between 800 -1000 µmol photon m^{-2} s^{-1}.

2.2. Pesticide treatment

Widely used pesticide Endosulfan [hexachloro hexahydro-methanobenzodioxathiepine-Oxide] 35% EC was selected for the treatment which was manufactured by Hindustan Pulversing Mills, Industrial Growth Center Samba Jammu (J&K)**.** Its various concentrations 0, 25, 50, 100, 200, 400 ppm in nutrient medium were prepared for screening experiment.

2.3. Estimation of fresh and dry weight

The method was cumulated with Rabinowich *et al.* (1985). The azolla fronds were blotted on tissue paper after 6 days of experimental period, and immediately weighed, each measurement was done in three replicates. Fronds were placed in petridsihes for 24 hr at 60^0C temperature. Again the dried samples were weighted after deducting the plate weight reading was recorded in milligrams.

2.4. Estimation of average root length and root number

Average root length and root number were determined by methods of Kurth *et al.,* (1986) and Ge-Shi-An *et al.*, (1980) respectively.

2.5. Estimation of doubling time and relative growth rate

Doubling time is the time in days needed for the production of next generation or needed for the doubling of the *Azolla* biomass, which is calculated as follows:

Doubling time = t/r

Where t = experimental period

$$r = \log \frac{(W_1 - W_0)}{0.301}$$

W_1 = weight after t days

W_0 = weight initial sample.

Relative growth rate (RGR) is basic component of growth analysis. RGR is defined at any instant of time as the increase in dry weight per unit dry material present. This is expressed in grams per gram per day or µg/g/ day. Protocol of Subudhi and Watanable (1981) was followed

$$RGR = \frac{0.693}{DT} \quad \mu \text{ g/g/day}$$

DT = doubling time.

2.6. Estimation of photosynthetic pigments

The major photosynthetic pigment chlorophyll was determined according to (Lichtenthaler and Welburn, 1983). Total carotenoids (B-carotene + xanthophyll) estimation requires simultaneous estimation of chlorophyll a and chlorophyll-b. Carotenoid was estimated by the method given by Lichtenthaler and Welburn (1983). The values obtained are in μgm^{-1} of plant.

2.7. Estimation 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 ^{o} C for 25 min as described by Heath and Packer 1968 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} as per by Heath and Packer (1968) method.

3. Results and Discussion

3.1. Growth in terms of fresh and dry weight

Pesticide exposure can lead to various physiological and biochemical changes within plant cells causing numerous changes in the structure and function. Present study deals with the effect of Endosulfan, on growth and Lipid peroxidation in *Azolla microphylla*. The results of fresh weight and dry weight at the 6th day are graphically depicted in Figure 1. Endosulfan treated plants shows continuous decrease from 25 ppm onward as compare to control. Maximum reduction in fresh weight was observed at 400 ppm (62%). Dry mass decreased in dose dependent manner of endosulfan. Growth measured as increment in dry weight, decreased by 8, 19 and 28% at 25, 50 and 100 ppm respectively. Further dose dependent decrease was observed when concentration of insecticide was increased. The reduced growth in response to higher concentration of melathion may result from reduction in protein and DNA content (Sengupta, *et al*., 1986). Reduction in fresh weight and dry weight was clear after five days of incubation at different concentration in ppm of endosulfan. Kalita (1997) demonstrated that higher concentrations of Melathion inhibit the growth of *Azolla pinnata*. Similar results were obtained by (Raja *et al.*, 2012a) with increasing dose of monocrotophos toxicity on *Azolla microphylla*, fresh weight and dry weight decreases in dose dependent manner.

3.2. Average root number and root length

Average root number decrease by 15%, 30% and 33% at 25ppm, 50ppm and 100ppm. Maximum reduction of 62% was observed at 400 ppm. Roots were physiologically inactive, brown and almost dilapillated. From observations, it is clear that the root length decreases by 10%, 16% and 38% at 25ppm, 50ppm and 100ppm respectively. From 100 ppm onwards, there was continuous decrease in root length, with maximum reduction of 67% at 400ppm. Data depicting average root number and root length are presented in Table 1. Pesticides have adverse effect on root length which results in vulnerable effect on plant growth and development (Luscombe *et al*., 1995; Jaleel, *et al*., 2007). Further the reduction in root length due to Endosulfan could also be explained on the basis of inhibition in the activity of 4-hydroxyphenyl pyruvate deoxygenase (HPPD), and enzyme needed for meristematic tissue as suggested by (Luscombe *et al*., 1995) following insecticide isoxafluote treatment in plant.

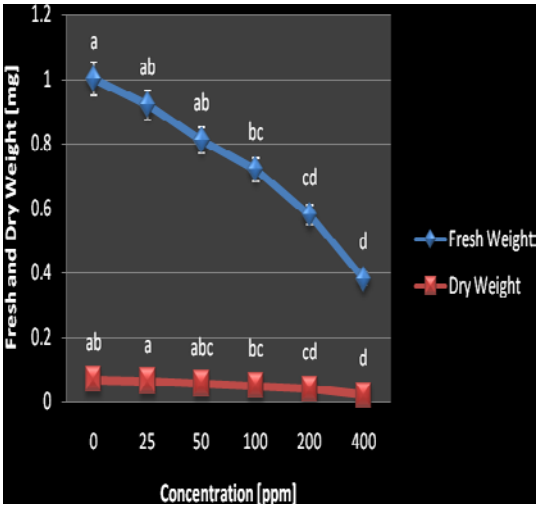

Figure1. Effect of different concentrations of Endosulfan on fresh weight and dry weight of Azolla microphylla. Data are means ± standard error of three replicates. Bars followed by different alphabets show ssignificant difference at P<0.05 significance level according to Duncan's multiple range test.

3.3. Doubling time and relative growth rate

Influence of different concentrations of Endosulfan on doubling time and relative growth rate are depicted in Table 1. Our observations showed that, as concentration of Endosulfan increases, doubling time also increases significantly. The doubling time increases by 20%, 42%, and 53% at 25, 50, and 100 ppm respectively. Thus, Endosulfan has vulnerable effect on plant growth and development. Dry matter accumulation per unit dry weight per unit time is also depicted in Table 1. From the results it may be concluded that, as the concentration of endosulfan increases, relative growth rate decreases. At lower concentration there was little effect but at higher concentration the effect was more vulnerable. The highest decrease of 52% was shown at 400 ppm. Our results are in accordance with prior studies (Arora and Singh, 2003) which showed less biomass and more doubling time in *Azolla* sp. treated with different concentration of sodium chloride. Recent studies on effect of municipal effluents by Arora and Saxena (2005) and monocrotophos toxicity (Chris *et al.*, 2011; Raja *et al.*, 2012b) on *Azolla microphylla* also showed similar trends.

Table1. Effect of different concentrations of Endosulfan on root number, root length, doubling time and relative growth rate of *Azolla microphylla*.

Endosulfan (ppm)	Root Number	Root Length (cm)	Doubling Time (Days)	RGR (µg/g/day)
0	7.3±0.201[a]	2.3±0.157[a]	2.5±o.256[c]	0.0758±0.017[a]
25	6.2±0.438[b]	2.0±0.114[a]	3.0±0.305[c]	0.0704±0.013[a]
	(-15.06)	(-13.04)	(+20.0)	(-7.01)
50	5.1±0.172[c]	1.9±0.265[a]	3.5±0.256[c]	0.0674±0.013[a]
	(-30.13)	(-17.39)	(+42.0)	(-11.1)
100	4.9±0.401[c]	1.4±0.185[b]	3.8±0.401[c]	0.0614±0.019[a]
	(-32.87)	(-39.13)	(+53)	(-19.03)
200	4.2±0.324[c]	1.1±0.151b[c]	5.7±0.305[b]	0.0469±0.009b[c]
	(-42.46)	(-52.17)	(+130)	(-38.12)
400	2.8±0.365[d]	0.76±0.074[c]	8.6±0.649[a]	0.0364±0.008[c]
	(-61.64)	(-66.95)	(+245)	(-52.05)
CD	0.164	0.024	0.055	0.001
SE	1.287	0.663	0.469	1.502

Values in parenthesis are percent increase (+) and decrease (-) with reference to respective controls. Mean ±SE (n=3). Values are significant at P<0.05 (Analysis of variance).

3.4 Total chlorophyll

Total chlorophyll was evaluated on 6th day after endosulfan exposure and observed values are graphically depicted in Figure 2. As the concentration of endosulfan increased, significant reduction in total chlorophyll content was observed as follows: 12%, 20% and 31% at 20, 50 and 100 ppm. Summarized results, showed the detrimental impact of Endosulfan on *Azolla microphylla* and indicates an inverse relation between increased concentration of endosulfan and chlorophyll content. Mustafa *et al.(*2002) suggested that decrease in chlorophyll-a, carotenoid and phyco biliprotein contents might be ascribed to the inhibition of pigment synthesis directly by the insecticide or accelerated degradation of pigments due to increased Active Oxygen Species (AOS) formation at various sites of the photosynthetic electron transport chain during stress. Similar observations were also made by Prasad *et al. (*2004) while studying on the growth, photosynthesis, active oxygen species and antioxidants responses of paddy field cyanobacterium *Plectonema boryanum* to Endosulfan stress.

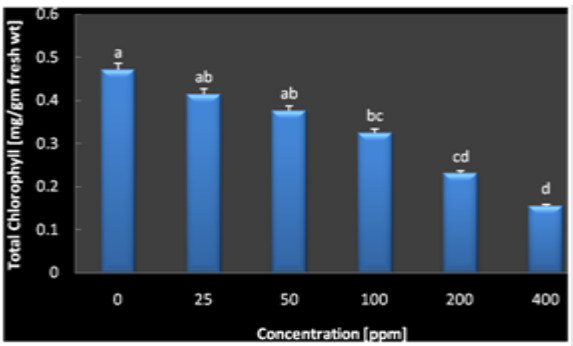

Figure 2. Effect of different concentrations of Endosulfan Total chlorophyll content of *Azolla microphylla*. Data are means ± standard error of three replicates. Bars followed by different letters show significant difference at P<0.05 significance level according to Duncan'smultiple range test.

3.4. Carotenoid content

The accessory photosynthetic pigments carotenoid was analyzed on 6th day and findings are graphically presented in Figure 3. Though it is non-enzymatic antioxidant, its content was adversely affected by the higher concentrations of Endosulfan. The carotenoid content decreased by 6%, 17% and 26% at 25ppm, 50ppm and 100ppm respectively. Prasad *et al*. (2005) showed that under stress conditions, carotenoid pigments are less affected than chlorophyll resulting in a low chlorophyll/carotenoid ratio. Our results are also in accordance with recent findings by Prasad *et al*. (2005), Chris *et al. (*2011), and Raja *et al. (*2012b and 2012c).

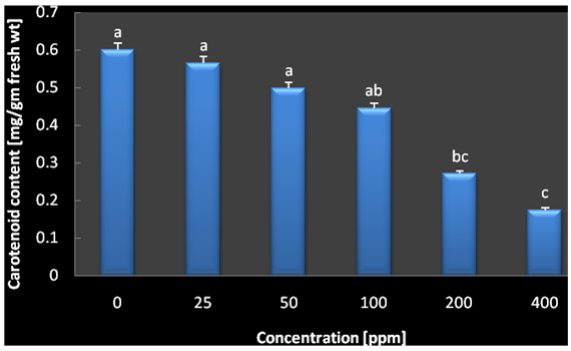

Figure 3. Effect of different concentrations of Endosulfan on Carotenoid content of *Azolla microphylla*. Data are means ± standard error of three replicates. Bars followed by different letters show significant difference at P<0.05 significance level according to Duncan's multiple range test.

3.5. . Lipid peroxidation

Malondialdehyde (MDA) accumulation is considered as important parameters to measure the rate of lipid peroxidation. The observed data of the present investigation are graphically depicted in Figure 4. Significant increment of 24%, 46% and 68% in MDA level was observed at 25ppm, 50ppm, 100ppm respectively. ROS are highly reactive and induces lipid peroxidation, thereby affecting the structural integrity and permeability of cellular membrane (Prasad *et al.*, 2005; Masood *et al.*, 2008).Lipid peroxidation could be a result of light dependent formation of singlet oxygen during stress conditions (Boo and Jung, 1999).

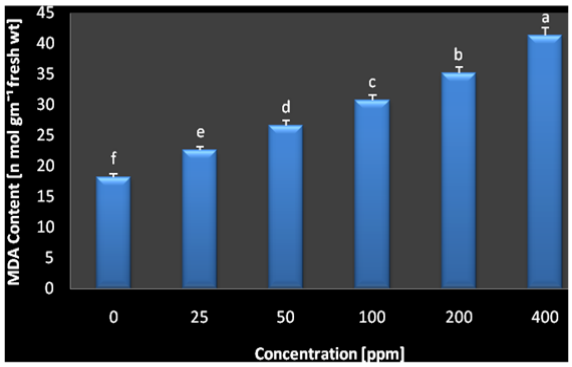

Figure 4. Effect of different concentrations of Endosulfan on Lipid peroxidation of *Azolla microphylla*. Data are means ± standard error of three replicates. Bars followed by different letters show significant difference at P<0.05 significance level according to Duncan's multiple range test

4. Conclusion

The situation of environmental changes mainly originates from anthropogenic activities, which are responsible for adverse plant growth. Since, plants are sessile organisms having limited mechanisms for insecticide avoidance. Thus, they need flexible means for acclimatization to changing environmental conditions. The choice of best insecticide, proper time of application and proper dose is an important consideration for lucrative returns. Hence in order to improve plant growth, this kind of researches may be fruitful in understanding the mechanisms contributing to effect of insecticide on plant system. Therefore, efforts need to be focused towards enrichment of indigenous *Azolla* populations, which are better adapted to the specific niche, through development of multiple inocula preparations on a regional basis. Research programs should be oriented towards agricultural practices including application of biofertilizers which need bio-insecticides thus enhances growth and proliferation of indigenous strains.

Acknowledgements

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

References

Anonymous. 1979. Report of working group on pesticides Industry for the plan (1978-79 to 1983-84) Ministry of Petroleum, chemicals and fertilizers, Government of India. India.

Arora A and Saxena S. 2005.Cultivation of *Azolla microphylla* biomass on secondary treatment Delhi municipal effluents. *J BiomBioen.*, **29**: 60-64.

Arora A and Singh AK. 2003. Comparison of biomass productivity and nitrogen fixing potential of *Azolla* spp. *Biom Bioen.*, **24**: 175 – 178.

Boo YC and Jung J. 1999.Water deficit-induced oxidative stress and antioxidative defenses in rice plants. *Plant Physiol*., **155**: 255– 261.

Chris A, Luxmisha G, Masih J and Abraham G. 2011. Growth, Photosynthetic and antioxidant responses of *Azolla filiculoides* to monocrotophos toxicity. *J Chem Pharm Res.*, **3**: 381-388.

Dobrovoljskiy GV and Grishina LA. 1985. *Ohrana poåv*. Kolos, Moskva. 224.

Forni C, Grilli CM and Gentili S. 1989. Bacteria in the *Azolla*–Anabaena symbiosis. In: Skinner FA, Boddey RM and Fendrik I (Eds.), **Nitrogen Fixation with Non-Legumes**, *Kluwer Academic Publishers, Dordrecht*. pp. 83–88.

Geshian S, Dai-Xing X and Zhi-Hao S. 1980.Salt tolerance of *Azolla filiculoides* and its effect on the growth of paddy in Yinwahaitu. *Zhejiang Nongyekexue*, **1**: 17-20.

Heath RL and Packer L. 1968. Photo-peroxidation in isolated chloroplast I kinetics and stoichiometry of fatty acid peroxidation. *Arch Biochem Biophys.*, **125**: 189-198.

Jaleel CA, Ragupathi G, Paramasivam M and Ranjaram P. 2007. Responses of antioxidant defense system of *Cathranthus roseus* to paclobutrazol treatment under salinity. *Acta Physiol Plant*., **29**: 205-209.

Kalita MC. 1997. Effect of malathion on growth, chlorophyll biosynthesis and total nitrogen accumulation of *Azolla,-Anabaena* symbionts. *J Ecotoxicol Environ Monit*. ,**7**: 59-63.

Kurth E, Cramer GR, Lauchli A and Epstein E. 1986.Effects of NaCl and $CaCl_2$ on cell enlargement and cell production in cotton roots. *Plant Physiol.*, **82**: 1102-1106.

Lichtenthaler HK and Welburn AR. 1983. Determination of total carotenoids and chlorophyll a and b of leaf extracts in different solvents. *Biochem Soc Transac.*,**11**: 591 – 592.

Luscombe BM, Pallett KE, Millet J, Melgorejo J and Vrabel TE. 1995. A novel herbicide for broad leaf and grass weed control in maize and sugarcane. In proceedings of the Brighton Crop Protect, *Corp Weeds*, **2**: 35-42.

Masood A, Zeeshan M and Abraham G, 2008. Response of growth and antioxidant enzymes in *Azolla* plants (*A. pinnata* and *A. filiculoides)* exposed to UV-B. *Acta Biol Hung.*, **59**: 247-257.

Mostafa F I and Helling CS. 2002. Impact of four pesticides on the growth and metabolic activities of two photosynthetic algae. *J Environ Sci Health*, **37**: 417- 444.

Peters GA and Meeks JC. 1989. The *Azolla–Anabaena* symbiosis: basic biology. *Ann Rev Plant Physiol Plant Mol Biol.*, **40**: 193-210.

Peters GA, Ray TB, Mayne BC and Toia RE. 1980. *Azolla – Anabaena* association: Morphological and physiological studies. In: Orne – Johnson, WH (Eds.), **Nitrogen Fixation**, University Park Press. Baltimore, pp 293 – 309.

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

Prasad SM, Kumar D and Zeeshan M. 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, Rathaur P, Ramteke PW and John SA. 2012a. Effect of monocrotophos toxicity on growth and some physiological variables in water fern *Azolla microphylla*. *J Chem Pharm Res.*,**4**: 1340-1348.

Raja W, Rathaur P, Ramteke PW and John SA. 2012b. *Azolla-Anabaena* association and its significance in supportable agriculture. *Hacettepe J Biol Chem.*, **40**: 1–6.

Raja W, Rathaur P, John SA and Ramteke PW. 2012c. *Azolla*: An Aquatic pteridophyte with great potential. *Inter J Res Biol Sci.*, **2(2):** 68-72

Rabinowich HD and Fridovich I. 1985.Cell content of superoxide dismutase and resistance to paraquat in *Chlorella sarabiniana. Planta*, **164** : 524.

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

Seulthorpe CD. 1967. **The Biology of Aquatic Vascular Plants**, Edward Arnold (Pub) Ltd. London , pp 610.

Subudhi BPR and Watanable I. 1981. Differential phosphorus requirements of *Azolla* species and strains in phosphorus limited continuous culture. *Soil Sci Plant Nutr.*, **27**: 37-47.

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