



Efficiency of *Azolla* as a biofertilizer for zooplankton production

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Abstract: An attempt has been made to study the efficiency of *Azolla* as a biofertilizer for production of natural fish food organisms, i.e. zooplankton for a period of 28 days. Zooplankton dynamics reveals that production is higher in culture water treated with *Azolla* as compared to control. The highest (1609 nos. L⁻¹) and lowest (1250 nos. L⁻¹) zooplankton population were observed in 3.0 and 1.0 ml L⁻¹ of *Azolla* respectively. Mean values of zooplankton population showed following trend for *Azolla* treated water: *Cyclops* > *Daphnia* > *Brachionus* > *Nauplii* > *Moina*. Results indicate that *Azolla* can be used as a pond fertilizer to augment the aquaculture production as it is cost effective, eco-friendly and may supplement organic and chemical fertilizers.

Key words: *Azolla*, Biofertilizer, Zooplankton

Introduction

In recent year's biofertilizer play a significant role in maintaining soil fertility, water quality and enhancing productivity as they are inexpensive, eco-friendly and supplement chemical fertilizers. In plant production, role of biofertilizer as a productivity enhancer have been well established (Behera, 1982; Ali and Malik, 1987; Bhattcharya *et al.*, 2004). However, reports on the impact of biofertilizer on zooplankton production in aquaculture are scanty (Garg *et al.*, 1998; Nigam *et al.*, 2007). Therefore, an attempt has been made to study the efficacy of *Azolla* as a biofertilizer on zooplankton production. *Azolla* is a globally distributed small aquatic fern found in symbiotic association with cyanobacterium, *Anabaena azollae* in its leaves which is known to fix atmospheric nitrogen.

Materials and Methods

Culture of *Azolla* was developed and maintained in cemented tank using FYM fertilized water. Maximum biomass of *Azolla* was observed during the month of September, November, February and March. Zooplankton culture was developed separately in a large rectangular syntax tank of 100 L capacity filled with FYM fertilized water at the rate of 500 mg L⁻¹. Concentrate of zooplankton was collected from Tekari pond of live stock farm for inoculation to develop the culture. The swarm of zooplankton appeared within a week and was maintained by adding adequate amount of FYM fertilized water.

Plastic tanks of 15 litres capacity were used for culture trials. They were first filled with filtered borewell water and then fertilized with a constant dose of FYM, i.e. 500 mg L⁻¹. The fertilized water was allowed to stand undisturbed for a period of seven days. Thereafter it was treated with selected doses of *Azolla* with three sets of replicates. Doses of *Azolla* as ml L⁻¹ of water were applied on the basis of results obtained from preliminary and/ or screening

trials. Doses selected as 00 (control), 1.0, 2.0, 3.0, 4.0 and 5.0 ml L⁻¹ were applied fortnightly intervals. The culture water fertilized with varying levels of *Azolla* was also analyzed initially and during the experimental trials for various physico-chemical characteristics following standard methods of APHA (2005).

Zooplankton samples from each treatment were collected weekly in the morning by filtering 500 ml of water and preserved in 4 % formalin and glycerin solution (8:2) for quantitative estimation of different zooplankton population. Species - wise population density of zooplankton were estimated under zoom trinocular microscope at various magnifications using sedwickrafter cell counts and circular disc and expressed as nos. L⁻¹. Data were analyzed statistically as per methods suggested by Steel and Torrie (1982).

Results and Discussion

Response of different zooplankton groups in culture water treated with varying levels of *Azolla* have been presented and compared in Tables 1-3. Significant zooplankton population of 1609 nos. L⁻¹ (Table 2) was observed in 3 ml L⁻¹ of *Azolla* on 28th day. Zooplankton population showed an increasing trend in all treatments of *Azolla*. However, comparing the population on last day, it showed gradual increase from treatments 0.0 (control) to 3.0 ml L⁻¹ (782.5 to 1114.0 nos. L⁻¹) and then declined to treatment of 5.0 ml L⁻¹ (952.75 nos. L⁻¹). Decreasing order of treatments in terms of total zooplankton population on final day of experiment was observed as: 3.0 > 2.0 > 4.0 > 5.0 > 1.0 > 0.0 (control). Grading of zooplankton species on the basis of mean values of population in *Azolla* treated water at different time intervals showed the following trend: *Cyclops* > *Daphnia* > *Brachionus* > *Nauplii* > *Moina*.

No significant variations in physico-chemical characteristics of water were observed during culture trials with *Azolla* (Table 4). The pH of culture water was observed in the range of 7.8 to 8.4.

Table - 1: Population response of different zooplankton groups in culture water treated with varying levels of *Azolla* at different durations

Treatment of <i>Azolla</i> (ml L ⁻¹)	<i>Daphnia</i> population (nos. L ⁻¹)			
	7 th Day	14 th Day	21 st Day	28 th Day
Control (0.0)	113	137 ^b	174 ^b	216 ^c
1.0	113	169 ^b	204 ^b	258 ^c
2.0	148	212 ^a	264 ^a	298 ^b
3.0	171 ^a	229 ^a	287 ^a	371 ^a
4.0	154	215 ^a	269 ^a	337 ^b
5.0	163	204 ^a	266 ^a	312 ^b
Mean	147.00	194.33	244.00	298.78
SEm +	12.70	12.70	12.70	12.70
CD (5%)	NS	36.20	36.20	36.20
Treatment of <i>Azolla</i> (ml L⁻¹)	Cyclops population (nos. L⁻¹)			
Control (0.0)	117	142 ^b	176 ^c	221 ^c
1.0	141	182 ^b	252 ^b	308 ^b
2.0	154	221 ^a	273 ^b	331 ^b
3.0	181 ^a	227 ^a	317 ^a	389 ^a
4.0	144	216 ^a	267 ^b	317 ^b
5.0	148	187 ^a	239 ^b	287 ^c
Mean	147.50	195.83	254.00	308.00
SEm +	11.20	11.20	11.20	11.20
CD (5%)	NS	31.8	31.8	31.8
Treatment of <i>Azolla</i> (ml L⁻¹)	<i>Moina</i> population (nos. L⁻¹)			
Control (0.0)	108	134 ^a	163 ^a	186 ^b
1.0	115	151 ^a	181 ^a	217 ^b
2.0	128	156 ^a	193 ^a	266 ^a
3.0	144 ^a	179 ^a	220 ^a	287 ^a
4.0	121	154 ^a	192 ^a	232 ^b
5.0	101	129 ^a	140 ^a	180 ^c
Mean	119.50	150.50	181.61	228.00
SEm +	9.75	9.75	9.75	9.75
CD (5%)	NS	27.70	27.70	27.70
Treatment of <i>Azolla</i> (ml L⁻¹)	<i>Brachionus</i> population (nos. L⁻¹)			
Control (0.0)	114	147 ^a	192 ^a	209 ^b
1.0	152	174 ^a	207 ^a	224 ^b
2.0	164	188 ^a	222 ^a	260 ^b
3.0	156 ^a	208 ^a	243 ^a	287 ^a
4.0	132	177 ^a	224 ^a	255 ^a
5.0	124	161 ^a	212 ^a	246 ^a
Mean	140.33	175.83	216.67	246.94
SEm +	11.30	11.30	11.30	11.30
CD (5%)	NS	32.20	32.20	32.20
Treatment of <i>Azolla</i> (ml L⁻¹)	Nauplii population (nos. L⁻¹)			
Control (0.0)	87	131 ^b	168 ^c	195 ^c
1.0	98	158 ^b	205 ^b	243 ^b
2.0	104	161 ^b	211 ^b	261 ^b
3.0	101 ^a	185 ^a	237 ^a	297 ^a
4.0	99	148 ^b	211 ^b	252 ^b
5.0	87	156 ^b	203 ^b	241 ^b
Mean	103.83	156.50	205.83	248.17
SEm +	9.85	9.85	9.85	9.85
CD (5%)	NS	28.00	28.00	28.00

Initial population : *Daphnia* – 42 nos. L⁻¹; *Cyclops* – 64 nos. L⁻¹; *Moina* – 41 nos. L⁻¹; *Brachionus* – 58 nos. L⁻¹; Nauplii – 34 nos. L⁻¹

Means having different alphabets differ significantly.

Table - 2: Total zooplankton population in FYM fertilized water treated with *Azolla*

Treatment of <i>Azolla</i> (ml L ⁻¹)	Zooplankton population (nos. L ⁻¹)				Average plankton population (nos. L ⁻¹)
	7 th Day	14 th Day	21 st Day	28 th Day	
Control (0.0)	539	691	873	1027	782.50
1.0	639	834	1049	1250	943.00
2.0	698	938	1163	1417	1054.00
3.0	763	799	1285	1609	1114.00
4.0	652	910	1161	1393	1029.00
5.0	635	837	1064	1275	952.75

Initial population of zooplankton : 239 nos. L⁻¹

Table - 3: Mean values of different zooplankton species in culture water treated with *Azolla*

Duration (days)	<i>Daphnia</i>	<i>Cyclops</i>	<i>Moina</i>	<i>Brachionus</i>	<i>Nauplii</i>
7	147.00	147.50	119.50	140.33	103.83
14	194.33	195.83	150.50	175.83	156.50
21	244.00	254.00	181.61	216.67	205.83
28	298.78	308.83	228.00	246.94	248.17
Mean	221.03	226.54	169.90	194.94	178.58
SEm+	5.20	4.57	3.98	4.63	4.02
CD (5%)	14.78	13.00	11.32	13.16	11.44
Initial population	42	64	41	58	34

Table - 4: Physico-chemical characteristics of the FYM based culture water treated with *Azolla*

Characteristics	Ranges	Average
pH	7.8- 8.4	8.1 ± 0.3
Temperature (°C)	26.5 – 29.5	28 ± 1.5
Dissolved oxygen (mg L ⁻¹)	5.0 – 6.5	5.7 ± 0.75
Nitrate (mg L ⁻¹)	1.0 – 1.8	1.20 ± 0.20
Phosphate (mg L ⁻¹)	0.03 – 0.05	0.04 ± 0.01
Alkalinity (mg L ⁻¹)	242-262	252 ± 10

Overall temperature was about 28°C (+1.5°C). Alkalinity of the water was observed to fluctuate between 242 to 262 mg L⁻¹. Dissolved oxygen values ranged between 5.0 to 6.5 mg L⁻¹. Phosphorus levels was recorded in a very narrow range of 0.003 to 0.005 mg L⁻¹. Nitrate values were found to be ranging from 1.0 to 1.8 mg L⁻¹.

In the present study higher zooplankton population were also observed in all the treatments of *Azolla* as compared to control. However, maximum zooplankton population of 1609 nos. L⁻¹ was recorded in culture water treated with 3.0 ml L⁻¹ of *Azolla* on 28th day. The number of zooplankton in *Azolla* treated water was found to increase from 0.0 (control) to 3.0 ml L⁻¹ but declined gradually towards 5.0 ml L⁻¹. Nigam *et al.* (2007) also studied the impact of blue green algae (BGA) on zooplankton production and reported highest population of 1707 nos. L⁻¹ with 0.6 ml L⁻¹ for 28 days.

Results of the present study suggest that like various organic manures, *Azolla* may also be used for better zooplankton

production in fish culture management as it is less expensive as compared to organic and chemical fertilizers. Liu *et al.* (1982) introduced rice – fish – *Azolla* culture in China to enhance the farmer's income through increased fish production and reduced consumption of fertilizers and pesticides. Present results are also in agreement with the above findings regarding the use of *Azolla* as a biofertilizer to reduce the cost on organic and chemical fertilizers in aquaculture.

It is well known that addition of manures also cause depletion in dissolved oxygen of water during zooplankton culture (Sharma *et al.*, 1984; Shevgoor *et al.*, 1994; Qin *et al.*, 1995). However, contrary to this, in the present study application of *Azolla* increases the dissolved oxygen of the water. Stumm and Morgan (1970) suggested that addition of chemical fertilizers increases alkalinity of water which is favorable for plankton growth. In the present study application of *Azolla* led to an increase in pH, *i.e.* medium turned to alkaline. Total alkalinity was recorded in a favorable range (242-262 mg L⁻¹) for plankton growth. Peters *et al.* (1980) reported optimum temperature for *Azolla* species between 25 to 30°C. Present investigation are also in agreement with the above findings as the temperature range of 26.5 to 29.5 °C was helpful in increasing zooplankton population in culture water treated with *Azolla*. Sirgur (1988) reported that pH values ranging from 8.4 to 8.7 are optimum for the development of zooplankton. In the present investigation pH values were recorded in the range of 7.8 to 8.4, *i.e.* average 8.1 ± 0.3. This suggests that application of *Azolla* increases alkalinity of the culture water which had a positive effect on zooplankton production.

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