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Research Article

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Adverse Effects of Monocrotophos Toxicity on Growth and Some Physiological Variables in Water Fern *Azolla microphylla.*

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ABSTRACT

The response of the rice field biofertilizer Azolla microphylla to different concentrations of the insecticide monocrotophos (25 – 400 ppm) was premeditated on 5th day after insecticide exposure with respect to growth and physiology under laboratory conditions. Insecticide exposure resulted in the reduction of relative growth rate and biomass accumulation at all the concentrations. An increase in the doubling time was also noticed which is an indication of delayed growth. Along with inhibition in growth insecticide application also resulted in reduced chl. a, chl. b, total chlorophyll and carotenoid contents of A. microphylla. Reduction in the heterocyst frequency was also observed under similar conditions. Protein content was also reduced on increasing insecticide exposure. The preliminary studies on the effect of the insecticide show adverse effect on the growth and physiology of A. microphylla which is a non-target organism in the rice fields. 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: *Monocrotophos, Chlorophyll, Carotenoid, Heterocyst frequency, doubling time.*

INTRODUCTION

Biofertilizers have acquired an increasing importance in recent years. They offer an economically attractive and ecologically sound means of reducing external input and improving the quality and quantity of internal resources. The name *Azolla* is derived from Greek word *azo* (to dry) and allyo (to kill) meaning that plant dies when it dries. *Azolla* is used as a biofertilizer and produces around 300 tones of green bio-hectare per year under normal subtropical climate which is comparable to 800 kg of nitrogen (1800 kg of urea). 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 benefits of *Azolla* application in the rice field are the following:

- Basal application of green manure @ 10-12 tones/hectare increases soil nitrogen by 50-60 kg/ha and reduces 30-35 kg of nitrogenous fertilizer requirement of rice crop.
- Under low land conditions a thick mat does not allow weeds to grow in rice field thus *Azolla* suppress the weed growth and creates congenial condition for rice production .*Azolla* reduces evaporation from water surface increases water use efficiency in rice.

- It may also be used for the production of hydrogen fuel, the production of biogas, the control of weeds, the control of mosquitoes, and the reduction of ammonia volatilization that accompanies the application of chemical nitrogen fertilizer

The application of insecticides, a group of pesticides in crop fields for selective control of pests has led to serious environmental contamination resulting in greater loss in crop productivity and growth of many beneficial microorganisms [1].The removal of these insecticides from soil and aquatic system has become a difficult problem [2].The indiscriminately applying pesticides in pretext of controlling insect pests like yellow stem borer, leaf roller, blue beetle, caterpillar, aphids etc. on paddy crop. Some of the pesticides applied on paddy crop expected to have adverse effect on growth of *Azolla* [3]. Though pesticides are applied only on the paddy crop to combat the pests and diseases, the floating fern in paddy field became the non-target victim of such applied pesticides. Detrimental effect of pesticides on the growth of aquatic macrophytes are generally known [4], but still the knowledge of the indirect effect of applied pesticides like monocrotophos on soil micro and macro flora is fragmentally and partly outdated [5]. The influence of pesticides on soil and aquatic algae including *Azolla* has been a growing concern [6].

When such insecticides (monocrotophos) are applied indiscriminately beyond certain limit. It adversely affects both plants and animals physiologically and biochemically. Pesticides (herbicides, fungicides and insecticides) adversely affect all aspects of primary and secondary metabolism in crops and animals when applied in agricultural field [7, 35]. Physiological expressions like wilting, drying, root damage could be recognized easily but it is not the case with biochemical changes which could be noticed only by conscious observations and experimental findings Nitrogen metabolism is also known to be affected by this stress [7]. A number of studies have indicated that various degrees of oxidative cellular damage in plants, exposed to abiotic stress are controlled by the capacity of oxidative-defence system [8, 9, and 10]. Plant exposed to stress shows an increased accumulation of proline [11, 12]. Under stress conditions plant stimulate the formation of reactive oxygen species (ROS) at various sites of respiratory and photosynthetic electron transport chain [13]. The studies exposed to stress shows reduced growth and reduction in protein and DNA content [14]. Studies also showed that organophosphorus insecticides interfere with carbohydrate accumulation in paddy seeds [15, 36]. Monocrotophos is an organophosphate insecticide which is systematic in action, penetrates plant tissue rapidly. It controls the broad spectrum of pests including sticking, chewing, boring and spider mites. It has highly fumigant action. Since *Azolla microphylla* is an important plant from an agronomic point of view due to its ability to fix atmospheric nitrogen and pesticides like monocrotophos are also used to check the pest of paddy like leaf hoppers, white flies spider mites etc. so definitely these pesticides influence the growth, biomass property of *Azolla* .*Azolla* occupy an important position in food web, and loss of Azolla biomass may seriously affect soil fertility through nitrogen and carbon fixation. They are also important as bioremediation agent in cleaning up the environment and thereby reducing pollution load, therefore it becomes much imperative to investigate the adverse effect of Monocrotophos on growth and some physiological variables in *Azolla microphylla.*

EXPERIMENTAL SECTION

Organism and growth conditions

Azolla microphylla was isolated from rice fields near Allahabad and was maintained in the tubs of 35 cm diameter and 12 cm depth. Each tub was filled with 3.5 kg sterilized rice field soil and mixed with single super phosphate of about 300 mg and water is allowed to stand up to 4 cm above the soil in the tub were put in open air in the field of biological sciences. Each tub was inoculated with 5 gm. *Azolla* fronds. After 12 days a thick mass of *Azolla* covered the entire water surface of the tub. From these tubs *Azolla* fronds were taken out to conduct the lab studies. For the growth and pesticide treatment solution of *Azolla microphylla* under laboratory conditions modified nitrogen free nutrient medium has been used and its composition and preparation were according to Peters and Mayne [16].

Estimation of dry weight (gm)

Dry weight was estimated by method of Robinson et al. [17]. The *Azolla* fronds were blotted on tissue paper and immediately weighed, each measurement was done in two replicates. To get the dry weight, fronds were placed in petridsihes for 24 hr at 60^0C temperature. Again the dried samples were weighted after deducting the plate weight and readings were recorded in grams.

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 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. To calculate the RGR of *Azolla* Subudhi and Watanable [18] protocol was followed, which was as follows:-

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

DT = doubling time.

Estimation of Photosynthetic pigments

The major photosynthetic pigment chlorophyll was determined according to Litchenthaler and Welburn method [19]. Total carotenoids (B-carotene + xanthophyll) estimation requires simultaneous estimation of chlorophyll a and chlorophyll-b carotenoid was estimated by the method given by Litchenthaler and Welburn method [19]. The values obtained are in $\mu g m^{-1}$ of plant.

Estimation of Protein and Heterocyst frequency

Protein was estimated by method given by Lowery et al. method [20]. The protein content was determined by the standard curve prepared out of the Bovine serum albumin protein. The number of heterocysts per hundred vegetative cells is referred to as heterocyst frequency and determined by the method of Fogg [21].

Statistical analysis

Duncan's new multiple range test ($P<0.05$ and $P< 0.01$) was used for data statistics of each treatment and indicated statistical significance. The results presented are the means of three replicates.

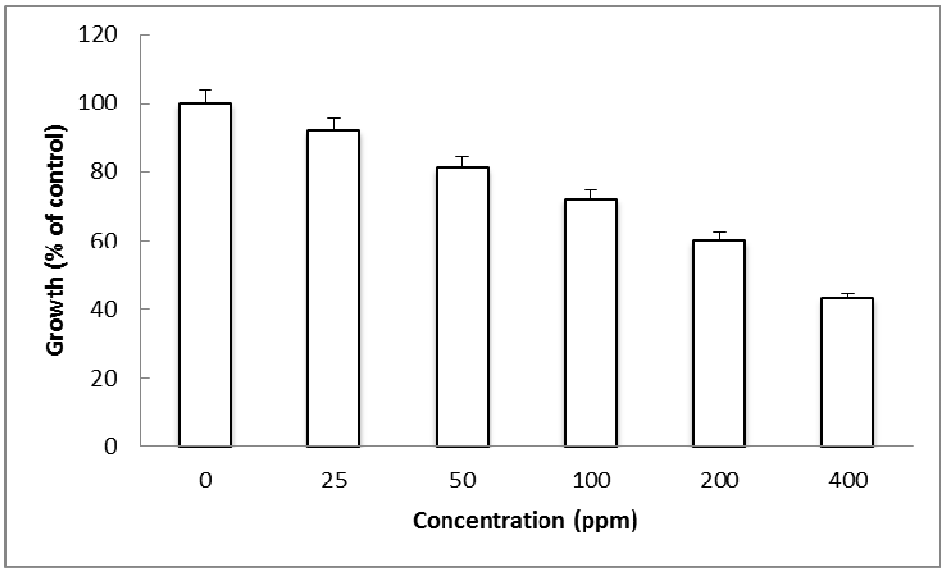

Fig.1: Effect of monocrotophos on dry weight (Dry Weight in untreated control was 0.089±0.1gm). All values are mean ± S.E. of three replicates. Values are significantly different at P<0.01 and *P<0.05 from control.

RESULTS

Growth

The growth of Azolla was studied on 5th day after insecticide exposure and results are presented in fig 1.growth measured as increment in dry weight decreased by 9, 21 and 31% at 25, 50 and 100 ppm respectively. Further dose dependent decrease was observed when concentration of insecticide was increased.

Doubling Time

The 5th day after insecticide exposure and data on doubling time of *Azolla microphylla* under the influence of insecticide at different level of concentration are graphically depicted in Fig.2. From the figure it is clear that as the concentration of chemicals increases the doubling time also increases significantly. The doubling time increases about 3 folds. So, pesticide has adverse effect which results in vulnerable effect on plant growth and development.

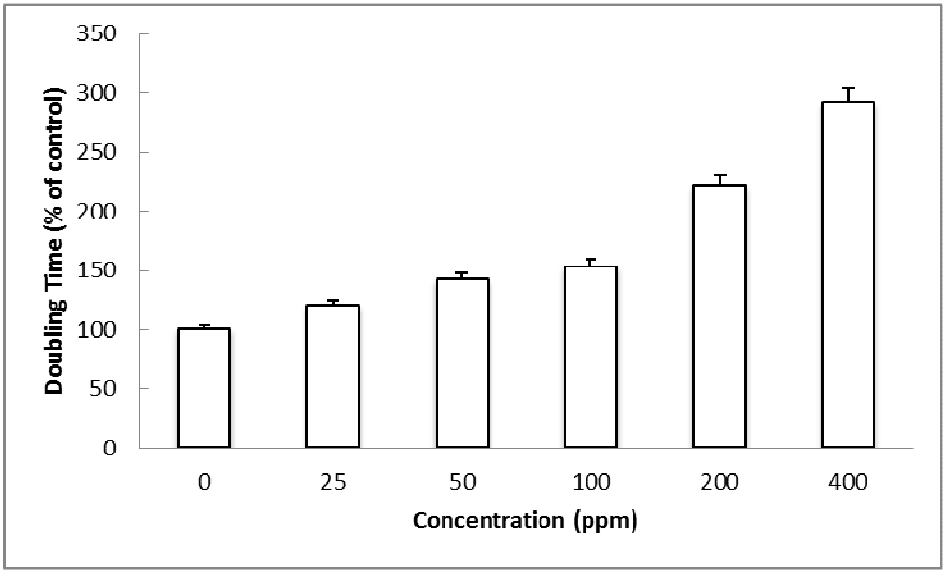

Fig. 2: Effect of monocrotophos on doubling time (Doubling time in untreated control was 2.70 days). All values are mean ± S.E. of three replicates. Values are significantly different at P<0.01 and *P<0.05 from control.

Relative growth rate

Dry matter accumulation per unit dry weight per unit time is graphically depicted in Fig.3. From the figure it is clear that as the concentration of chemical increases relative growth decreases. At lower concentration there was little effect but at higher concentration the effect was adverse. The highest decrease was shown at 400 ppm which was about 54%.

Photosynthetic Pigments

Chlorophyll a, b and total chlorophyll

Photosynthetic pigments, chlorophyll-a, chlorophyll-b and total chlorophyll was evaluated on 5th day after insecticide exposure, and their observed values are graphically depicted in fig.4. As the concentration increased, progressive decrease was recorded in the chlorophyll-a, chlorophyll- b and total chlorophyll. When different concentration of monocrotophos was applied there was a significant decrease in chlorophyll-content. In this study it was observed that chlorophyll- a content decreased by 16%, 26% and 30% beyond 100ppm there was further decrease in chlorophyll-a and same results were obtained in case of chlorophyll-b as chlorophyll-b content decreases by 10%, 15% and 38%. Further there was decrease in chlorophyll-b as concentration of pesticide increases. Thus there was significant decrease in total chlorophyll content as pesticides decreased the total chlorophyll content by 14%, 22% and 31% Further there was a gradual decrease in total chlorophyll as the concentration of pesticide increases. Thus summing up results, effect of monocrotophos on *Azolla microphylla* is detrimental and there was an inverse relation between concentration of pesticide and chlorophyll content.

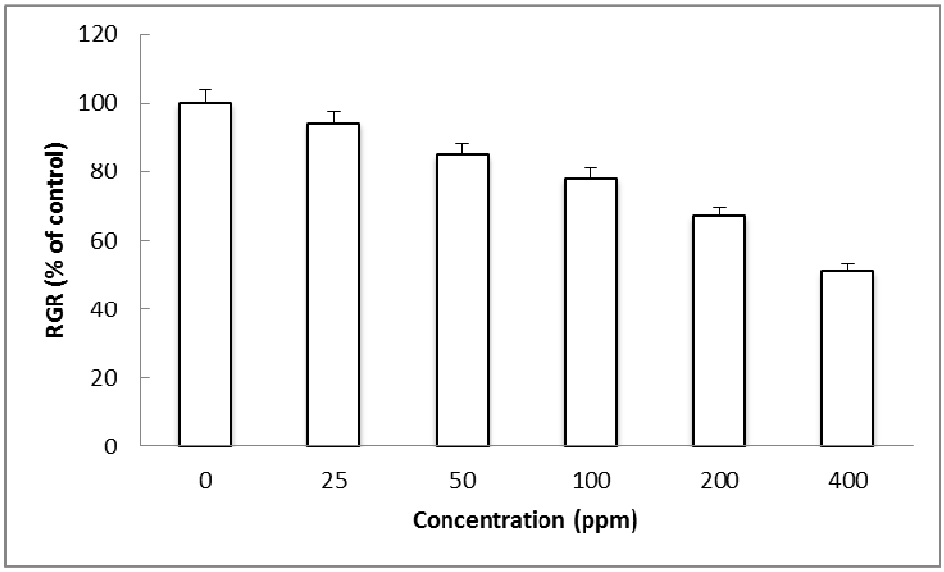

Fig. 3: Effect of monocrotophos on relative growth rate (relative growth rate in untreated control was (0.257±0.1 µg/g/day). All values are mean ± S.E. of three replicates. Values are significantly different at P<0.01 and *P<0.05 from control.

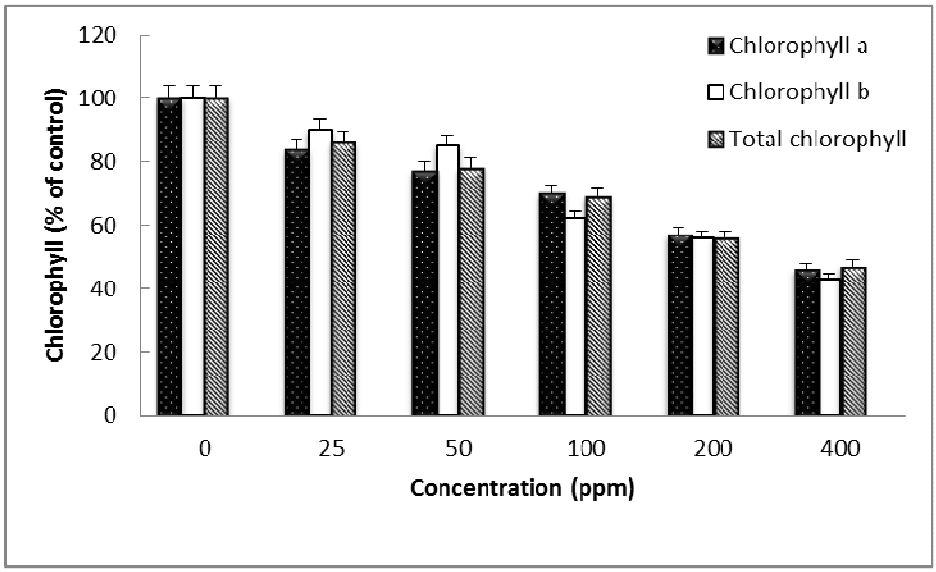

Fig. 4: Effect of monocrotophos on chlorophyll content (chlorophyll – a content in untreated control was 0.179±0.0 mg/gm. fresh weight, chlorophyll – b content in untreated control was 0.076±0.1 mg/gm. fresh weight and total chlorophyll content in untreated control was 0.255±0.1 mg/gm. fresh weight) All values are mean ± S.E. of three replicates. Values are significantly different at P<0.01 and *P<0.05 from control.

Carotenoid

The accessory photosynthetic pigments carotenoid was analyzed on 5th day after insecticide exposure and their findings are graphically depicted in fig.5.Though it is non-enzymatic antioxidant its content adversely affected by the higher concentration. The carotenoid content decrease by 7%, 18% and 29% at 25ppm, 50ppm and 100ppm. Further there was a gradual decrease in carotenoid content as the concentration increases.

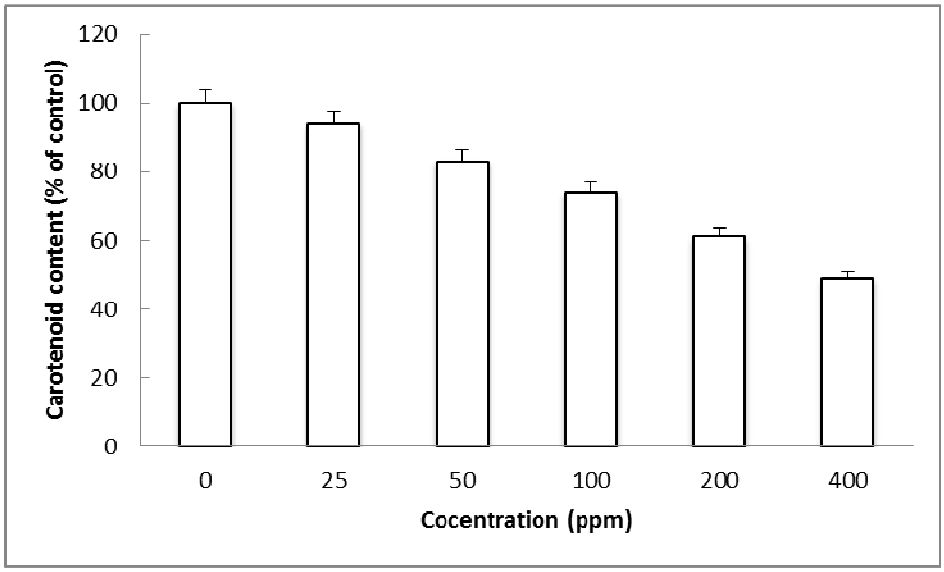

Fig.5: Effect of monocrotophos on carotenoid content (Carotenoid content in untreated control was 0.881±0.6 mg/gm. fresh weight). All values are mean ± S.E. of three replicates. Values are significantly different at P<0.01 from control.

Protein

Being essential macromolecules of living cells proteins plays paramount role in metabolic pathway to understand the effect of monocrotophos on *Azolla microphylla*. Analysis of protein was done on 5th day after insecticide exposure. The data is graphically depicted in fig.6. The concentration of protein was maximum in control showed a considerable decrease with increasing concentration. Protein content reduces by 7%, 12% and 20% at 25ppm, 50ppm and 100ppm. Further there was gradual decrease in protein content as concentration increases.

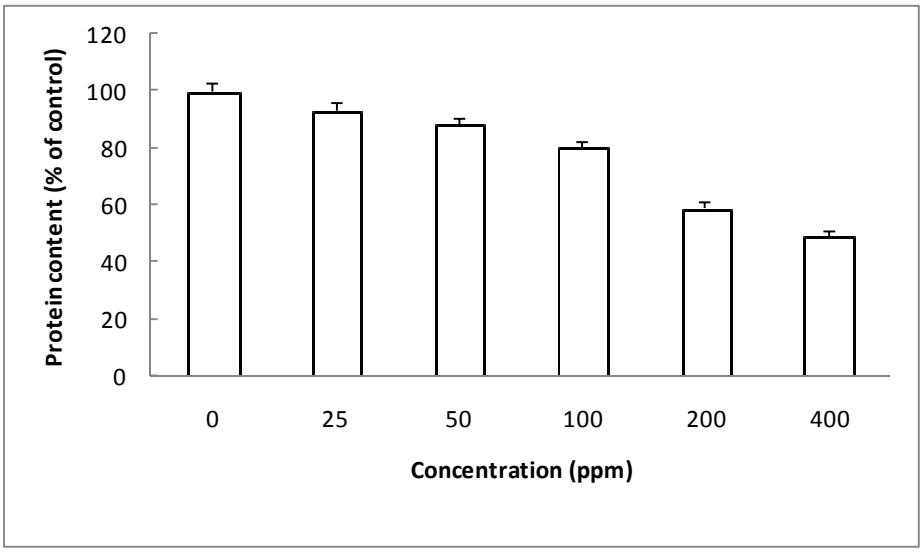

Fig. 6: Effect of monocrotophos on protein content (Protein control was 1.006±0.4 mg/gm. fresh weight). All values are mean ± S.E. of three replicates. Values are significantly different at P<0.01 and *P<0.05 from control.

Heterocyst frequency

The data observed on heterocyst frequency on 5th day after insecticide exposure is graphically depicted in fig.7 Heterocyst frequency also showed a considerable decrease with increasing concentration of monocrotophos. The reduction followed a regular pattern in the order of control> 25 ppm> 50ppm>100ppm> 200ppm>400ppm respectively with the values of 100%, 95%, 89% 81%, 69% and 58%.

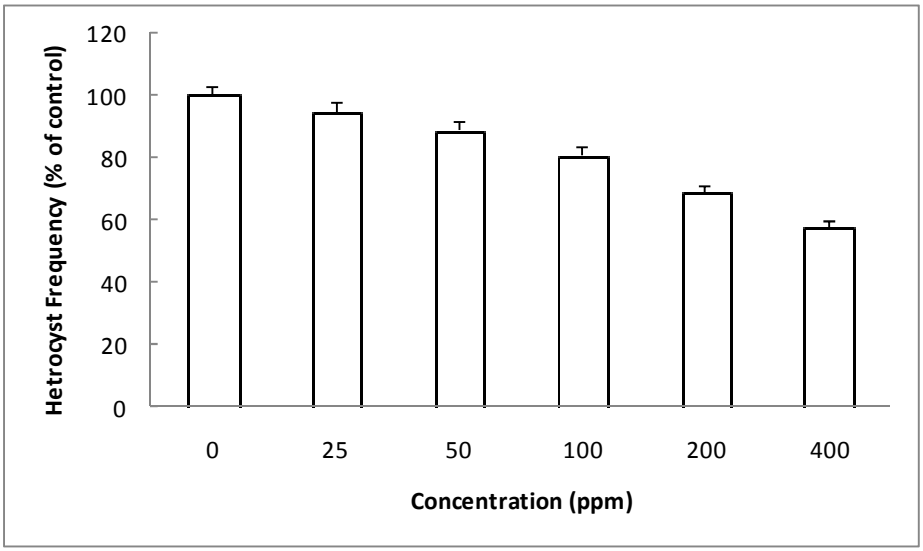

Fig. 7: Effect of monocrotophos on heterocyst frequency (Heterocyst Frequency in untreated control was 7.80±1.6). All values are mean ± S.E. of three replicates. Values are significantly different at P<0.01 and *P<0.05 from control.

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 dry weight was clear after five days of incubation at different concentration in ppm of monocrotophos. [22] has demonstrated that high concentration of melathion inhibit the growth of *Azolla pinnata*. The reduction in dry weight by monocrotophos might be due to chemical which affects 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 [23]. The reduced growth in response to higher concentration of melathion may result from reduction in protein and DNA content [14].

Doubling time as well as relative growth rate was influenced by pesticides. Formulation time and formulation X time interaction significantly infused doubling time. [24]. While studying phosphorus removal in *Azolla caroliniana* found that its growth rates were influenced by plant density, temperature, nutrient composition and solar radiation. Our results could be supported with the result of Arora and Singh [25] who had shown less biomass and more doubling time in *Azolla* sp. treated with different concentration of sodium chloride. Recently and [26] again got same results in *Azolla microphylla* treated by municipal effluents of Delhi.

The damaging effect of monocrotophos on photosynthetic pigments of *Azolla microphylla* was noticed after five days of treatment of pesticides from 25 ppm to 400 ppm (Fig 4). 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 [27]. 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 to the pesticides and are in consequence with earlier findings.[28].Since carotenoids are less affected it also act as an antioxidant metabolite[29,12] it protects chlorophyll and photosynthetic membrane from oxidative damage, therefore decline in carotenoids could have serious concequence on chlorophyll as well as thylakoid membrane which may lead to

reduction in photosynthetic capability of *Azolla microphylla*. To cope with such damage cells have been naturally equipped with an efficient antioxidant system which consists of enzymatic and non-enzymatic antioxidants [37].

Like chlorophyll, protein of the *Azolla microphylla* was also inhibited by enhanced doses of pesticide which is in agreement with results of earlier work of [30, 31] and recently by [38] 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 [32]. Recently [10] have shown reduction in growth (Protein) of cyanobacterium *Plectonema boryanum* under monocrotophos stress which is in agreement with our findings. The reduction in heterocyst frequency is an indirect evidence for reduced nitrogen fixation. The heterocyst frequency is known to control the growth rate and nitrogen fixation in *Azolla* fronds [33]. It may be inferred that monocrotophos at higher concentration hastens the onset of senescence in plants leading to loss of chlorophyll and decreasing biomass yield and reduction in nitrogen fixing ability. The nitrogen starvation in the cells would result in the reduction of protein synthesis [34] and ultimately decrease in growth of cyanobacterial population. [12]

CONCLUSION

In the present study the deleterious effect of 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 monocrotophos. However, more study to conform our findings at molecular level is suggested.

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