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GROWTH AND BIOCHEMICAL RESPONSES OF *AZOLLA MICROPHYLLA* KAULFUSS UNDER RECOMMENDED DOSES OF CHLORPYRIFOS, BUTACHLOR AND PRETILACHLOR PESTICIDES USED FOR AGRICULTURAL CROPS OF PUNJAB

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ABSTRACT

Growth and biochemical responses of *Azolla microphylla* Kaulfuss under recommended doses of chlorpyrifos (insecticide), butachlor and pretilachlor (herbicides) pesticides used for agricultural crops of Punjab were investigated. *Azolla microphylla* was grown, multiplied and maintained in plastic trays using Espinase and Watanable medium. In chlorpyrifos and butachlor treatments, freshmass, drymass, relative growth rate, total chlorophyll, carotenoid content and protein content of *Azolla microphylla* were maximum at 0.5 ppm on 10th, 20th and 30th day harvesting. For pretilachlor treatment, these parameters were maximum at 0.25 ppm on 10th, 20th and 30th day harvesting. Chlorpyrifos, butachlor and pretilachlor under recommended doses of 0.5 ppm, 1.5 ppm and 0.75 ppm, respectively pose no threat to the growth, metabolic activity and survival of *Azolla microphylla*. It is, therefore, suggested that, these pesticides are completely safe for field application for successful and efficient management of rice crop ecosystem coupled with *Azolla* as dual crop.

Keywords: Chlorpyrifos, butachlor, pretilachlor, *Azolla microphylla,* rice crop ecosystem.

INTRODUCTION

Pesticides play a vital role for the successful and efficient multiplication of *Azolla,* a nitrogen fixing fern grown along with rice as dual crop, since its growth is highly affected by pests. Moreover, pesticides considered for use on *Azolla* should be tested under field conditions before recommendations are given. *Azolla* is sensitive to many pesticides, so the testing of pesticides and of its various dilutions is essential. The main objective of the present study is to testify whether the recommended doses of pesticides for field application in rice-crop ecosystem has any effect on the growth of *Azolla* or not, since *Azolla* is used as biofertilizer as dual crop with rice. Of the different pesticides practiced for agricultural crops in Punjab, three widely used pesticides namely, chlorpyrifos (insecticide), butachlor and pretilachlor (herbicides) were selected to achieve this objective. In the proposed plan of work, *Azolla microphylla* is used as a study material due to its advantage over other species of *Azolla* since it is highly adapted to high temperature (>35°C) whereas species such as *A. pinnata* is comparatively sensitive to high temperature. Also, *A. microphylla* is of particular importance because of its high adaptability and capacity to grow in adverse conditions and shows luxuriant growth between pH 4-8.

MATERIALS AND METHODS

Azolla microphylla was procured from Centre for Conservation and Utilisation of Blue Green algae, Indian Agricultural Research Institute, New Delhi andwas cultured in Espinase and Watanabe medium (Singh *et al*., 2000) in plastic trays in tropical house of Botanic Gardens, Punjabi University, Patiala. Recommended doses of chlorpyrifos (0.5 ppm), butachlor (1.5 ppm) and pretilachlor (0.75 ppm) were made according to Bajwa (2012). Six concentrations each for chlorpyrifos (0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 ppm), butachlor (0.5, 0.75, 1.0, 1.25, 1.5 and 1.75 ppm) and pretilachlor (0.25, 0.5, 0.75, 1.0, 1.25 and 1.5 ppm) supplemented with E & W medium, were used along with E & W medium as

control. Plastic pots (12 cm diameter and 14 cm depth) containing 1L of E & W medium in each beaker were used for experimental studies. The set were made for 10^{th}, 20^{th} and 30^{th} day harvesting. For every concentration, the beakers were used in triplicate. Two grams of *Azolla microphylla* were sprinkled in each beaker before the start of the experiment. The level of E & W medium was maintained by adding distilled water to prevent *Azolla* from evaporative losses. The quantitative growth and biochemical parameters observed were; freshmass and drymass (Singh and Srivastava, 1985); relative growth rate (Kannaiyan *et al.,* 1989); total chlorophyll (Arnon, 1949); carotenoid content (Wellburn, 1994) and protein content (Lowry *et al*., 1951). The data generated, were subjected to the statistical analysis in accordance with the procedure given by Gomez and Gomez (1984) and were analyzed as per completely randomized design (Snedecor and Cochran, 1967) to test the significance of differences between the treatments. Coefficient of variation was calculated using method given by Burton and Devane (1953).

RESULTS AND DISCUSSION

For chlorpyrifos and butachlor treatments, freshmass, drymass, relative growth rate, total chlorophyll, carotenoid content and total protein content of *Azolla microphylla* were maximum in 0.5 ppm on 10^{th}, 20^{th} and 30^{th} day harvesting. For pretilachlor treatment, freshmass, drymass, relative growth rate, total chlorophyll, carotenoid content and total protein content of *Azolla microphylla* were maximum in 0.25 ppm on 10th, 20th and 30th day harvesting (Figs. 1-18).

The inhibitory effects of chlorpyrifos have been reported in *Synechococcus leopoliensis* (Van Donk *et al.,* 1992), *Anabaena sphaerica, Nostoc hatei,* and *Westiellopsis prolifica* (Jha and Mishra, 2005), *Phormidium valderianum* (Palanisami *et al.,* 2009) and *Spirulina platensis* (Thengodkar and Sivakami, 2010). Reduction in the growth of *A. pinnata* due to cypermethrin and cholrpyrifos may be attributed to the inhibition of normal cell division and arrest of key physiological and biochemical processes such as photosynthesis (Prasad *et al.,* 2015) as reported in *A. pinnata* and *A. microphylla* under pretilachlor treatment (Prasad and Kumar, 2011). Reduction in chlorophyll *a* contents at high concentrations of chlorpyrifos may be due to the inhibition of their biosynthesis, breakdown of pigments or their precursors as suggested by Mishra *et al.*, (2008) and Sresen *et al.,* (2000).

The inhibitory effect of butachlor on photosynthetic pigments of *Plectonema boryanum* was found to be dose dependent and the deleterious effect was more pronounced on chlorophyll *a* followed by carotenoids. Such decrease in chlorophyll *a* and carotenoid contents may be ascribed to the inhibition of pigment synthesis directly by herbicide or accelerated degradation of pigments due to increased reactive oxygen species formation at the various sites of the photosynthetic electron transport chain during stress (Kumar and Vikash, 2012). Supplementation of butachlor (8-20 mg L^{-1}) in culture medium of *Nostoc muscorum* resulted in decrease of protein content by 27-89% whereas 14 and 63% decrease in protein content in 5 and 8 mg Thiobencarb L-1 was reported (Dowidar *et al*., 2010).The herbicides shown to affect growth of cyanobacteria include Butachlor, Bensulfuron-methyl and Dimethoate in *Nostoc* (Selvakumar *et al*., 2002; Chen, 2007). Glyphosate in *Anabaena* sp., *Leptolyngbya boryana*, *Nostoc punctiforme, Microcystis* sp. and *Microcystis aeruginosa* (Rodas *et al.,* 2006; Forlani *et al.,* 2008), Molinate and Bentazon in *Anabaena cylindrica* and *Nostoc muscorum* (Galhano

et al., 2010a; Galhano *et al.,* 2010b), Atrazine and DCMU in *Anabaena variabilis* (Singh *et al.,* 2011), Anilofos in *Oscillatoria simplicissima* (Singh and Sandhu, 2010) and *Anabaena torulosa* (Singh *et al.,* 2012).

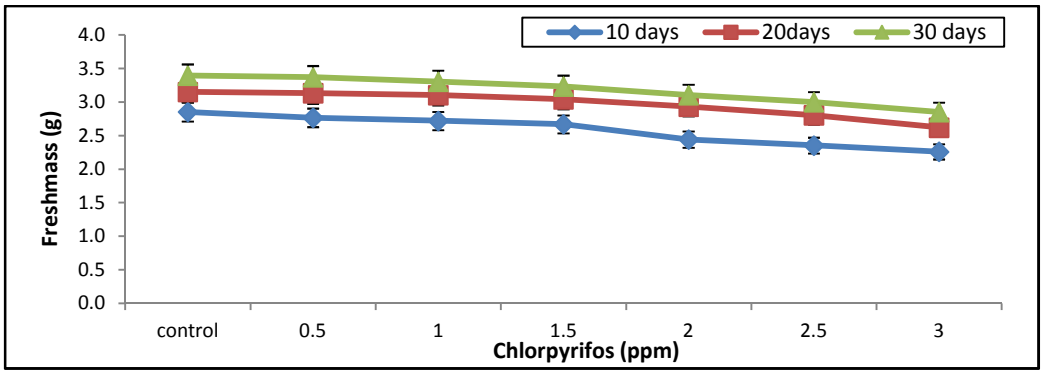

Fig. 1. Freshmass of *Azolla microphylla* on 10th day, 20th day and 30th day harvesting under different concentrations of chlorpyrifos in E & W medium

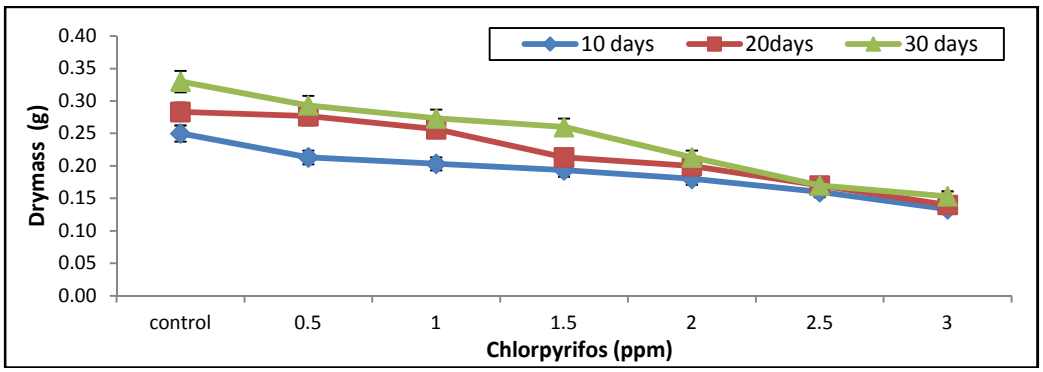

Fig. 2. Drymass of *Azolla microphylla* on 10th day, 20th day and 30th day harvesting under different concentrations of chlorpyrifos in E & W medium

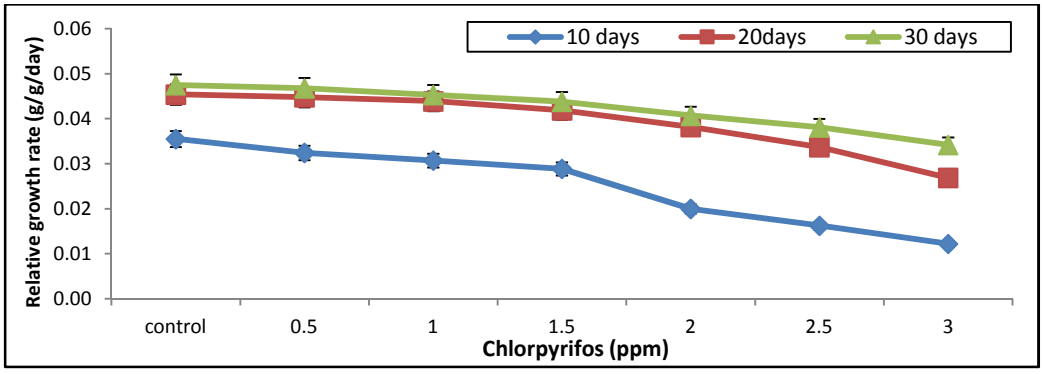

Fig. 3. Relative growth rate of *Azolla microphylla* on 10th day, 20th day and 30th day harvesting under different concentrations of chlorpyrifos in E & W medium

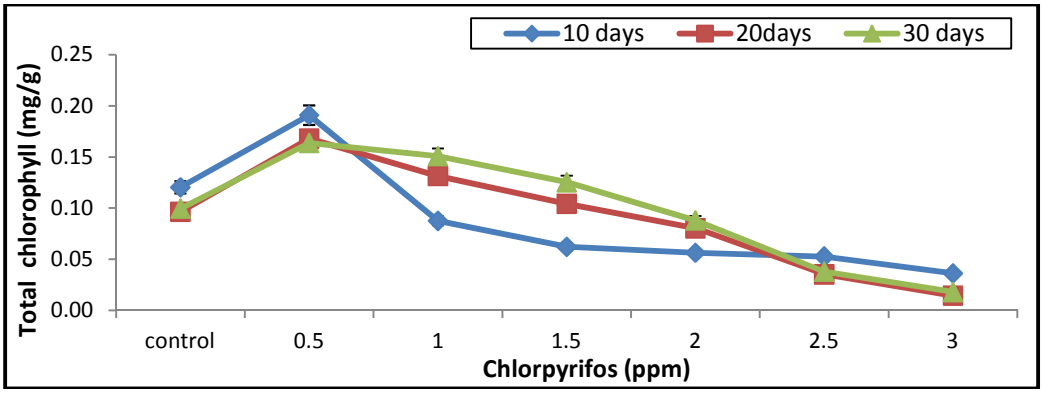

Fig. 4. Total chlorophyll content of *Azolla microphylla* on 10th day, 20th day and 30th day harvesting under different concentrations of chlorpyrifos in E & W medium

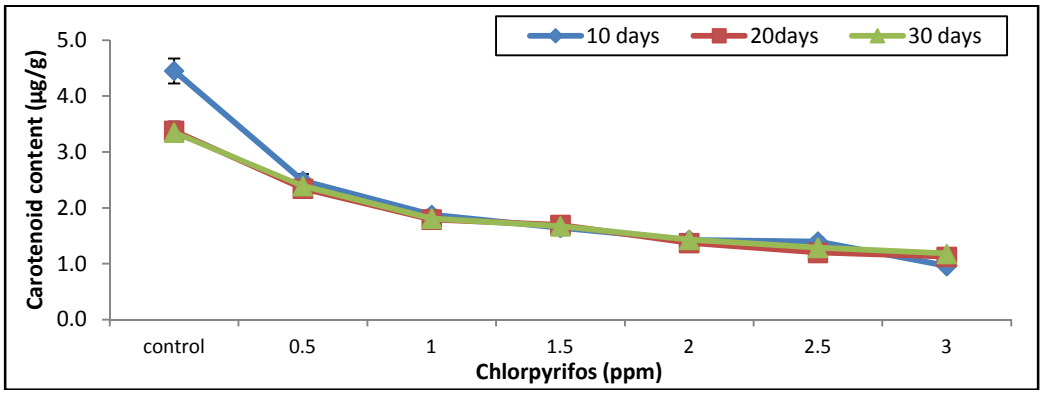

Fig. 5. Carotenoid content of *Azolla microphylla* on 10th day, 20th day and 30th day harvesting under different concentrations of chlorpyrifos in E & W medium

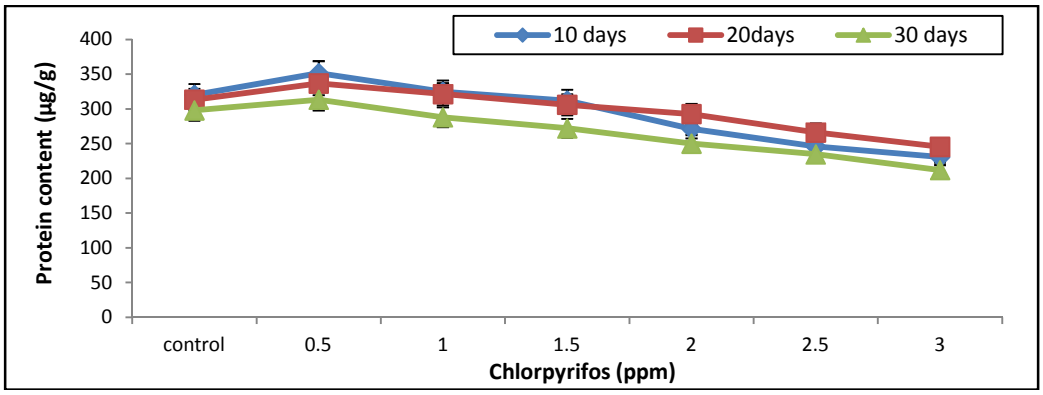

Fig. 6. Protein content of *Azolla microphylla* on 10th day, 20th day and 30th day harvesting under different concentrations of chlorpyrifos in E & W medium

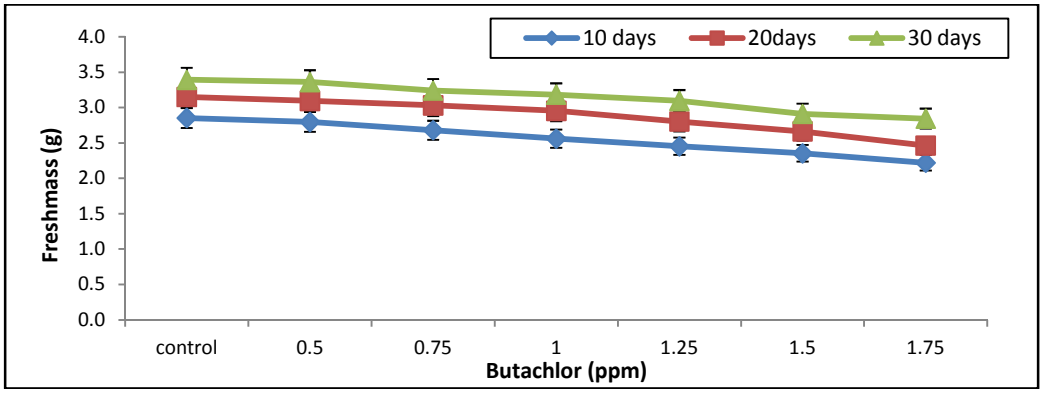

Fig. 7. Freshmass of *Azolla microphylla* on 10th day, 20th day and 30th day harvesting under different concentrations of butachlor in E & W medium

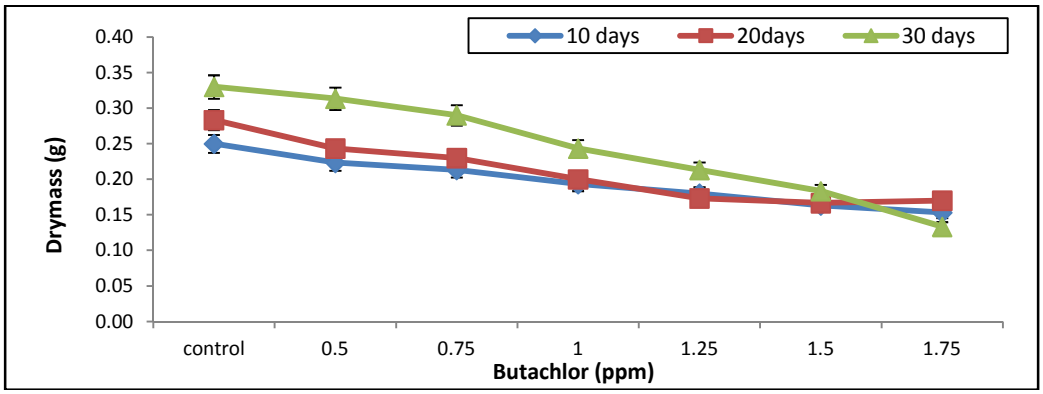

Fig. 8. Drymass of *Azolla microphylla* on 10th day, 20th day and 30th day harvesting under different concentrations of butachlor in E & W medium

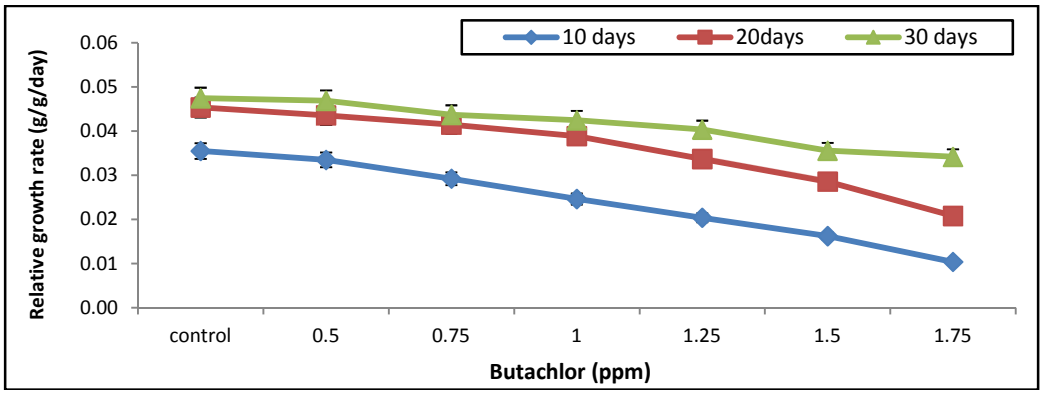

Fig. 9. Relative growth rate of *Azolla microphylla* on 10th day, 20th day and 30th day harvesting under different concentrations of butachlor in E & W medium

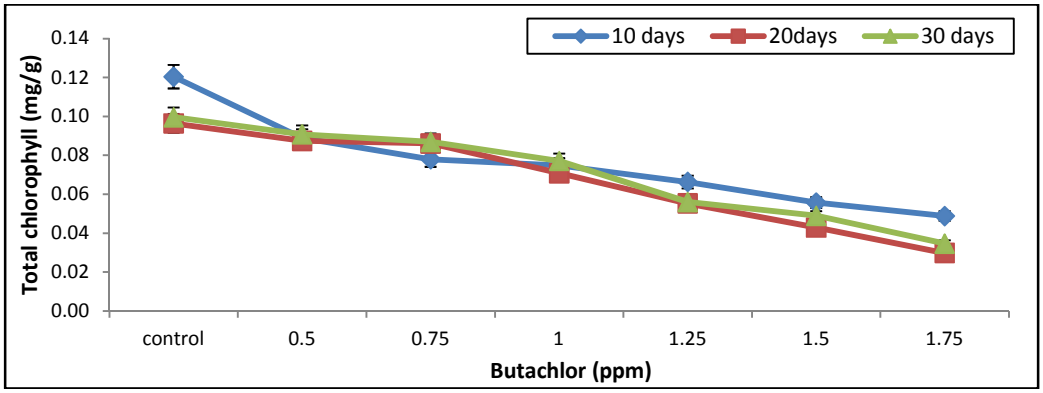

Fig. 10. Total chlorophyll content of *Azolla microphylla* on 10th day, 20th day and 30th day harvesting under different concentrations of butachlor in E & W medium

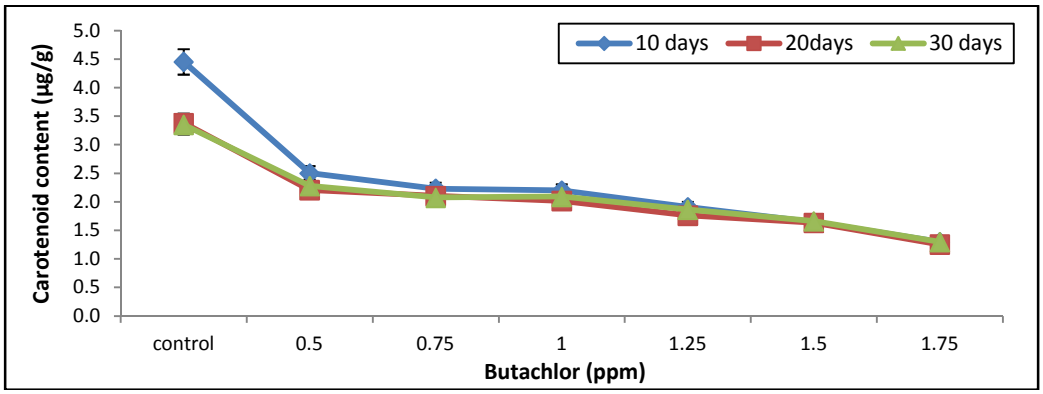

Fig. 11. Carotenoid content of *Azolla microphylla* on 10th day, 20th day and 30th day harvesting under different concentrations of butachlor in E &W medium

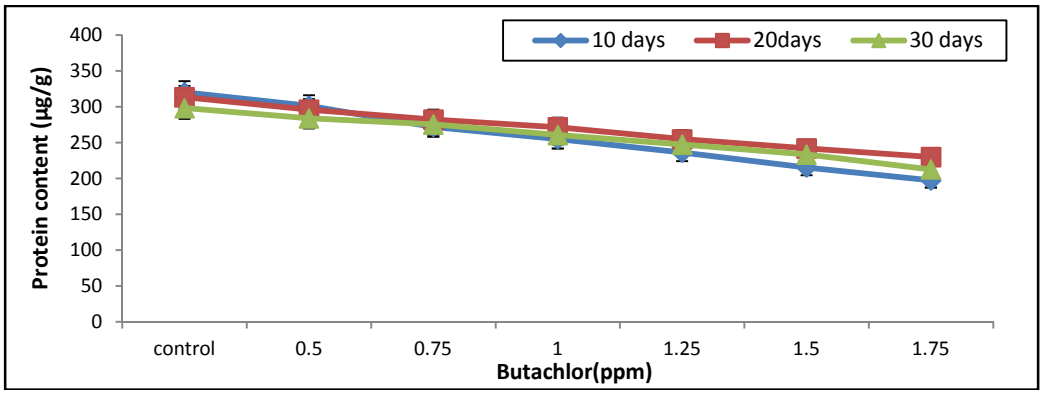

Fig. 12. Protein content of *Azolla microphylla* on 10th day, 20th day and 30th day harvesting under different concentrations of butachlor in E & W medium

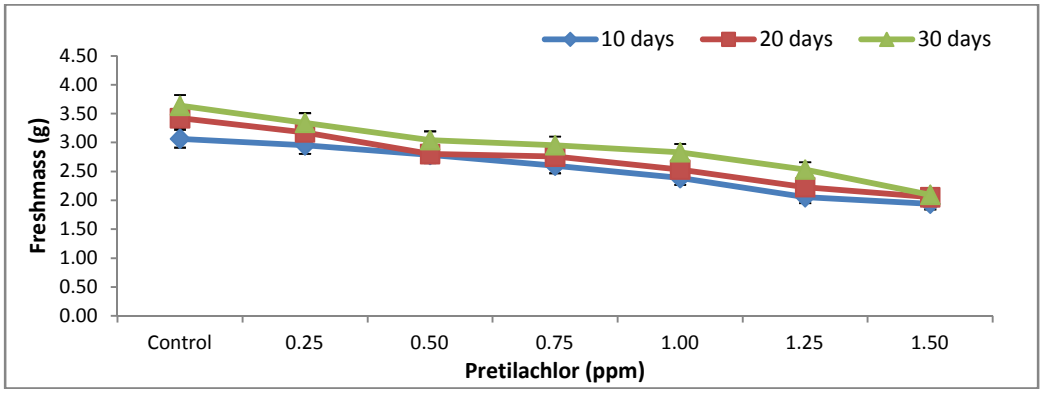

Fig. 13. Freshmass of *Azolla microphylla* on 10th day, 20th day and 30th day harvesting under different concentrations of pretilachlor in E & W medium

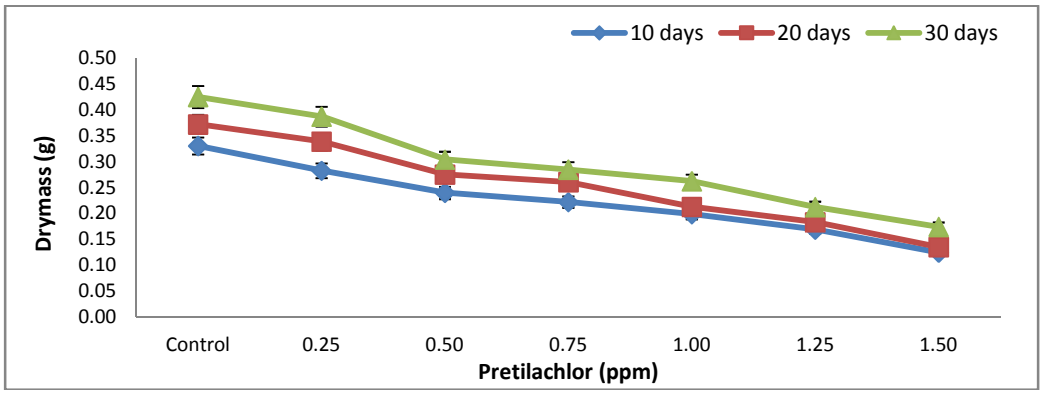

Fig. 14. Drymass of *Azolla microphylla* on 10th day, 20th day and 30th day harvesting under different concentrations of pretilachlor in E & W medium

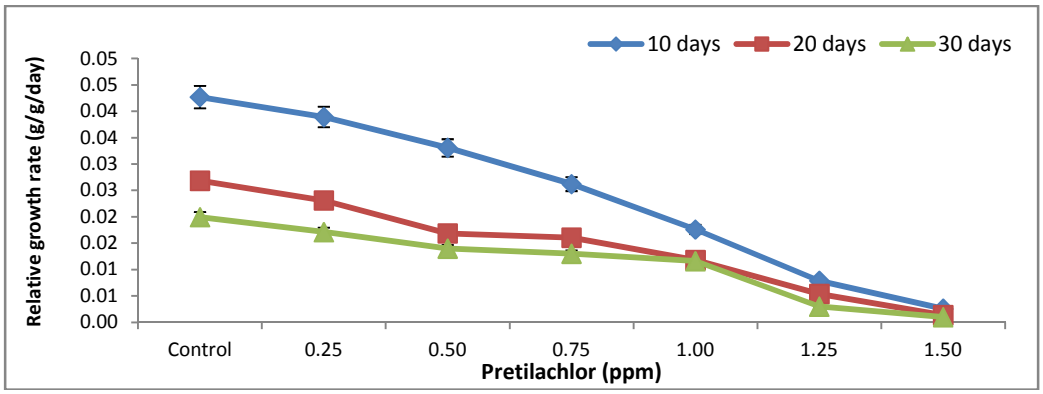

Fig. 15. Relative growth rate of *Azolla microphylla* on 10th day, 20th day and 30th day harvesting under different concentrations of pretilachlor in E & W medium

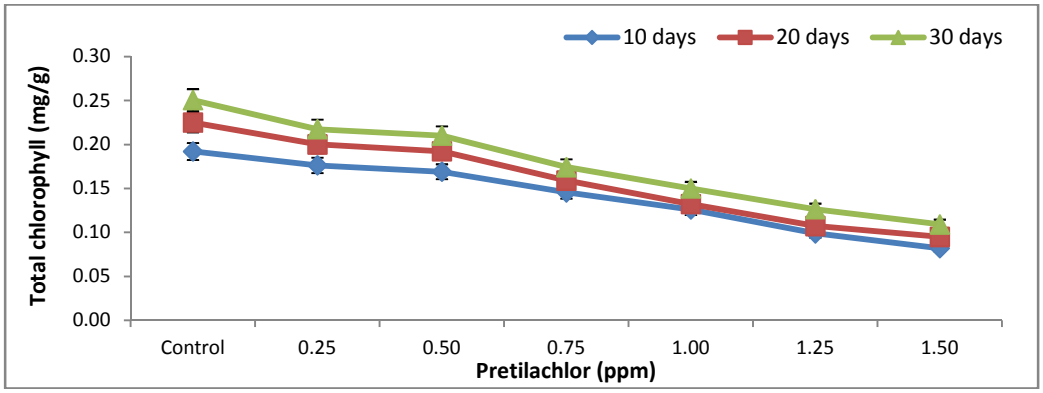

Fig. 16. Total chlorophyll content of *Azolla microphylla* on 10th day, 20th day and 30th day harvesting under different concentrations of pretilachlor in E & W medium

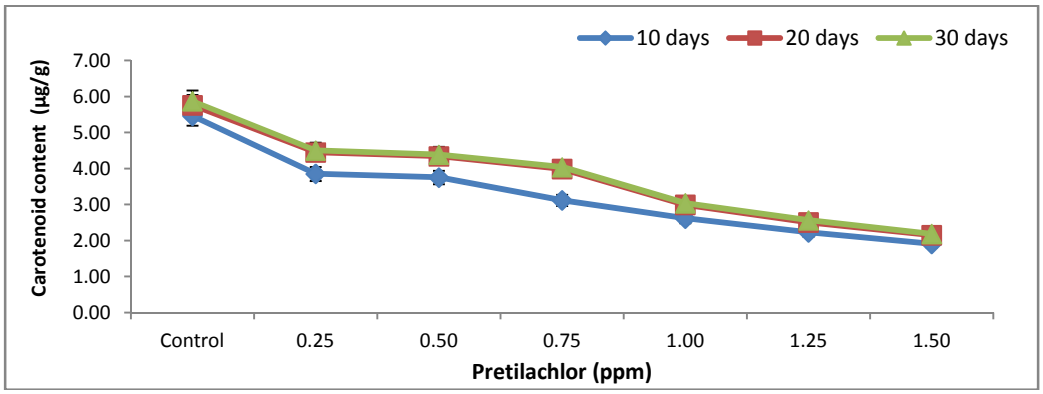

Fig. 17. Carotenoid content of *Azolla microphylla* on 10th day, 20th day and 30th day harvesting under different concentrations of pretilachlor in E &W medium

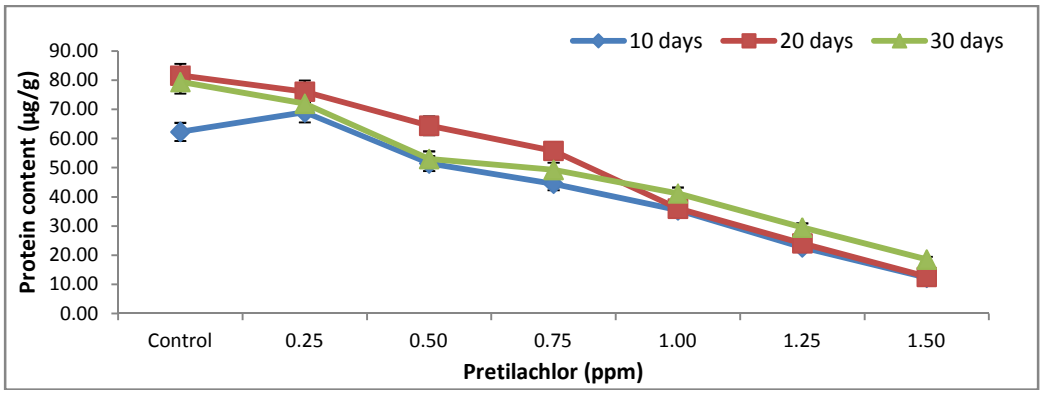

Fig. 18. Protein content of *Azolla microphylla* on 10th day, 20th day and 30th day harvesting under different concentrations of pretilachlor in E & W medium

Inderjit and Kaushik (2010) studied the toxic effect of three herbicides propanil, pretilachlor and glyphosate with different mode of action on chlorophyll *a*, dry weight and protein content of *Anabaenafertilissima.* Dharumarajan *et al.,* (2008) studied the persistant of pretilachlor in coastal rice ecosystem and concluded that pretilachlor (40 ppm) and glyphosate (80 ppm) exhibited toxicity at higher doses than propanil (1.5 ppm). Pretilachlor at 0.75 and 1.5 kg ha^{-1} dissipated to below detectable level at 60 days after application. Prasad and Kumar (2011) worked on the differential responses of growth, antioxidant enzymes and oxidative stress in *Azolla microphylla* and *Azolla pinnata* exposed to pretilachlor and enhanced UV-B radiations and concluded that drymass decreased in dose dependent manner of pretilachlor and UV-B exposure. In *A. microphylla* pretilachlor at 5, 10 and 20 µg ml^{-1} decreased dry mass by 10, 14 and 20%, while in case of *A. pinnata* it declined by 17, 24 and 33%, respectively over the values of respective controls. Reduction in growth may be attributed to inhibition in normal cell division, as reported in barley plant under pretilachlor treatment. Toxic effect was considerably higher in *A. pinnata* than *A. microphylla*. Reduction in growth may be attributed to inhibition in normal cell division, as reported in barley plant under pretilachlor treatment (Srivastava *et al.,* 2008). Pretilachlor caused a significant decrease in chlorophyll *a*, carotenoids, phycocyanin, allophycocyanin and phycoerythrin content in a dose dependent manner compared to control with more pronounced effects at higher concentrations of pretilachlor (Singh *et al.,* 2016). Herbicides inhibit photosynthesis mainly by preventing electron flow from PS-II both in algae as well as in higher plants (Tischer and Strotmann, 1977; Pfister and Arntzen, 1979; Allen *et al.,* 1983).

It is concluded from the results and discussion that chlorpyrifos, butachlor and pretilachlor under recommended doses of 0.5 ppm, 1.5 and 0.25 ppm, respectively pose no threat to the growth, metabolic activity and survival of *Azolla microphylla*. It is, therefore, suggested that these pesticides are completely safe for field application under recommended doses for the successful and efficient management of rice-crop ecosystem coupled with *Azolla* as dual crop.

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