

## Studies on the sensitivity of *Azolla* species to rice herbicides

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**Abstract:** Laboratory and green house experiments were conducted during 2002 to find out the sensitivity of different *Azolla* species to rice herbicides. From the screening experiment in the laboratory, three herbicides viz. anilofos 0.4 kg ha<sup>-1</sup>, butachlor 1.25 kg ha<sup>-1</sup> and clomazone 0.3 kg ha<sup>-1</sup> and four species of *Azolla* viz., *A. microphylla*, *A. filiculoides*, TNAU hybrid and Rong Ping hybrid at 500 kg ha<sup>-1</sup> were chosen and compared independently and in different combinations in the pot culture experiment. Rice var. CO 47 was raised under green house conditions and the experiment was carried out under FRBD with 3 replications. The growth, yield and yield parameters were recorded after imposing the treatments. The results revealed that combination of clomazone 0.3 kg ha<sup>-1</sup> and *A. microphylla* performed significantly superior with respect to plant growth, yield attributes and yield. Among the herbicides tried, clomazone application at 0.3 kg ha<sup>-1</sup> showed an increase in plant growth characters, yield attributes and yield. Between the individual *Azolla* species, *A. microphylla* recorded the highest grain yield and was on par with *A. filiculoides*.

**Key words :** Clomazone, *Azolla* species, Growth, Yield parameters, Yield.

### Introduction

Worldwide, India stands first in rice area with 43 million hectare and second in rice production (199 million tonnes), after China (Anon, 2001) contributing 31.0 per cent of global rice production (Brar and Walia, 2001). Demand for rice is increasing and an additional 300 million tonnes per year has to be produced by 2025 (Swaminathan, 1998). Approaches to achieve this demand include expanding the rice area (horizontal expansion) increasing the yield (vertical expansion), shrinking the yield gap and reducing yield losses. Weed infestation is one of the major constraints responsible for low yields of rice in India. Several studies conducted in India and abroad indicate that weed incidence in low land situation accounts for a yield loss of 11-12 per cent in transplanted rice (Ghosh and Moorthy, 1998). Reliance on herbicides for weed control is expected to continue because there is no other attractive superior technology available (Singh and Bhan, 1998).

*Azolla* is a free floating aquatic fern that assimilates nitrogen in symbiotic association with the cyanobacterial symbiont, *Anabaena Azolla*. Biological nitrogen fixation (BNF) through *Azolla-Anabaena* complex could contribute 40-60 kg N ha<sup>-1</sup> in one crop of rice and is considered

to be a potential biological system for increasing rice yield at comparatively low cost in lowland rice cultivation. The important factor in using *Azolla* as biofertilizer for rice crop, is its quick decomposition in soil and better availability of nitrogen. Britto and Seethalakshmi (2002) reported that increased concentration of 2,4-D decreased the biomass and chlorophyll content of two *Azolla* species viz. *A. microphylla* and *A. filiculoides*.

The integration of *Azolla* and herbicides in rice cultivation was found to be superior over other treatments, which registered the synergistic interaction or improved weed reduction or yield increment when used in combination (Kathiresan *et al.* 2002). Hence the present investigation was undertaken to study the sensitivity of *Azolla* species to rice herbicides.

### Materials and Methods

A laboratory study was carried out for fixing the treatment combination for pot culture experiment, by using five herbicides (anilofos, butachlor, clomazone, 2,4-D and metsulfuran methyl) at recommended (x) and double the recommended (2x) dose compared with control and with four species of *Azolla* grown under water (50 ml water + 0.5 g *Azolla*) and soil

Table 1. Effect of herbicides and *Azolla* species on biomass (g) grown under water and soil medium

Treatment details		Water medium				Soil medium			
		Control	Normal dose	Higher dose	Mean	Control	Normal dose	Higher dose	Mean
<i>Herbicide</i>									
H <sub>1</sub> Anilofos	a <sub>1</sub>	0.230	0.450	0.540	0.407	0.645	0.630	0.605	0.627
	a <sub>2</sub>	0.690	0.840	0.695	0.742	0.670	0.665	0.760	0.698
	a <sub>3</sub>	0.500	0.430	0.580	0.503	0.630	0.635	0.585	0.617
	a <sub>4</sub>	0.505	0.635	0.840	0.660	0.795	0.580	0.765	0.713
	Mean	0.481	0.589	0.664		0.685	0.627	0.679	
H <sub>2</sub> Butachlor	a <sub>1</sub>	0.235	0.535	0.475	0.415	0.740	0.970	0.480	0.730
	a <sub>2</sub>	0.740	0.825	0.655	0.740	0.725	0.785	0.515	0.675
	a <sub>3</sub>	0.595	0.495	0.535	0.542	0.740	0.765	0.705	0.737
	a <sub>4</sub>	0.830	0.585	0.770	0.728	0.690	0.740	0.535	0.655
	Mean	0.600	0.610	0.609		0.724	0.815	0.559	
H <sub>3</sub> Clomazone	a <sub>1</sub>	0.330	0.460	0.565	0.452	0.500	0.480	0.675	0.552
	a <sub>2</sub>	1.045	0.580	0.685	0.770	0.830	0.625	0.580	0.678
	a <sub>3</sub>	0.650	0.425	0.485	0.520	0.950	0.670	0.640	0.753
	a <sub>4</sub>	0.590	0.870	0.680	0.713	0.670	0.565	0.830	0.688
	Mean	0.654	0.584	0.604		0.737	0.585	0.681	
H <sub>4</sub> 2,4-D	a <sub>1</sub>	0.180	0.370	0.495	0.348	0.880	0.630	0.695	0.735
	a <sub>2</sub>	0.785	0.565	0.705	0.685	0.780	0.735	0.695	0.737
	a <sub>3</sub>	0.535	0.570	0.785	0.630	0.325	0.590	0.615	0.510
	a <sub>4</sub>	0.625	0.545	0.810	0.660	0.780	0.525	0.875	0.727
	Mean	0.531	0.512	0.699		0.691	0.620	0.720	
H <sub>5</sub> Metasul-furan methyl	a <sub>1</sub>	0.335	0.500	0.570	0.468	0.275	0.775	0.995	0.682
	a <sub>2</sub>	0.645	0.660	0.775	0.693	0.270	0.975	0.525	0.590
	a <sub>3</sub>	0.435	0.505	0.480	0.473	0.485	0.630	0.540	0.557
	a <sub>4</sub>	0.545	0.640	0.880	0.688	0.675	0.690	0.735	0.700
	Mean	0.490	0.576	0.676		0.426	0.767	0.699	

	SEd	CD (0.05)	SEd	CD (0.05)
H	0.006	0.012	0.015	0.029
a	0.005	0.010	0.013	0.026
d	0.005	0.009	0.011	0.023
Ha	0.012	0.014	0.029	0.058
ad	0.009	0.018	0.023	0.045
Hd	0.010	0.020	0.025	0.051
Had	0.020	0.041	0.051	0.101

a<sub>1</sub> - *A. microphylla*, a<sub>2</sub> - *A. filiculoides*, a<sub>3</sub> - TNAU hybrid, a<sub>4</sub> - Rong Ping hybrid

(20 g soil + 50 ml water + 0.5 g *Azolla*) medium in petri plates with two replications. From the screening study, three herbicides (anilofos, butachlor, clomazone) with four species of *Azolla* were screened for pot culture experiment. Pot culture experiment was conducted during February 2002 at Department of Soil Science and Agricultural

Chemistry, TNAU, Coimbatore with CO-47 cultivar of rice in FRBD with three replications. The treatments include the three selected herbicide at recommended and double the recommended doses and four *Azolla* species at 500 kg/ha alone and in combination constituting twenty treatments. Common recommended fertilizer dos

TABLE 2. MEAN PLANT HEIGHT AND NUMBER OF PRODUCTIVE TILLERS OF *Azolla* SPECIES ON PLANT GROWTH CHARACTERISTICS

Treatment	Active tillering stage					Flowering stage						
	a <sub>1</sub>	a <sub>2</sub>	a <sub>3</sub>	a <sub>4</sub>	a <sub>5</sub>	Mean	a <sub>1</sub>	a <sub>2</sub>	a <sub>3</sub>	a <sub>4</sub>	a <sub>5</sub>	Mean
H <sub>1</sub>	49.50	53.50	53.20	51.40	51.20	51.76	74.20	82.40	82.10	80.00	80.20	79.78
H <sub>2</sub>	48.90	52.60	52.20	51.40	50.33	51.08	73.40	81.60	81.00	80.40	79.80	79.24
H <sub>3</sub>	50.10	57.03	56.20	55.00	54.10	54.48	76.00	85.20	84.80	83.90	83.20	82.62
H <sub>4</sub>	45.80	48.20	48.06	47.30	47.00	47.27	68.40	72.80	72.50	71.00	70.10	70.96
Mean	48.57	52.83	52.41	51.27	50.65		73.00	80.50	80.10	78.82	78.32	
SED	H					Ha	H					Ha
CD (0.05)	0.29					0.66	0.21					0.48
	0.60					1.35	0.43					0.97
	a						a					
	0.33						0.24					
	0.67						0.49					
	Number of tillers											
H <sub>1</sub>	4.66	6.80	6.66	5.66	5.90	5.94	4.66	5.66	6.00	6.00	6.33	5.73
H <sub>2</sub>	4.70	6.20	6.20	5.80	6.00	5.78	4.66	5.66	5.66	5.66	4.66	5.26
H <sub>3</sub>	5.50	7.50	7.40	7.00	7.10	6.90	4.66	8.00	8.00	7.33	5.66	6.73
H <sub>4</sub>	4.20	4.90	4.70	4.60	4.50	4.58	3.00	4.66	4.33	4.00	3.00	3.80
Mean	4.76	6.35	6.24	5.76	5.87		4.25	6.00	6.00	5.75	4.91	
SED	H					Ha	H					Ha
CD (0.05)	0.16					0.36	0.55					1.24
	0.32					0.73	1.12					2.52
	a						a					
	0.18						0.62					
	0.36						1.26					
H <sub>1</sub> - Anilofos 0.4 kg ha <sup>-1</sup>	H <sub>2</sub> - Butachlor 1.25 kg ha <sup>-1</sup>					H <sub>3</sub> - Clomazone 0.3 kg ha <sup>-1</sup>					H <sub>4</sub> - Control	
a <sub>1</sub> - Control	a <sub>2</sub> - <i>A. microphylla</i>					a <sub>3</sub> - <i>A. filiculoides</i>					a <sub>4</sub> - TNAU hybrid	
											a <sub>5</sub> - Rong Ping hybrid	

was applied (120:38:38 kg ha<sup>-1</sup>) to all the treatments.

The experimental soil was moderately alkaline in reaction (pH 8.0) and low in soluble salt content (EC < 0.45 dSm<sup>-1</sup>). The available nitrogen status was low with medium available P and K. To assess the sensitivity of *Azolla* species to rice herbicides, plant growth parameters, yield attributes and yield were recorded.

### Results and Discussion

#### Sensitivity of *Azolla* to herbicides

Significant effect of herbicides on *Azolla* species on biomass (g) grown under water and soil medium were found out through screening study. Among the herbicides butachlor and clomazone performed well and were on par with each other followed by anilofos, 2,4-D and metsulfuran methyl (Table 1). Hence, three herbicides viz. anilofos, butachlor and clomazone were selected for pot culture study. Among the *Azolla* species all the four species performed well and are tolerant to

Table 3. Effect of herbicides and *Azolla* species on yield and yield attributes

Treatment	Panicle length (cm)					Mean	100 grain weight (g)					
	a <sub>1</sub>	a <sub>2</sub>	a <sub>3</sub>	a <sub>4</sub>	a <sub>5</sub>		a <sub>1</sub>	a <sub>2</sub>	a <sub>3</sub>	a <sub>4</sub>	a <sub>5</sub>	Mean
H <sub>1</sub>	20.60	22.66	22.40	21.33	21.13	21.62	1.44	1.54	1.52	1.48	1.45	1.48
H <sub>2</sub>	20.36	22.16	21.73	21.40	20.46	21.22	1.47	1.50	1.49	1.44	1.44	1.47
H <sub>3</sub>	20.43	25.26	23.23	23.23	22.86	23.00	1.44	1.64	1.59	1.56	1.52	1.55
H <sub>4</sub>	17.16	18.46	18.50	18.36	18.23	18.14	0.92	1.41	1.39	1.36	1.33	1.28
Mean	19.64	22.14	21.46	21.08	20.67	18.14	1.32	1.52	1.50	1.46	1.43	1.43
SEd	H						H					Ha
CD (0.05)	0.37			a		0.84	0.02			a		0.05
	0.76			0.42		1.71	0.04			0.05		0.10
				0.85								
Treatment	Straw yield (g/pot)					Mean	Grain yield (g/pot)					
	a <sub>1</sub>	a <sub>2</sub>	a <sub>3</sub>	a <sub>4</sub>	a <sub>5</sub>		a <sub>1</sub>	a <sub>2</sub>	a <sub>3</sub>	a <sub>4</sub>	a <sub>5</sub>	Mean
H <sub>1</sub>	96.66	120.00	113.33	108.33	105.00	108.66	40.00	53.33	51.66	50.00	50.00	49.00
H <sub>2</sub>	95.00	111.66	111.66	103.33	103.33	105.00	40.00	51.66	50.00	50.00	48.33	48.00
H <sub>3</sub>	100.00	145.00	128.33	125.00	125.00	124.66	41.66	55.00	55.00	53.33	53.33	51.66
H <sub>4</sub>	93.33	91.66	90.00	81.66	80.00	87.33	23.30	38.33	38.33	36.66	28.33	32.99
Mean	96.25	117.08	110.83	104.58	103.33	108.66	36.24	49.58	48.75	47.50	45.00	45.00
SEd	H						H			a		Ha
CD (0.05)	4.49			a		10.05	2.99			3.35		6.70
	9.09			5.02		20.34	6.06			6.78		13.56
				10.17								
H <sub>1</sub> - Anilofos 0.4 kg ha <sup>-1</sup>												
a <sub>1</sub> - Control												
H <sub>2</sub> - Butachlor 1.25 kg ha <sup>-1</sup>												
a <sub>2</sub> - <i>A. microphylla</i>												
H <sub>3</sub> - Clomazone 0.3 kg ha <sup>-1</sup>												
a <sub>3</sub> - <i>A. filiculoides</i>												
H <sub>4</sub> - Control												
a <sub>4</sub> - TNAU hybrid*												
a <sub>5</sub> - Rong Ping hybrid												

herbicides without any ill effects on its growth. So all the species of *Azolla* were chosen for pot culture experiment. The results of screening study showed that in higher dose of herbicides on the four *Azolla* species were able to survive but the growth was good only in recommended dose. Hence, recommended doses were selected for pot culture experiment.

The results of growth and yield parameters due to the effect of combination of herbicides with *Azolla* are presented in Table 2 and 3.

#### Effect on plant growth characters

##### Plant height (cm)

Significant differences in plant height at different stages were observed due to various treatments. At tillering stage among the herbicides tried, clomazone at 0.3 kg ha<sup>-1</sup> recorded the highest plant height (54.5 cm) followed by anilofos at 0.4 kg ha<sup>-1</sup> (51.7 cm). Regarding the *Azolla* sp. the highest plant height was observed in *A. microphylla* and was found to be on par with *A. filiculoides*. The interaction effect was also significant among the treatments. The highest plant height was observed in (clomazone

*A. microphylla*) (57.0 cm) and this was on par with (clomazone + *A. filiculoides*). The lowest plant height was observed in absolute control (48.6 cm). Similar results were observed in flowering stage.

#### Number of tillers

On perusal of the data, the number of tillers was in the range of 4.2 to 7.3. The maximum number of tillers were observed in clomazone applied pot (6.9) and among the *Azolla* species, *A. microphylla* recorded the highest number of tillers (6.3). The interaction effect was found to be non-significant. At flowering stage, significant effect due to interaction was observed among herbicides and *Azolla* species. The treatment which received clomazone + *A. microphylla* recorded the highest number of tillers (12.0) and the lowest was found in absolute control (7.0).

The data on productive tillers showed significant effect of treatments. The highest productive tillers were observed (6.7) in clomazone at 0.3 kg ha<sup>-1</sup> followed by (5.7) anilofos at 0.4 kg ha<sup>-1</sup>. Among the *Azolla* species, the highest number of productive tillers were observed in *A. microphylla* (6.0). The interaction effect was non-significant among the herbicides and *Azolla*.

#### Yield Attributes

The various treatments significantly influenced the yield attributes (Table 3). The highest panicle length and test weight were recorded in clomazone applied pots (23.0 cm, 1.55 g). Among the *Azolla* species, *Azolla microphylla* recorded the highest panicle length and test weight (22.1 cm, 1.52 g). The highest panicle length and test weight was observed in clomazone + *A. microphylla* followed by clomazone + *A. filiculoides*.

#### Yield

The grain and straw yields ranged from 23.3 to 55.0 g pot<sup>-1</sup> and from 93 to 145 g pot<sup>-1</sup> respectively. The lowest yield was observed in absolute control. The maximum grain and

straw yield was observed in clomazone + *A. microphylla* (55.0 g). The concomitant use of herbicides + *Azolla* may augment the grain yield by protecting the crop from weeds and probably by increasing the N fixation by *Azolla* species. The results of *Azolla* inoculation improving rice yields are in consonance with the reports of Nazeer and Prasad (1984) and Singh and Singh (1995). Among the herbicides tried, clomazone at 0.3 kg ha<sup>-1</sup> increased the grain yield.

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