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# Differential Sensitivity of Nitrogen-Fixing, *Azolla Microphylla* to Organochlorine and Organophosphate Insecticide

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**ABSTRACT:** The development of the intensive agriculture in our country between 1960 and 1990 totally over passed the aspect connected with the negative impact of the toxic chemical compounds on the air, water and soil. Using chemical products as nutrients, fertilizers and pesticides, we believe that we attack our safety and we must know the effects of pesticides from these compounds. Application of pesticides in the paddy fields has deleterious effects on non-target organisms including Azolla which are photosynthesizing and nitrogen fixing micro-organisms contributing significantly towards soil fertility and crop yield. Pesticide contamination in the paddy fields has manifested into a serious global environmental concern. Present study was aimed to study the comparative effect of two such pesticides, a well-known species of Azolla, Azolla Microphylla were selected for their stress responses to an Organochlorine insecticide - Endosulfan, and Organophosphate insecticide-Monocrotophos with reference to their growth, Free radicals, Antioxidant enzymes and metabolites. Azolla microphylla strains were adversely affected by the insecticide doses and inhibition was dose dependent. But the highest decrease was seen in case of organochlorine insecticides. Pesticide treatment with increasing doses accelerated the formation of reactive oxygen species progressively, whereby an enhanced Antioxidant enzymes and metabolites were noticed in A. microphylla. On the other hand, increased amount of proline in all the insecticide treated concentrations was indicative of stressed activities of the organisms. In this work the effect of the insecticides on Azolla microphylla resulted in growth inhibition, a decline of physiological and biochemical activities but the highest effect was shown in case of organochlorine insecticide which is commonly used in the rice fields. 

Baron Justus von Liebig, a German scientist in the mid-19th century, showed that nutrients are essential for plant life. Knowing the nutrients required to grow plants is only one aspect of successful crop production. Optimum yield also requires knowing the rate to apply, the method and time of application, the source of nutrients to use, and how the elements are influenced by soil and climatic conditions. The development of the intensive agriculture in our country between 1960 and 1990 totally over passed the aspect connected with the negative impact of the chemical compounds toxic on the plants, air, water and soil. As one of the consequences of pesticide pollution in soil, water and air, plants are contaminated by pesticides. Many authors examined the inhibitory effect of pesticide compounds on growth and the performance of photosynthetic apparatus of plants. The inherent nitrogen fixing capacity of

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indigenous Azolla is one of the most important factors aiding in the process of biological nitrogen fixation in rice field ecosystems (Kaushik 1978).Due to their distinctiveness of atmospheric nitrogen fixation, these organisms form an excellent material for investigation by ecologists, physiologists, biochemists and molecular biologists. 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 (Waseem Raja *et al.,* 2012). Moreover, pesticides are mainly synthetic organic compounds that are introduced into the environment to control selected pests (Mellanby 1978). Although pesticides are indispensable to the modern agricultural practice, the biological use of these pesticides over the years have resulted in problems caused by their interactions with the biological systems in the environment and have deleterious effects on algae by influencing soil algal growth, photosynthesis, nitrogen fixation, biochemical composition, and metabolic activities ( Pankratz *et al 2003*). Pesticides are often used in agriculture to protect human beings from the insect vectors of disease-causing pathogens, to protect crop plants from competition with abundant but unwanted other plants species, and to protect crop plants and livestock from diseases and depredations by fungi, insects, mites, and rodents (Freedman, 1995). However, an undesirable side effect from the use of pesticides is that they enter into freshwater ecosystems by spray, drift, leaching, run-off, or accidental spills (Van der Werf, 1996). It is therefore important to assess the adverse effects the pesticides may have on non-target organisms in aquatic ecosystems (Peterson et al., 1994).

At present many progressive farmers and non-governmental organizations are employing *Azolla* as an invaluable input in agriculture and some of them are actively engaged in its popularization as biofertilizer, green manure, poultry feed and fodder. However, certain key scientific issues need to be addressed to make the system more compatible with the present day need and demand. *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 (Wagner 1973) because it is economically important as an animal feed, medicine (Wagner 1973), hydrogen fuel, biogas producer, weed Controller as well as a biofertilizer. *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. (Waseem Raja *et al.,* 2011)Although, numbers of studies have been carried out regarding physiological and biochemical effects of pesticide individually on cyanobacteria, algae and higher plants and animals, however, their comperative effects are still poorly known. The major contribution of *Azolla* is in nitrogen economy in paddy field, and its sensitivity towards pesticide and 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 other properties of *Azolla* when the two stresses are imposed. Therefore, an attempt was made to investigate the effect of Endosulfan and monocrotophos on Biomass, free radicals and antioxidant metabolism of *Azolla microphylla*.

## MATERIALS AND METHOIDS

*Plant material Organism and growth conditions: Azolla microphylla* were collected locally from paddy fields near Allahabad. Plants were washed and cleaned of contamination organisms. The plants were surface sterilized with a solution of mercuric chloride (0.1% for 30 min) and were dipped immediately into a large volume of sterile distilled water. Plants were then transferred

into dishes containing combined-N free 2/5 strength sterile Hoagland's medium (Peters and Mayne, 1980) and 0.04mM ferrous ion as Fe-EDTA, pH 5.6.The cultures were grown at 26 $^{o}$ C under a 16:8 (light: dark) photoperiod with light from a combination of incandescent and cool white light fluorescent lamps at a photon fluence rate of 95 µmol $m^{-2}s^{-1}$. Fronds were routinely transferred into fresh medium twice a week to maintain plants in a sterile state. Log phase plants were used for experiments.

*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$^{0}$C for 24 h to a constant weight.

*Estimation of superoxide radical:* Superoxide ($O_2^-$) 10 n was measured as described by (Elstner and Heupel 1976) by monitoring the nitrite formation from hydroxylamine in the presence of $O_2^-$ *Azolla* fronds of 0.2 gm were weighed and crushed in 2 ml of 65 mM potassium phosphate suffer (Ph 7.8) and centrifuged at 5000 rpm for 10 minutes. The reaction mixture contained 0.9 ml of 65 mM phosphate buffer, 0.1 ml of 10 mM hydroxylamine hydrochloride and 1 ml of the supernatant. After incubation of 25$^{0}$C for 20 min, 17 mM sulfanilamide and 7 mM α-nephtylamine were added to the incubation mixture ethyl ether in some volume was added and centrifuged once again at 1500 rpm for 5 min. the absorbance in the aquous solution was read at 530 nm.

*Estimation of Lipid peroxidation :* For the determination of lipid peroxidation value in *Azolla microphylla* (Heath and Packer 1968) method was followed. 0.3 gm *Azolla* frond was cursched in 5 ml of 50 ml phosphate buffer and then centrifuged. To the 1 ml supernant 4 ml TCA-TBA solution was added (24 gm TCA + 0.6 gm TBA + 120 ml D.W). Than the reaction mixture was heated on 90$^{0}$C water bath for 20 minutes then quickly cooled in ice bath.Then again centrifuged for 10 minutes in high speed centrifuge. Then the absorbance was read at 532 and 600 nm.

*Estimation of Electrolyte leakage*: It is indispensable to any biological system to have electrolytes dissolve in their cell fluid and it is also essential to the cells to protect them any leakage. But when a cell experience any sort of stress the protective membrane lasts its capacity results in leakage of electrolytes, the electrolyte leakage has evaluated according to (Dionisio Sese and Tobita 1998) procedure was followed.

*Estimation of Proline*: Proline content in treated and untreated fronds was estimated according to the method of (Bates *et al.,* 1973). 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.

*Estimation of Ascorbic acid :* 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).

*Estimation of flavonoids*: The flavonoids were determined according to the method of (Mirecki and Teramura 1984). The fronds (0.2gm) were weighed and extracted in acidified methanol: HCl (99:1) the homogenate was incubated at $4^0$C for 24 hrs. The homogenate was centrifuged for 10 minutes and absorbance of extract was measured at 530 nm.

*Estimation of Superoxide dismutase (E.C. 1.15.1.1):* Suproxide dismutase (SOD) activity was assayed according to method of (Giannopolitis and

Ries 1977). SOD catalyze the dismulation of suproxide radical ($O_2$) to hydrogen .0.2 gm of *Azolla* was weighed and crushed in 100 mm phosphate buffer. The homogenate was centrifuged for 15 minutes at 800 rpm. The supernant was used as the enzyme source. The reaction mixture (3 ml) contained 63rm P-nitroblue, tetrozolium chloride, 0.05 m $Na_2 CO_3$ (Ph10.2); 13 mm L-methlonine; 1.3 µm riboflavin and 0.1 ml of crude enzyme mixture. The reaction was carried out at $25^0$C under fluorescent lamp. The rate of reaction was measured by the difference in increase in absorbance at 560 nm in the presence or absence of enzyme. The unit of suproxide dismutase activity was defined as the amount of enzyme which caused at 50% inhibition of the reaction observed in absence of enzyme. For the blank the reaction was run in dark.

*Estimation of Peroxidase activity (E.C 1.11.1.7):* Peroxidiase activity (POD) was assayed by the method of different (Gahagen ,*et al.,* 1976). 0.2 gm treated and untreated fronds were weight and crushed in 2 ml of 100 mm phosphate buffer. The homogenate was centrifuged at 8000 rpm for 15 minutes. The supernant contained the enzyme extract. The reaction mixture (3 ml) was made up of 1 ml 25 mM $H_2O_2$, 1 ml, 100 mM pyrogallol prepared in distilled water and 1 ml enzyme extract. After mixing with the reaction mixture change in optical density was recorded at 430 nm for 2-3 minute.

*Estimation of Catalase activity (E.C 1.11.1.6):* Catalase (CAT)activity was determined polarographically by the method of (Egashira *et al.,* 1989) *Azolla* fronds were weighed and crushed in 50 mM phosphate buffer and centrifuged at 8000 rpm for 10 minutes. In each sample catalase activity was determined by following $O_2$ evolution for 1 min after the addition of 1 ml phosphate buffer 50 mM containing 50mM $H_2 O_2$. To this 1 ml extract was added then absorbance was read at 240 nm for 1 – 2 minutes.

*Statistical analysis:* All the data obtained of *Azolla microphylla* in terms of growth, free radicals, antioxidant enzymes and metabolites in response to different levels of endosulfan and Monocrotophos 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.

## RESULT AND DISCUSSION

An attempt was made in this part to study the effect of various concentrations of endosulfan and monocrotophos on Az0lla Microphylla. Final results are represented in the histogramic figures. Insecticides exposure can lead to various physiological and biochemical changes within plant cells causing numerous changes in the cell structure and function. The growth (dry weight) of *Azolla microphylla* was drastically inhibited at 400 ppm. But the highest reduction of growth was seen in case of endosulfan at 400 ppm in comparison to monocrotophos. at 800 ppm after 6 days of inoculation. The data on dry weight is graphically depicted in fig.1. Endosulfan and monocrotophos treated plants shows

reduction in dry weight on increasing concentration as compare to control. Dry weight was reduced by 7%, 13% and 25% at 25ppm, 50ppm and 100ppm in case of endosulfan and by 6%, 10% and 20% in case of monocrotophos at same concentration. Further there was gradual decrease in dry weight as concentration of pesticide increases. In this study also endosulfan has more deterimental effect than monocrotophos.. Reduction in fresh weight and dry weight was clear after six days of incubation at different concentration in ppm of endosulfan and monocrotophos. Kalita (1997), has demonstrated that high concentration of melathion inhibit the growth of *Azolla pinnata*. and recently by (Waseem Raja *et al* 2012). Has shown the reduction of growth by monocrotophos toxicity. The reduction in dry weight and fresh weight by endosulfan and monocrotophos 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).

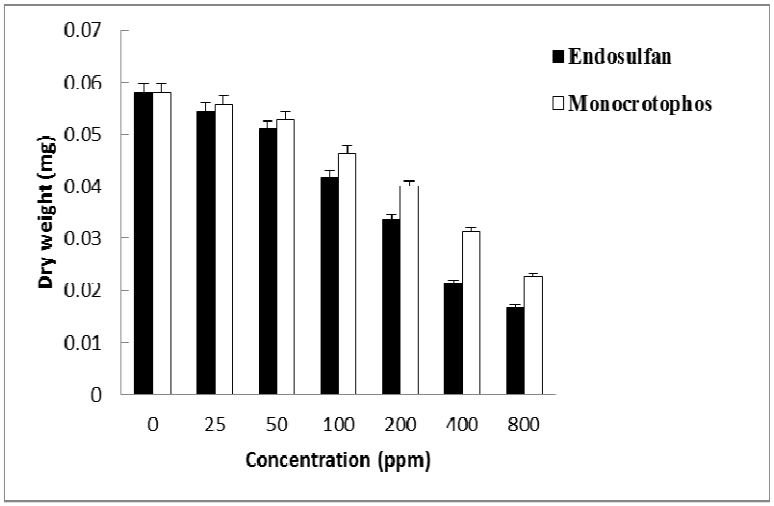


**Fig.1:** Effect of Endosulfan and Monocrotophos toxicity on Dry Weight of *Azolla microphylla*. Data are means ± standard error of two independent experiments and all the values are significant at P<0.05

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The result of superoxide radicle analysis are graphically depicted in the fig.2.The cellular level of superoxide was analyzed in *Azolla microphylla* treated with endosulfan and monocrotophos. The $O_2^-$ level increases by 6%, 15% and 24% at 25ppm, 50ppm and 100ppm and by 2%, 8%, and 13% at same concentrations in case of endosulfan and monocrotophos respectively. Further there was a gradual increase in $O_2^-$ as concentration increase. A significantly higher levels of accumulation was noticed in endosulfan treated plants as it increase $O_2^-$ level by 90% and 61% at 800ppm in endosulfan and monocrotophos respectively. In this study increased free radical generation was found in *Azolla microphylla* under endosulfan and monocrotophos stress as indicating in the malonedialdehyde production which is similar to the effect of pesticide and heavy metal stress in higher plants. (Alia and Pardha Saradhi, 1991., Somashekaraiah *et al*., 1992. Mahalingam and Fedoroff, 2003., Jaleel, *et al*., 2008).

Malondialdehyde (MDA) accumulation is considered as an important parameters to measure the rate of lipid peroxidation. The observed data of the present investigation are graphically depicted in fig.3, a

significant increase in MDA level was observed in both endosulfan and monocrotophos treated plants as compare to control.The MDA content of endosulfan treated plant increased by 44%, 73% and 105% at 25ppm, 50ppm, 100ppm however in monocrotophos treated plants, the increase in MDA content increase by 14%, 45% and 88% at same concentration. Further there was a gradual increase in MDA concentration as the concentration of pesticide increase. The study reveals that endosulfan exert more stress on *Azolla microphylla* and increase MDA content more as compare to monocrotophos. During the stress conditions, the polyunsaturated fatty acids (PUSFAs) of the membrane were peroxidised due to ETS dependent formation of reactive oxygen species and produced MDA (Boo and Jung, 1999). (Fig.3)shows that MDA content was highest in highest doses of pesticides indicating a higher degree of lipid per oxidation under pesticide stress.

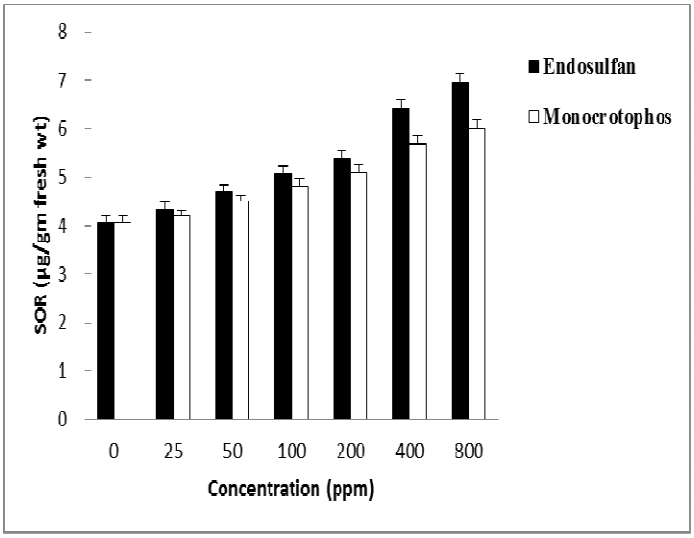


**Fig.2:** Effect of Endosulfan and Monocrotophos toxicity on Superoxide Radicle content of *Azolla microphylla*. Data are means ± standard error of two independent experiments and all the values are significant at P<0.05.

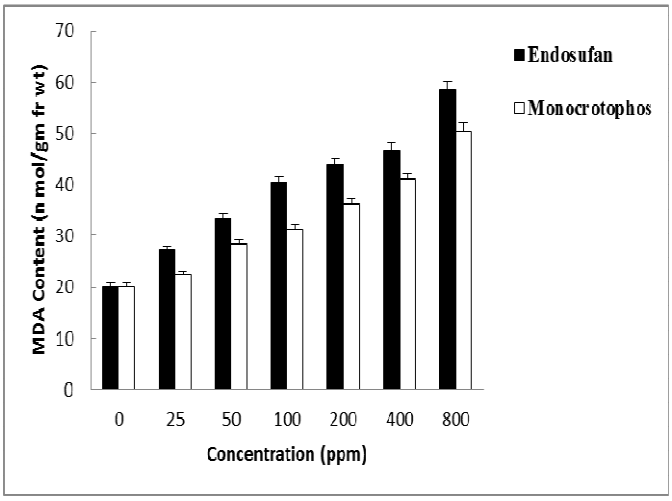


**Fig.3**: Effect of Endosulfan and Monocrotophos toxicity on Lipid peroxidation of *Azolla microphylla*. Data are means ± standard error of two independent experiments and all the values are significant at P<0.05.

Membrane stability is the widely used criterion to assess the damage due to pesticide induced stress. The percentage of electrolyte leakage is graphically depicted fig.4. Electrolyte leakage was found to increase significantly with increasing concentration of endosulfan and monocrotophos maximum leakage was observed at 800ppm. However, the percentage of electrolyte leakage was higher in endosulfan treated plants (1 to 4 folds) as compared to monocrotophos (1 to 3 folds). Thus enhanced lipid peroxidation lead to increased. Electrolyte leakage due to cell membrane damage (Rai, *et al*., 1998). It was presumed that the extinct of membrane damage was so severe in such species where electrolyte leakage was highest (in percentage) under stress condition. Low level of electrolyte leakage and MDA content at low doses of pesticides (in ppm) may be one of the reasons for the observed tolerance of *Azolla microphylla*.

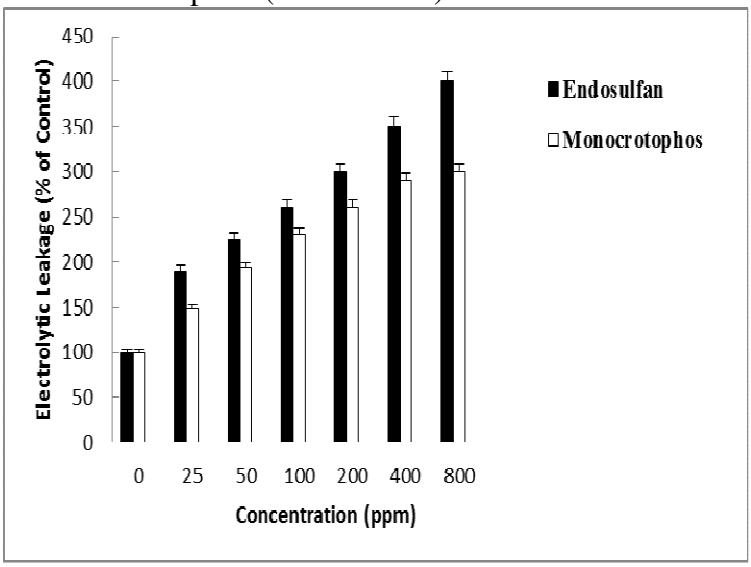


**Fig.4:** Effect of endosulfan and monocrotophos toxicity on Electrolytic leakage (Electrolyte leakage in untreated control was 12%). Data are means ± S.E. of two independent experiments and all values are significant at P<0.05.

Proline is an important non-enzymatic antioxidant compound and accumulation of proline is considered as a physiological response due to many environmental stresses. Proline was analyzed at the 6$^{th}$ days in plant treated with different concentration of endosulfan and monocrotophos and experimental findings are graphically arranged in fig.5. As the concentration of endosulfan and monocrotohpos increase, there is a significant and progressive increase in proline accumulation as compare to control. The cellular proline content of endosulfan treated plants was increased upto 309% as compared to an increase of 247% in monocrotophos treated plants at 800 ppm. The present study help to percieve that the higher concentration of endosulfan is exerting more stress on *Azolla microphylla* and as a result the proline accumulation is increased more in endosulfan as compare to monocrotophos treated plants. The aromatic amino acid proline act as a free radical scavenger to overcome the oxidative stress by preventing the membrane damage and protein denaturation (Reddy, *et al*., 2004). Hyper accumulation of proline in plants can be co-related with detoxification against any stress induced oxidative stress (Foyer, *et al*.,1994).

The data on ascorbic acid content is graphically depicted in Fig.6. Ascorbic acid content increases as the concentration of

pesticides increases. The ascorbic acid content increase by 10%, 90%, 30% and 42% in case of endosulfan at 25ppm, 50ppm, 100ppm and 200ppm and by 5%, 12%, 23% and 31% in case mococrotophas at same concentration. Beyond 200ppm there was a gradual decrease in both the pesticide. This study reveals that ascorbic acid increases up to certain limit and then decreases. 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 hydroperoxidases (Reddy *et al*. 2004). Triazole-treatment increased the ascorbic acid content in tomato seedlings (Senaratna, *et al* .,1988)

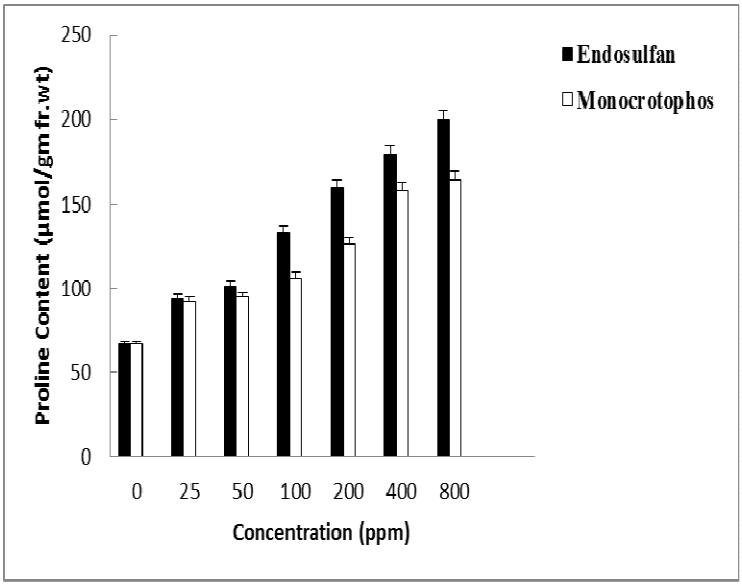


**Fig.5:** Effect of Endosulfan and Monocrotophos toxicity on Proline content of *Azolla microphylla*. Data are means ± standard error of two independent experiments and all the values are significant at P<0.05.

Increase or decrease in the amount of flavonoids may be favourable to an organisms in an environment like pesticide exposure. Flavonoid content was analysed at the 6$^{th}$ day in plants treated with different concentration of endosulfan and monocrotophos and the observation are arranged graphically in fig.7.From the observed data it is evident that as the concentration of endosulfan and monocrotophos increase there is gradual increase in flavonoid content by 10%, 21% and 35% at 25ppm, 50ppm and 100ppm and by 6%, 17% and 26% at same concentration. Further there was gradual decrease in flavonoid content as the concentration of pesticides increases so flavonoid content increases up to certain limit of concentration. Since these also act as antioxidant metabolite. Bores, *et al.,* (1990) reported effective free radical capacity of flavonoids.. Boling *et al*. (2001) observed increased tolerance to high light stress in pea and bean plants due to increase in flavonoid content. Brawn (1991) reported that the epidermal layer of oat seedling accumulated large amount of UV abserving pigment flavonoid and anthocyanin during early development which gave a better protection against UV-B.

The conversion of superoxide anion to $H_2O_2$ facilitated by the enzyme superoxide dismutase. In the current investigation the data observed for SOD is graphically depicted in fig.8. As the endosulfan and monocrotophos stress increases the SOD concentration also increase progressively by

8%, 88%, 31% and 45% at 25ppm, 50ppm, 100ppm and 200ppm and by 5%, 13%, 25% and 33% at same concentration respectively. But beyond 200ppm there was a gradual decrease in SOD concentration by both the pesticide as compared respectively as compared to 200ppm. This study reveals that effect on SOD concentration is in dose dependent manner. Both the pesticides were found to enhance the activity of superoxide dismutase which catalises the disproportunate of $O_2^-$ to $O_2$ and $H_2O_2$ and is considered often to be the first line of defence against reactive oxygen species Superoxide dismutase activity is also considered to be an indirect measure to $O_2^-$ production and hence the extent of oxidative damage. Superoxide dismutase is prominent biomarker of defense against oxidative stress. The increase superoxide dismutase activity after pesticide treatment can be explained in the light of the report of Rabinowich and Fridovich (1985) and Li *et al*.(2005

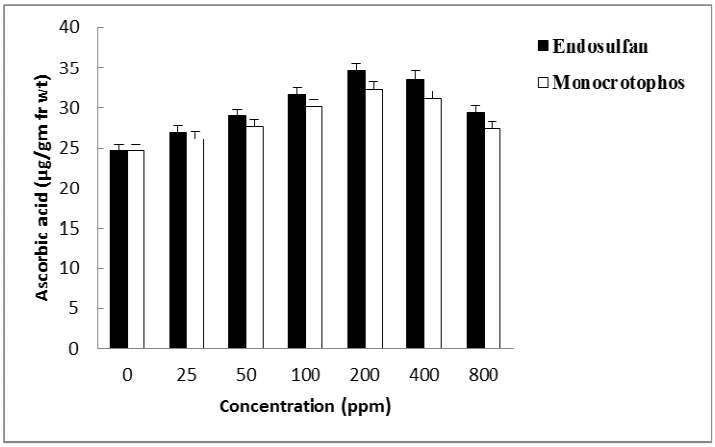


**Fig.6:** Effect of Endosulfan and Monocrotophos toxicity on Ascorbic acid content of *Azolla microphylla*. Data are means ± standard error of two independent experiments and all the values are significant at P<0.05.

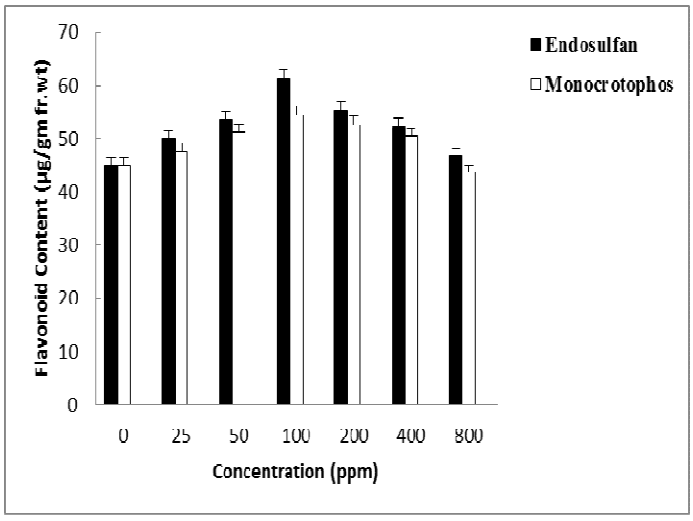


**Fig.7:** Effect of Endosulfan and Monocrotophos toxicity on Flavonoid Content of *Azolla microphylla*. Data are means ± standard error of two independent experiments and all the values are significant at P<0.05

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The result of this enzymes analyzes are graphically depicted in fig.9. The peroxidase activity increases progressively by 15%, 30% and 55% at 25ppm, 50ppm and

100ppm and by 10%, 23% and 42% in case of monocrotophos at same concentration. Beyond 100ppm there was a gradual decrease in peroxidase activity as concentration increases so effect on POD content is in dose dependent manner. The peroxidase activity decreases by 5% in compression to control in case of endosulfan. So effect of endosulfan was more determental than mnocrotophos. The treatment of endosulfan and monocrotophos might have resulted into the formation of (ROS) reactive oxygen species. Our result is in agreement with the results of Halliwell and Gutterdg (1994), Die *et al*. (1997) and Die *et al.(* 2006) as they have worked on different stresses.

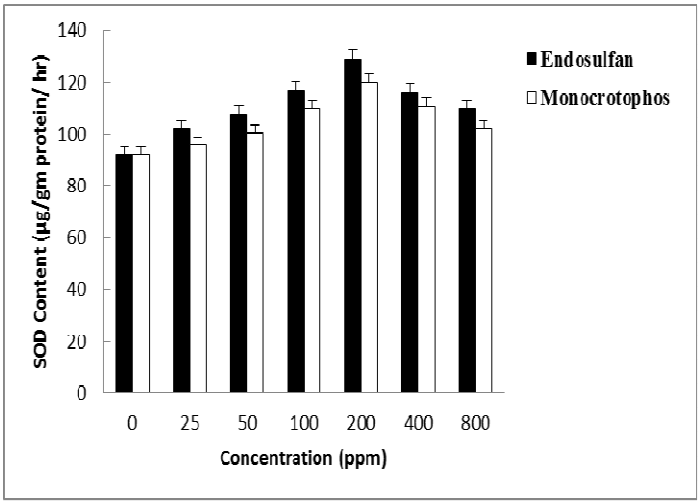


**Fig.8:** Effect of Endosulfan and Monocrotophos toxicity on Superoxide Dismutase content of *Azolla microphylla*. Data are means ± standard error of two independent experiments and all the values are significant at P<0.05.

The data on catalase activity is graphically depicted in fig.10. Catalase activity increase due to increase in the concentration of endosulfan and monocrotophos but the catalase increase up to certain concentration, then it decreases. The catalase activity increases by 3%, 10% and 17% at 25ppm, 50ppm and 100ppm in endosulfan and by 0%, 7% and 12% at same concentration in case of monocrotophos. In both the cases catalase decrease beyond 100ppm as the concentration of endosulfan and monocrotophos increases. Reduction was maximum at 800ppm as catalase activity reduced up to 5% and 10% respectively in comparison to control. A significant rise of catalase activity of *Azolla microphylla* following pesticide treatment suggest that experimental organism synthesize a good amount of catalase to scavenge the excess of $O_2^-$ and $H_2O_2$. The catalase activity under pesticide treatment in our findings was also supported by Zeeshan (2002) and Prasad *et al*.(2005)

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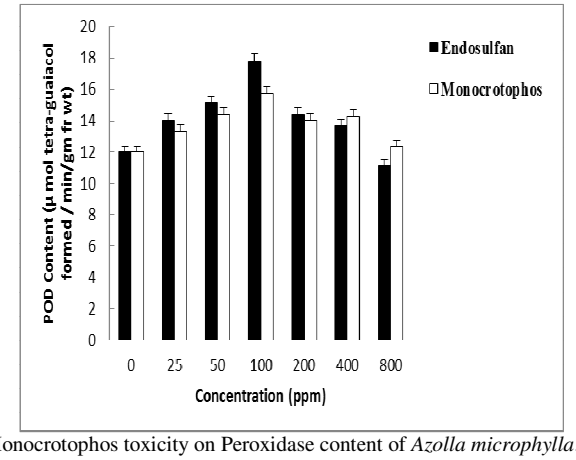


**Fig.9:** Effect of Endosulfan and Monocrotophos toxicity on Peroxidase content of *Azolla microphylla*. Data are means ± standard error of two independent experiments and all the values are significant at P<0.05.

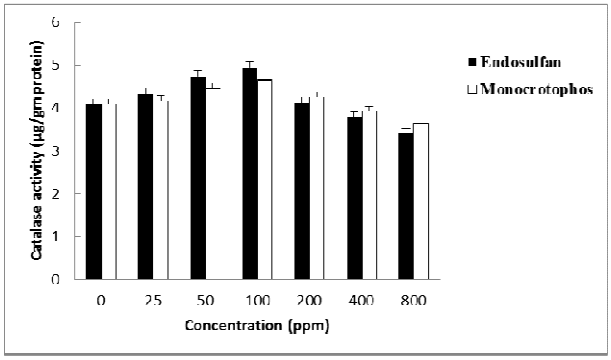


**Fig.10**: Effect of Endosulfan and Monocrotophos toxicity on Catalase activity of *Azolla microphylla*. Data are means ± standard error of two independent experiments and all the values are significant at P<0.05.

*Conclusion:* In the present study the deleterious effect of endosulfan (chlorinated insecticide) was found to be more than monocrotophos (organophosphorus insecticide) with respect to overall growth of *Azolla microphylla*. The *Azolla microphylla* although shows reduction in growth it is quite good in resisting stress caused by endosulfan and monocrotophos. The reactive oxygen species and activity of antioxidants have enhanced the resistance capacity of *Azolla microphylla* to insecticides (endosulfan and monocrotophos). The superoxide dismutase, catalase and peroxidase activities are stimulated by insecticide treatment so that these can be used as sensitive biomarker for early warning of insecticide pollution. The protective action of proline was enhanced at all treatments 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. Under the present study the role of antioxidants in increasing the resistance of *Azolla microphylla* to endosulfan and monocrotophos is evident, however more study to conform our findings at molecular level is suggested.

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