



Evaluation of Temperature - Tolerant *Azolla* Strains Suitable for Tiruvallur District

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ABSTRACT

The survey on *Azolla* diversity revealed that *Azolla pinnata* and *Azolla microphylla* are the two species found throughout the agro-climatic zones of Tamil Nadu. The pot-culture experiments to screen the strains for local adaptability for the Tiruvallur district revealed that during cold months (Nov-Dec), most of the species grow with high growth rate (5 to 7 days doubling time) and during Jan-Feb, the doubling time increased from 7.5 to 9.5 days. Among the different *Azolla* species studied, *A. pinnata* and *A. microphylla* recorded a high growth rates and higher biomass production at elevated temperatures. Selected *Azolla* strains were highly suitable to grow well under open field conditions. *Azolla pinnata* and *Azolla microphylla* strains suitable for high-temperature zone district of Tamil Nadu (Tiruvallur) were identified. These strains can withstand the temperature up to 37 °C in the months of July – Aug and produce high biomass and application of *Azolla microphylla* (Dual crop) with a 75 per cent recommended dose of NPK recorded as the maximum grain and straw yield of rice followed by the *Azolla pinnata* (dual crop) with 75 per cent recommended dose of NPK. These *Azolla* strains could be used as animal feed and also as green manure.

Key words: Rice; *Azolla pinnata*; *Azolla microphylla*; Biomass; Growth; Yield

INTRODUCTION

Azolla is widely used as a potential biofertilizer for increasing the grain yield of rice in India, China, Vietnam, Thailand, Philippines, Korea, Sri Lanka, Bangladesh, Pakistan, Nepal, Burma, Indonesia, Brazil and West Africa (Diara et al., 1987; Kannaiyan, 1987; Loudhapasitiporn and Kanareugsa, 1987; Mabbayad, 1987; Watanabe, 1987a). 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 plants. In tropical rice soils, the applied *Azolla* mineralizes rapidly, and its nitrogen is made available to rice crops quickly. Inoculation of *A. microphylla* on 10 DAT at 1 t ha⁻¹ and incorporation at 30 DAT increased the soil organic carbon content and available N status (Thamizhvendan et al., 1998).

Incorporation of *Azolla* into soil increased grain yield by 20 per cent in field experiments at 12 sites in South Asian countries (Watanabe, 1982). A positive rice crop response with *Azolla* inoculation was reported in four different sites of Tamil Nadu viz., Coimbatore, Aliyarnagar, Ambasamudram and Tirurkuppam (Kannaiyan et al., 1983b). *Azolla pinnata* inoculation at 10 t ha⁻¹ as fresh biomass and a combination of *Azolla pinnata* inoculation with fertilizer nitrogen at 30 kg N ha⁻¹ recorded increased grain and straw yield of rice (Mathewkutty and Sreedharan, 1983). Reynaud and Franche (1986) reported that *Azolla* inoculation always increased the grain yield of rice. The increase was 30-40 per cent when fresh *Azolla* was incorporated before or after transplanting and 54 per cent when incorporated both before and after transplanting.

Shanmugasundaram and Kannaiyan (1994) found that *Azolla* hybrid Rong Ping along with fertilizer nitrogen increased the grain yield and yield components of rice. Even though *Azolla* has high-potential as bioinoculant, it is sensitive to high temperature, salinity, sunlight, pests, and diseases. Among the several abiotic factors controlling the growth of *Azolla*, temperature is one of the most sensitive factors. Tiruvallur district of Tamil Nadu is one of the largest wetland rice growing areas of Tamil Nadu and hence using *Azolla* as bioinoculant will enhance the yield along with soil fertility sustainability. As native, *Azolla* species could not tolerate the high temperature prevailing in this region (> 40 °C in summer) which leads to poor performance and maintenance of *Azolla*. Hence, it is essential to assess the high temperature tolerant strains suitable for this locality.



MATERIALS AND METHODS

The native *Azolla* strains from different agro-climatic zones of Tamil Naduviz., Pollachi, Anaimalai, Coimbatore, Madurai, Needamangalam, Kancheepuram, Pondicherry, Tiruvallur, Nellai, and Kanniyakumari were collected and maintained in cement tanks (1 x 1 x 0.5 m). The species identification was performed based on the morphological features and trichome morphology (Dunham and Fowler, 1987). The growth rate, doubling time, biomass production, and carbon:nitrogen ratio of different *Azolla* strains were assessed as per the procedures described by Kannaiyan (1992). The relative growth rate was calculated from growth rate using the formula as described by Kannaiyan and Kumar (2005).

$$\text{Relative growth rate (g/g/day)} = \frac{0.639}{\text{Doubling time}}$$

Where, 0.639 is the growth factor constant, and doubling time refers to the time in days required for *Azolla* to double its biomass.

The field experiment was conducted at Rice Research Station, Tirur (13°06'32.0"N, 79°57'37.2"E) to assess the impact of temperature-tolerant *Azolla* spp. on the growth and yield of rice (ADT45). The field experiment was conducted in August, 2016 under the SRI method of rice cultivation. The treatments include *Azolla microphylla*(Green manure) + 75% NPK; *Azolla microphylla*(Dual crop) + 75% NPK; *Azolla pinnata*(Green manure) + 75% NPK; *Azolla pinnata*(Dual crop) + 75% NPK; 100 % recommended dose of NPK. The *Azolla* spp. were incorporated as green manure (6.25 t/ha) or as dual crop (500 kg/ha) depending upon the treatment. The experiment was conducted in a Randomized Block Design with three replications. The plant height, tillers production, panicle length, grain and straw yield, and nitrogen uptake of rice were recorded at the time of harvest.

The data were subjected to statistical analysis using analysis of variance, and critical difference at $p < 0.05$ was used to differentiate the means (Gomaz and Gomaz 1984).

RESULT AND DISCUSSION

Effect of application of temperature-tolerant *Azolla* strain *Azolla microphylla* and *Azolla pinnata* on the growth and yield of rice (ADT45) under natural field conditions

The growth rate, biomass production and nutrient content of the *Azolla* strains were assessed. The results revealed that all the *Azolla* cultures had maximum biomass production and relative growth rate during November- December (Table 1) than in other months. The results also indicated that apart from temperature, relative humidity, wind velocity and evaporation would have a significant role of *Azolla* biomass production. Among the four *Azolla* strains *A. pinnata* has recorded maximum biomass production when compared to other strains. However, the usual rate of multiplication of *Azolla* is about five fold over period of five weeks (Table 2). The carbon and nitrogen contents of the *Azolla* species revealed that *Azolla pinnata* had high carbon (42.78%) and N content (3.62%) than other species (Table 3).

Table 1. Growth of different strains of *Azolla* from different agro-climatic zones of Tamil Nadu during different months at RRS, Tirur

Locations	<i>Azolla</i> species	Growth parameters							
		July – Aug		Sep-Oct		Nov-Dec		Jan - Feb	
		Doubling time (Days)	RGR (g/g/day)	Doubling time (Days)	RGR (g/g/day)	Doubling time (Days)	RGR (g/g/day)	Doubling time (Days)	RGR (g/g/day)
Pollachi, Anaimalai	<i>A.pinnata</i>	8.00	0.0866	7.00	0.099	5.50	0.126	7.00	0.099
	<i>A.microphylla</i>	7.50	0.0924	6.50	0.106	5.00	0.138	6.50	0.011
Coimbatore	<i>A.pinnata</i>	7.50	0.0924	6.50	0.106	5.00	0.138	6.50	0.011
	<i>A.microphylla</i>	7.50	0.0924	7.50	0.092	5.00	0.138	7.00	0.099
	<i>A.filiculoides</i>	8.00	0.0866	8.00	0.086	6.00	0.115	7.50	0.092
	<i>A.rongping</i>	9.50	0.0729	8.00	0.086	7.00	0.099	8.50	0.082
Madurai	<i>A.pinnata</i>	7.50	0.0924	6.50	0.106	5.00	0.138	6.50	0.011
Needamangalam	<i>A.pinnata</i>	7.50	0.0924	7.50	0.092	5.00	0.138	6.50	0.011
Kancheepuram	<i>A.pinnata</i>	7.50	0.0924	7.00	0.099	5.00	0.138	6.50	0.011
	<i>A.microphylla</i>	8.00	0.0866	6.50	0.106	5.50	0.126	7.00	0.099



Tiruvallur	<i>A.pinnata</i>	7.50	0.0924	6.50	0.106	5.00	0.138	6.50	0.011
	<i>A.microphylla</i>	8.00	0.0866	7.00	0.099	5.50	0.126	7.00	0.099
Pondicherry	<i>A.pinnata</i>	7.50	0.0924	7.50	0.092	5.00	0.138	5.00	0.138
Nellai	<i>A.pinnata</i>	7.50	0.0866	7.50	0.086	5.50	0.126	5.50	0.126
Kaniyakumari	<i>A.microphylla</i>	7.50	0.0924	6.00	0.115	5.00	0.138	5.00	0.138
	<i>A.pinnata</i>	8.00	0.0866	7.00	0.099	5.00	0.138	5.00	0.138
	<i>A.microphylla</i>	7.50	0.0924	7.50	0.092	5.00	0.138	5.00	0.138
CD (5%)		0.42	0.0012	0.41	0.021	0.31	0.005	0.45	0.004

Doubling time refers the days to double the biomass under natural field conditions; RGR refers the relative growth rate of Azolla (g of biomass produced per g of Azolla per day).

Table 2. Effect of temperature on the growth of Azolla

Locations	Azolla species	27 °C/ 23 °C (Day/ Night)		32 °C/ 25 °C (Day/ Night)	
		Doubling time (Days)	Biomass (gf.wt)	Doubling time (Days)	Biomass (gf.wt)
Pollachi, Anaimalai	<i>A. pinnata</i>	5.00	250	7.50	250
	<i>A. microphylla</i>	5.50	250	8.00	250
Coimbatore	<i>A. pinnata</i>	5.00	250	7.50	250
	<i>A. microphylla</i>	5.00	250	7.50	250
	<i>A. filiculoides</i>	6.00	250	8.00	250
	<i>A.rong ping</i>	7.00	250	9.50	250
Madurai	<i>A.pinnata</i>	5.00	250	7.50	250
Needamangalam	<i>A.pinnata</i>	5.00	250	7.50	250
Kanchipuram	<i>A.pinnata</i>	5.00	250	7.50	250
	<i>A.microphylla</i>	5.50	250	8.00	250
Tiruvallur	<i>A.pinnata</i>	5.00	250	7.50	250
	<i>A.microphylla</i>	5.50	250	8.00	250
Pondicherry	<i>A.pinnata</i>	7.50	250	7.50	250
Nellai	<i>A.pinnata</i>	7.50	250	7.50	250
	<i>A. microphylla</i>	7.50	250	7.50	250
Kaniyakumari	<i>A.pinnata</i>	8.00	250	8.00	250
	<i>A. microphylla</i>	7.50	250	7.50	250
CD (5%)		0.46	NS	0.44	NS

Doubling time refers the days to double the biomass under natural field conditions; RGR refers the relative growth rate of Azolla (g of biomass produced per g of Azolla per day)

Table 3. C: N ratio of Azolla species grown at RRS, Tirur during 2016

Azolla strains	C%	N%	C:N ratio	C%	N%	C:N ratio
	July- Aug			Nov- Dec		
<i>A.pinnata</i>	32.76	3.26	10.04	33.70	3.36	10.02
<i>A.microphylla</i>	40.78	3.52	11.58	42.78	3.62	11.81
<i>A.filiculodes</i>	36.76	3.41	10.78	37.70	3.51	10.74
<i>Rong ping</i>	32.42	2.86	11.33	42.78	3.62	11.81
CD (5%)	1.25	0.52	0.55	4.27	0.38	0.36

Effect of application of temperature -tolerant Azolla strain *Azolla microphylla* and *Azolla pinnata* on the growth of rice

Application of temperature-tolerant *Azolla* strains viz., *Azolla microphylla* and *Azolla pinnata* significantly influenced the plant height of ADT 45 rice (Table 4). The maximum plant height was obtained due to the application *Azolla microphylla* (Dual crop) with 75 percent recommended dose of NPK followed by the *Azolla pinnata* (Dual crop) with 75 percent recommended dose of NPK at all growth stages, during which observations were recorded. The highest number of tillers and productive tillers were registered due to the application of *Azolla microphylla* (Dual crop) with 75 percent recommended dose of NPK followed by the *Azolla pinnata* (Dual crop) with 75 percent recommended dose of NPK both on 25 DAP (14.000) and 45 DAP (20.50) which was however statistically on par with 100 per cent recommended dose of NPK (Table 4).

Table 4. Effect of application of temperature tolerant *Azolla* strain *Azolla microphylla* and *Azolla pinnata* on the growth parameters in rice

Treatments	Plant height (cm)				Tiller number		Productive tillers
	25 DAP	45 DAP	65 DAP	85 DAP	25 DAP	45 DAP	65 DAP
<i>Azolla microphylla</i> (Green manure) + 75% NPK	54.78	65.55	75.10	82.18	12.50	16.50	13.50
<i>Azolla microphylla</i> (Dual crop) + 75% NPK	58.43	73.73	79.98	86.53	14.00	20.50	17.50
<i>Azolla pinnata</i> (Green manure) + 75% NPK	53.35	63.95	74.00	81.33	12.00	16.50	13.50
<i>Azolla pinnata</i> (Dual crop) + 75% NPK	54.68	67.58	75.83	83.13	13.00	18.50	15.50
100 % recommended dose of NPK	53.35	63.95	74.00	81.33	12.50	16.00	13.50
	SEd		CD (5%)		SEd		CD (5%)
Treatments	0.01581		0.03149		3.60500		7.22187
Days	0.01414		0.02817		2.79242		5.59404
Interaction	0.03162		0.06298		6.24404		12.50865

Effect of application of temperature-tolerant *Azolla* strain, *Azolla microphylla* and *Azolla pinnata* on the yield parameters of rice

A significant increase in the yield components viz., panicle length, grains per panicle and 1000 grain weight over control was observed due to the application of *Azolla microphylla* (Dual crop) with 75 percent recommended dose of NPK followed by the *Azolla pinnata* (Dual crop) with 75 percent recommended dose of NPK. The panicle length, grains per panicle and 1000 grain weight were the lowest in the control, which received 72 kg N ha⁻¹ as 100 per cent recommended dose of NPK (Table 5).

Table 5. Effect of application of temperature-tolerant *Azolla* strain *Azolla microphylla* and *Azolla pinnata* on the yield parameters of rice

Treatments	Panicle length (cm)	Grains per panicle	1000 Grain weight (g)
<i>Azolla microphylla</i> (Green manure) + 75% rec NPK	19.60	124.00	16.65
<i>Azolla microphylla</i> (Dual crop) + 75% rec NPK	23.40	149.50	18.55
<i>Azolla pinnata</i> (Green manure) + 75% rec NPK	19.00	124.00	16.13
<i>Azolla pinnata</i> (Dual crop) + 75% rec NPK	20.40	128.75	17.13
100 % recommended dose of NPK	19.40	124.00	16.00
	S E d		CD (5%)
Treatments	0.01265		0.02534
Days	0.00980		0.01963
Interaction	0.02191		0.04389



Effect of application of temperature - tolerant *Azolla* strain *Azolla microphylla* and *Azolla pinnata* on the straw, grain yield and N uptake of rice of rice

Among the treatments, application of *Azolla microphylla* (Dual crop) with 75 percent recommended dose of NPK recorded the maximum N uptake followed by the *Azolla pinnata* (Dual crop) with 75 percent recommended dose of NPK (Table 6).

Table 6. Effect of application of temperature - tolerant *Azolla* strain, *Azolla microphylla* and *Azolla pinnata* on the straw& grain yield and N uptake of rice.

Treatments	Straw yield (kg ha ⁻¹)	Grain yield (kg ha ⁻¹)	N uptake by plant (mg plant ⁻¹)
<i>Azollamicrophylla</i> (Green manure) + 75% rec NPK	6312	5290	1.26
<i>Azollamicrophylla</i> (Dual crop) + 75% rec NPK	6586	5480	1.60
<i>Azollapinnata</i> (Green manure) + 75% rec NPK	6144	5120	1.20
<i>Azollapinnata</i> (Dual crop) + 75% rec NPK	6420	5350	1.27
100 % recommended dose of NPK	6060	5050	1.00
Sed	50.91	40.84	0.26
CD (5%)	107.93	86.59	0.59

CONCLUSION

The present survey on *Azolla* diversity reveals that *Azolla pinnata* and *A. microphylla* are the two species found throughout Tamil Nadu agro-climatic zones. The pot-culture experiments to screen the strains for local adoptability for Tiruvallur district revealed that during cold months (Nov-Dec), most of the species grow with high growth rate (5 to 7 days doubling time) and during Jan-Feb, the doubling time increased from 7.50 to 9.50 days. Among the different *Azolla* species studied, *A. pinnata* and *A. microphylla* recorded high growth rate as well as higher biomass production at elevated temperature. Selected *Azolla* strains were highly suitable to grow well under open field conditions. *Azolla pinnata* and *Azolla microphylla* strains suitable for high-temperature zone district of Tamil Nadu (Tiruvallur) were identified. These strains can withstand the temperature up to 37°C in the months of July – Aug and produce high biomass and application of *Azolla microphylla* (Dual crop) with 75 per cent recommended dose of NPK recorded the maximum grain and straw yield of rice followed by the *Azolla pinnata* (dual crop) with 75 per cent recommended dose of NPK. These *Azolla* strains could be used as animal feed and also as green manure for rice specific to Tiruvallur district.

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