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Effect of butachlor and chlorpyrifos on the growth of *Azolla microphylla* Kaulfuss used as dual crop alongwith rice

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Abstract : The present investigation embodies the result of the effect of butachlor and chlorpyrifos on the growth of *Azolla microphylla* Kaulfuss used as dual crop alongwith rice. The plants (*Azolla microphylla*) were grown, multiplied and maintained in plastic trays using Espinase and Watanable medium in Tropical house, Botanic Gardens, Punjabi University, Patiala, which were later used for experimental studies. For butachlor treatment, freshmass, drymass, relative growth rate, total chlorophyll, carotenoid content, total protein content of *Azolla microphylla* and heterocyst frequency (of symbiont) were maximum in 0.5 ppm on 10th, 20th and 30th day harvesting. Doubling time was minimum in 0.5 ppm on 10th, 20th and 30th day harvesting. For chlorpyrifos treatment**,** freshmass, drymass, relative growth rate, total chlorophyll, carotenoid content, total protein content of *Azolla microphylla* and heterocyst frequency (of symbiont) were maximum in 0.5 ppm on 10th, 20th and 30th day harvesting. Doubling time was minimum in 0.5 ppm on 10th, 20th and 30th day harvesting. It is concluded from the results that butachlor (herbicide) and chlorpyrifos (insecticide) under recommended doses of 1.5 ppm and 0.5 ppm, respectively pose no threat to the growth, metabolic activity and survival of *Azolla microphylla*. Heterocyst frequency, too, is not affected. It is, therefore, recommended that both 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.

Key Words: Butachlor, chlorpyrifos, *Azolla microphylla*, dual crop and rice crop ecosystem.

INTRODUCTION

Azolla – Anabaena association is a favourite biofertilizer of crops, especially in rice fields because of its ability to fix nitrogen at high rates and low cost. In addition, *Azolla* is a suitable candidate as an animal feed, human food, water purifier, medicine, hydrogen fuel, biogas producer, weed controller, suppressor of weeds and reduces ammonia volatilization after chemical nitrogen application and rightly called as "green gold". 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 fields. Pesticides, in this context, play a vital role for the successful and efficient multiplication of *Azolla* since growth of the fern is highly affected by pests. The literature pertaining to effect of pesticides on the growth of *Azolla* is scanty (Holst *et al.*, 1982; Ferrari *et al.*, 2000; Britto and de Seethalakshmi, 2000; Aida *et al.,* 2004; Okon-Levy *et al.,* 2005; Aida *et al.,* 2006; EI-Shahate *et al.,* 2011; Chris *et al.,* 2011; Sood *et al.,* 2011; Raja *et al,.* 2012). Moreover, pesticides considered for use on *Azolla* should be tested under local conditions before recommendations are given. *Azolla* is sensitive to many pesticides, so the testing of pesticides and of its

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various dilutions are 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, two widely used pesticides namely, Butachlor (herbicide) and Chlorpyrifos (insecticide) were selected to achieve this objective which are till date not being used for any growth study pertaining to *Azolla* as evident by world literature. Butachlor [2 – chloro - 2, 6 – diethyl – N - (butoxymethyl) - acetanilide] belongs to the chloroacetanilide group of herbicides which inhibits protein synthesis in developing plant tissue, and is largely used for pre-emergence and/or early post-emergence control of a variety of undesirable grasses and selected broad-leaved weeds in rice, wheat, cotton, peanuts and several *Brassica* crops (Martin and Worthing, 1977; Hackett, 1998). Chlorpyrifos [O,O-diethyl O-(3,5,6- trichloro-2-pyridyl) phosphorothioate] is used worldwide as an agricultural insecticide. Chlorpyrifos is applied on a large scale in rice fields of Punjab state of India as a broad spectrum organophosphate insecticide for the control of foliar insects. In the present investigation, *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 to Punjab 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 and was identified using the standard literature (Lumpkin and Plucknett, 1982). *Azolla microphylla* was cultured in Espinase and Watanabe medium in plastic trays in tropical house of Botanic Gardens, Punjabi University, Patiala. It was also grown under natural conditions in cemented pots in Conservatory of Botanic Gardens, Punjabi University, Patiala. Espinase and Watanabe medium was prepared by using the method given by Singh *et al.,* 2000. Recommended doses of butachlor and chlorpyrifos were calculated according to the literature mentioned by Bajwa, 2012. The experiment was conducted in tropical house, Botanic Gardens, Punjabi University, Patiala. The recommended doses of butachlor and chlorpyrifos for rice-crop ecosystem were calculated, which were; 1.5 ppm and 0.5 ppm, respectively. Altogether, six concentrations for butachlor and similar concentrations for chlorpyrifos were made and mixed with E & W medium. Altogether, 117 plastic beakers of 12 cm diameter and 14 cm depth containing 1L E & W medium in each beaker were used for the experimental study. Three set, each having 39 beakers were made for 10th, 20th and 30th 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 observations were made daily. The level of E & W medium was maintained daily by adding distilled water to prevent *Azolla* from evaporative losses. The quantitative growth parameters observed were; freshmass, drymass, doubling time, relative growth rate, total chlorophyll, carotenoid content, total protein content and heterocyst frequency (of symbiont). Freshmass and drymass were calculated according to the method proposed by Singh and Srivastava, 1985. Doubling time, relative growth rate and heterocyst frequency (of symbiont) were calculated using the formula referred by Kannaiyan *et al.* 1989. Total chlorophyll was calculated using the formula given by Arnon, 1949. Carotenoid content was calculated according to the formula used by Wellburn, 1994.

Protein content was determined as per method followed by Lowry *et al*., 1951. The data obtained from present investigation was subjected to the statistical analysis in accordance with the procedure given by Gomez and Gomez (1984). The data were analyzed for a completely randomized design (Snedecor and Cochran, 1967) to test the significance of differences between the treatments. Co-efficient of variation was calculated using method given by Burton and Devane (1953).

RESULTS AND DISCUSSION

For butachlor treatment, freshmass, drymass, relative growth rate, total chlorophyll, carotenoid content, total protein content of *Azolla microphylla* and heterocyst

frequency (of symbiont) were maximum in 0.5 ppm on 10th, 20th and 30th day harvesting. Doubling time was minimum in 0.5 ppm on 10th, 20th and 30th day harvesting (Fig.1-8). For chlorpyrifos treatment, freshmass, drymass, relative growth rate, total chlorophyll, carotenoid content, total protein content of *Azolla microphylla* and heterocyst frequency (of symbiont) were maximum in 0.5 ppm on 10th, 20th and 30th day harvesting. Doubling time was minimum in 0.5 ppm on 10th, 20th and 30th day harvesting (Fig. 9-16).

Azolla can be used as a green manure as well as biofertilizer in rice crop. For using it as a green manure, the field should be well prepared and leveled properly. The water depth of 2 inches, be maintained, then fresh *Azolla* is inoculated @ 1 ton/ha. This can be reduced or enhanced depending on the growth condition. SSP is applied in split doses (25-50 kg/ ha). Furadon @ 2-3 kg/ ha can be applied for pest/ insect control. After 2-3 weeks a thick mat forms which can be incorporated in the soil and then rice can be transplanted. This accounts for 10-20 ton *Azolla* contributing 20-40 kg N/ ha. Fresh *Azolla* is incorporated @ 0.5 - 1.0 ton/ha to be applied in the field after 7-10 days of transplantation of rice as dual crop. SSP is applied @ 20 kg/ha in split doses. After 15-20 days, thick mat is formed. Incorporated *Azolla* decomposes within 8-10 days and releases nitrogen. Another crop of *Azolla* can be raised in a similar way during the crop cycle of rice. Each crop of *Azolla* during dual cropping with rice contributes on an average 30 kg N/ha. Looking at the growth data, viz. freshmass, drymass, doubling time, relative growth rate, total chlorophyll, carotenoid content, protein content and heterocyst frequency (of symbiont), (Fig. 1-8), it is obvious that there is no deleterious effect of butachlor (machete) on the growth and metabolic activity of *Azolla.* The fern is not only showing survival but is also growing well at the recommended dose of herbicide (butachlor), i.e., 1.5 ppm. It is noteworthy, that butachlor is basically pre-emergence herbicide and is applied after the crop has been planted, but before weed emergence. The degradation of half-lives of butachlor at the recommended doses in soil were calculated to be 12.5, 4.5 and 3.2 days for the first, second and third applications, respectively (Fang *et al.*, 2009). It is usually applied to the soil surface as granules (G) or emulsifiable concentrates (EC), Butachlor is used to prevent establishment of weeds right after planting and must not be toxic to the crop under recommended dose. Chlorpyrifos (dursban) is an insecticide mainly used for the eradication of brown plant hopper of rice @ 1.5 L/ ha as spray. It is not used as precautionary measure and is recommended only on the onset of the disease and applied when pest number reaches 1-2 per tiller and is repeated as necessary. The half-life of chlorpyrifos in soil is usually between 60 and 120 days (Howard, 1991; Wauchope *et al.*, 1992). Since chlorpyrifos is usually applied at tillering stage, about 35-40 days after rice transplantation, the growth of *Azolla* is not at all affected. Thus, the growth and survival of *Azolla* under recommended dose of chlorpyrifos, i.e., 0.5 ppm is not at stake as evident from growth data (Fig.9-16). It is concluded from the results that butachlor (herbicide) and chlorpyrifos (insecticide) under recommended doses of 1.5 ppm and 0.5 ppm, respectively pose no threat to the growth, metabolic activity and survival of *Azolla microphylla*. Heterocyst frequency, too, is not affected. It is, therefore, recommended that both 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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