Studies on the Adaptation of *Azolla mexicana* in the Aegean and the Mediterranean Regions

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Abstract : Azolla (*Azolla anabaena*) is an aquatic fern plant that can be used as a green manure and organic matter resource in many countries where irrigation water is not a problem. *A. mexicana* is one of the azolla species adapted to the environmental conditions of Izmir (Turkey). The objective of this research was to determine the adaptation and agronomic potential of azolla plants to the Mediterranean climate regions. The research was conducted at three locations (Izmir and Aydın located in the Aegean region and Antalya located in the Mediterranean region of Turkey) in 1999, 2000 and 2001. Fresh azolla was planted in April, May, June and October at the rate of 300 g m² at each application. The growing azolla plants were harvested 15 days after planting. The highest fresh yield (1481 g m²) was obtained in April at Izmir. The total N, P, and K values were between 2.93-3.31 %, 0.43-0.54 % and 1.10-1.37 % at Izmir, Aydın, and Antalya, respectively. The shortest time to reach a two-fold increase was 3.53 days at Izmir based on the average of three years.

Key words : Adaptation, Azolla mexicana, Dry weight, Fresh weight, Organic matter.

Azolla is an aquatic floating fern with a worldwide distribution. The plant consists of a short, branched, floating rhizome with small leaf-like fronds and has roots elongating into the water. A symbiotic blue-green algae, *Anabaena azolla* lives in cavities of azolla fronds, fixing atmospheric nitrogen. Azolla rapidly colonizes on the water surface and doubles its original fresh weight in 3 to 7 days under suitable environmental conditions (Hove, 1989; Thomas et al., 1982; Watanabe et al., 1977).

Normally, azolla in dual culture with rice can reach full bloom in 15 to 20 days and releases approximately 30 kg N ha⁻¹. Two or three cultures of azolla in one rice growing season would add 60 to 80 kg N per hectare. The biomass of azolla in pure culture in tropics could be around 200 tons per hectare per year with 22 harvests in 16-day intervals. This amount of azolla contained 480 to 500 kg nitrogen (Khan, 1987). Nitrogen in dry matter is approximately 4-6 % and changes to ammonium form which can be easily taken up by plants in 14 to 21 days and thus can be used as organic fertilizer (Hove, 1989; Watanabe et al., 1977; Wilbur and Watanable, 1980; Gevrek and Yagmur, 1996). A. filiculoides and A. mexicana grown with rice in California increased rice yield 23 % and 67 %, respectively (Talley et al., 1977).

Culture of azolla may be useful to rice farmers in the Mediterranean region where certain azolla species (*A. filiculoides, A. carolina* and *A. mexicana*) are adapted, with a higher growth rate and dry weight as compared

to the mediocre species adapted to the tropical regions.

It is known that *A. caroliana* and *A. filiculiadies* grow spontaneously in rice fields and wet-lands of Europe (Hove, 1989). Also, certain species of azolla plants are known to exist in certain areas of the northwestern region of Turkey and species determination is still underway (Unal and Uzen, 1996; Gevrek, 2000).

Since azolla grows only in wet or stagnant water conditions and rice fields, utilization of azolla in the Mediterranean type climate regions could be important for the production of organic matter and green manure. The Azolla plant shows a great specificity to its growing environment (Ashton, 1971; Hove, 1989). The air temperatures and relative humidity are the main factors for the adaptation of azolla strains to the region (Thomas et al., 1982).

There is little information available about A. mexicana growing in the Mediterranean countries. Therefore, in this preliminary adaptation study, strains of A. mexicana was collected from IRRI, Philippines. The agronomic potentiality of azolla plants was not previously studied in the Mediterranean regions. It is necessary to explore such potentiality for organic farming. The objective of this study was to evaluate adaptation and agronomic potential of A. mexicana-2026 strain in the Aegean and Mediterranean coastlines of Turkey.

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Table 1.	Minimum an	nd maximum	monthly air
temper	atures (° C) at	three location	s (Izmir, Aydın
and An	talya, Turkey) ii	n 1999, 2000, an	d 2001.

	Minimum monthly air temperatures (°C)										
	Locations										
		Izmir			Aydın			Antalya			
Month	1999	2000	2001	1999	2000	2001	1999	2000	2001		
1	1.9	-4.5	1.0	0.9	-4.5	0.3	7.5	7.0	7.1		
2	-0.9	-1.1	-0.5	-1.2	-1.0	1.0	7.3	7.1	7.0		
3	-0.5	-0.7	4.9	2.0	-0.4	2.3	8.5	9.0	9.1		
4	5.5	6.0	4.2	6.6	6.1	6.6	11.7	12.4	12.3		
5	8.1	5.4	7.5	9.2	7.8	14.9	16.4	15.7	15.5		
6	14.6	11.4	9.3	16.0	14.0	18.8	21.5	21.5	20.1		
7	17.6	13.7	16.8	19.1	18.2	22.4	24.1	24.2	20.4		
8	14.6	12.1	17.0	16.0	16.4	22.3	23.9	24.0	24.1		
9	10.5	11.5	10.8	14.0	10.2	17.7	20.0	20.4	22.1		
10	7.9	5.7	2.9	8.5	6.0	13.4	16.8	17.1	16.9		
11	0.5	4.4	-0.9	-0.4	6.2	9.3	11.8	12.2	12.7		
12	2.7	-0.4	-3.3	2.0	5.3	7.8	9.8	10.0	11.2		

	Maximum monthly air temperatures (°C)										
				L	ocations	6					
		Izmir			Aydın			Antalya			
Month	1999	2000	2001	1999	2000	2001	1999	2000	2001		
1	13.1	8.6	14.0	18.0	16.9	15.9	16.7	17.0	17.3		
2	13.0	13.0	14.0	19.5	20.1	15.7	16.7	17.3	16.9		
з	16.9	15.7	18.0	23.0	27.2	23.7	18.2	22.3	21.8		
4	22.5	22.4	23.0	30.3	29.5	23.0	21.8	21.3	22.3		
5	28.5	27.2	26.0	34.6	34.2	28.7	28.5	26.8	27.5		
6	31.3	32.1	31.0	37.0	41.0	34.7	32.5	34.1	31.4		
7	33.9	35.7	35.6	40.3	44.4	38.0	34.8	36.5	34.0		
8	33.6	34.3	36.4	41.0	39.8	37.1	33.8	34.3	34.0		
9	29.8	30.3	30.0	36.8	39.4	32.0	31.5	32.1	37.0		
10	25.0	26.0	25.0	33.0	26.0	28.5	27.8	28.2	27.3		
11	18.0	17.6	17.6	25.1	23.0	18.1	22.0	21.0	22.3		
12	15.6	13.0	10.8	21.7	15.8	12.0	14.3	14.6	15.0		

Materials and Methods

This research was carried out at three locations (Izmir and Aydın located in the Aegean region and Antalya located in the Mediterranean coast, Turkey) in 1999, 2000 and 2001. *A. mexicana*-2026 strain was used in this study.

Tables 1 and 2 show average and maximum air temperatures, water temperatures and relative humidity at each location. The air temperatures in April, May and June increase about 5.0 °C in each month (Table 1). Such a sudden increase in air temperature at three locations caused a drastic reduction in vegetative development and finally azolla sedimentation (Ashton, 1971; Thomas et al., 1982). Based on temperature range, azolla was inoculated only in April, May, June, and October at all locations.

Fresh azolla plants were inoculated at the rate of 300 g m⁻² into 4 m x 4 m (16 m⁻²) ponds. All ponds were constantly stagnant with a water level of about 20 cm. To promote the vegetative development of azolla plants and nitrogen fixation of Anabaena algae, we applied 0.20 g m⁻² of P_2O_5 to the ponds as triple superphosphate at seven-day intervals (Hove, 1989; Khan, 1987).

Fresh azolla plants were collected by hand 14 days after inoculation and fresh weight (g m⁻²), dry matter (g m⁻²), organic matter (%), C/N, water holding capacity were measured. Macro and micro nutrient elements in harvested azolla plants from the locations were also determined to evaluate the potentiality of the

Table 2.	Average monthly water temperature (° C) and
relati	ve humidity (%) values at three locations (Izmir,
Aydıı	n and Antalya, Turkey) in 1999, 2000, and 2001.

Average water temperatures (° C) ¹											
		Izmir			Aydır	1		Antalya	1		
Month	1999	2000	2001	1999	2000	2001	1999	2000	2001		
4	26.7	26.0	26.1	35.2	35.0	27.0	26.8	26.3	27.3		
5	33.4	33.1	33.4	38.7	38.2	32.6	32.5	35.8	37.5		
6	35.4	36.4	36.3	41.5	45.0	39.5	36.5	38.1	36.4		
7	36.7	37.4	38.6	44.6	47.1	42.0	38.8	39.5	40.0		
8	35.6	37.3	37.4	46.0	44.2	42.1	34.8	36.3	39.0		
9	32.4	33.3	34.4	40.4	40.2	36.0	31.5	32.1	34.0		
10	27.4	28.0	29.1	37.0	30.1	31.5	27.8	28.2	27.3		

			Aver	age rel	ative hi	umidity ((%)		
		Izmir			Aydır	1		Antalya	1
Month	1999	2000	2001	1999	2000	2001	1999	2000	2001
1	75.3	68.0	77.3	72.8	61.5	71.6	71.5	70.0	71.0
2	78.0	69.3	74.2	70.2	63.0	67.9	71.5	69.0	72.0
3	72.0	67.4	65.3	63.6	58.8	60.7	62.6	61.2	63.4
4	65.4	57.2	68.8	58.3	63.4	62.0	69.3	65. 9	69.3
5	53.5	58.9	59.1	49.5	52.3	56.1	59.6	57.3	58.1
6	54.0	46.5	48.2	47.1	45.7	41.2	52.9	42.6	42.3
7	53.8	46.6	49.7	54.6	47.5	44.8	56.6	53.3	69.2
8	53.8	49.3	56.1	50.1	51.2	53.8	59.2	58.7	68.6
9	60.9	59.1	65.7	57.1	49.8	54.2	55.6	60.5	67.7
10	69.0	60.0	61.0	60.5	61.7	54.3	55.9	55.0	55.5
11	67.1	72.0	78.4	63.6	61.3	68.7	53.4	64.0	67.9
12	77.4	80.0	81.3	71.4	68.7	77.0	70.1	72.0	71.7

¹ Average temperature values at 14⁰⁰ hour of the day below 10 cm water level.

Table 3. Selected physical and chemical properties of soil samples of the research fields at three locations (Izmir, Aydın, and Antalya).

Soil properties	Izmir	Aydın	Antalya
pН	7.0	7.6	7.9
Total salt (%)	0.10	0.04	0.03
Lime (%)	9.20	22.20	29.20
Texture	Silty loam	Sandy loam	Sandy loam
Organic matter (%)	1.40	1.30	2.40
Total N (%)	0.06	0.08	0.10
P (ppm)	3.70	4.60	1.80
K (ppm)	300.00	105.00	245.00

organic matter as a biofertilizer. Dry matter content was determined after drying at 65.0° C for 24 hours in an forced-air oven. The dried samples were ground in a grinding machine. The ground samples were mixed with HNO₃+HClO₄ and wet burning was done. K, Na, and Ca were measured by flame photometry and Mg, Fe, Zn, Mn and Cu by ASS (Kacar, 1972). Total P was measured by the vanado-molibdo phosphorile yellow colour method. Total nitrogen in the plant samples was measured by the modified Kjeldahl method (Kacar, 1972).

Table 3 shows the physical and chemical properties of the soil samples from research ponds. Surface soil (0-25 cm) was collected, air dried in the laboratory and then sieved through 2 mm sieves. PH values of the sieved soil samples were measured by pH-meter after saturation of soil with pure water (Jackson, 1962). Total water soluble salts of the same soil paste were

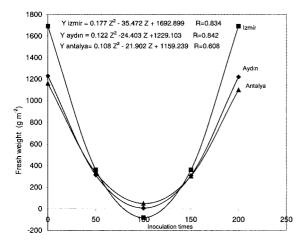


Fig. 1. Fresh weight as a function of inoculation days starting from April to October (0-200 days).

determined with the help of a reduction monogram after measuring the resistance with a conductometer. Lime of soil was measured by the volumetric method and texture of the soil was determined as hydrometric fractions. Texture properties were defined by using the soil triangle. Organic matter was determined by the fresh burning method and total nitrogen by using a modified Kjeldahl method (Bremner, 1965). Utilizable P was measured colorimetrically by the water extraction method, whereas K, Na, Ca and Mg were extracted with 1 M NH₄OAC. K, N and Ca were measured by flame photometry and that of Mg by atomic absorption spectroscopy (AAS) (Pratt, 1965). Usable Fe, Zn, Mn and Cu were measured by AAS after extraction with DTPA (Lindsay and Norwell, 1978). The soils in the locations were alkaline in reaction and silty-sandy loam in texture with no salt problems. Soils in Aydın and Antalya were highly calcareous.

The experiment was arranged in a 3 X 3 factorial design (location X time) replicated the three years (1999, 2000 and 2001). Results were analyzed statistically by the analysis of variance using the Statistical Analysis System (SAS) computer package (SAS Institute 1980). When analysis of variance showed significant treatment effects, the least significance difference (LSD) test was applied to make comparisons among means at the 0.05 level of significance (Steel and Torrie, 1980).

Results and Discussion

There were significant (P<0.05) differences among locations in the fresh weight values. The highest mean fresh weight (1299 g m⁻²) was obtained at İzmir (Table 4). No significant differences were observed among inoculation times; however, there was a significant location x time interaction. Regression analysis was done on means of fresh weight values from April to October (0 to 200 days) (Fig. 1). Regression coefficients developed from the fresh weight values

Table.4 Certain agronomical traits of *A. mexicana*-2026 grown at Izmir, Aydın and Antalya in 1999, 2000 and 2001.

		April	May	October	Mean		
Fresh weight(g m ⁻²)	Izmir	1481.00	1216.00	1200.00	1299.00	(LSD 0.05)
	Aydın	871.00	668.33	1094.00	877.98	Location (L)	258.22
	Antalya	680.00	997.00	811.00	829.33	Time (T)	ns
	Mean	1010.67	960.44	1035.20	1002.10	LxM	107.70
Doubling time of	lzmir	3.53	4.60	4.90	4.34	(LSD 0.05)
resh weight	Aydın	7.40	5.20	5.57	6.06	Location (L)	1.38
ncrease (days)	Antalya	11.00	6.00	8.67	8.56	Time (T)	ns
	Mean	7.31	5.27	6.38	6.32	LxT	2.16
Dry matter amount	lzmir	104.40	83.35	99.00	95.58	(LSD 0.05)
(g m²)	Aydın	61.37	53.01	68.03	60.80	Location (L)	18.59
	Antalya	69.50	64.97	73.87	69.45	Time (T)	1.905
	Mean	78.42	67.11	80.30	75.28	LxT	ns
Organic matter (%)	lzmir	54.33	53.87	58.00	55.40		LSD 0.05)
	Aydın	52.13	54.33	55.37	53.94	Location (L)	1.68
	Antalya	57.00	58.47	60.50	58.66	Time (T)	ns
	Mean	54.49	55.56	57.96	56.00	LxT	ns
C/N	Izmir	10.67	10.60	10.87	10.71	(LSD 0.05)
	Aydın	10.30	10.33	10.40	10.34	Location (L)	0.24
	Antalya	10.50	10.53	10.60	10.54	Time (T)	ns
-	Mean	10.49	10.49	10.62	10.53	LxT	ns
Water holding	lzmir	411.67	408.33	416.67	412.22	(LSD 0.05)
capacity (%)	Aydın	413.33	423.33	426.00	420.89	Location (L)	ns
	Antalya	420.00	416.67	415.00	417.22	Time (T)	ns
	Mean	415.00	416.11	419.22	416.78	LxT	ns
Total protein (%)	Izmir	17.04	16.72	17.61	17.12	(LSD 0.05)
	Aydın	16.74	17.36	17.61	17.24	Location (L)	0.41
	Antalya	17.95	18.28	18.85	18.36	Time (T)	0.46
	Mean	17.24	17.45	18.02	17.57		ns

ns refers to not significant.

for the locations were significantly different from zero. Estimated maximums for the fresh weight curves were 1692, 1229 and 1159 g m^2 for İzmir, Aydın and Antalya in April, respectively.

Of the two temperature factors important for the growth of azolla, water temperature is generally more important during summer, and air temperature is more important during winter. During the summer the mid-day temperature of surface water is often 3.0-5.0°C higher than that of the air temperature (Table 2). On very sunny summer days surface water temperatures may reach up to 42.0-46.0°C, while the same water in rice fields covered with Azolla only 39.0-40.0 °C. When temperatures exceed 30.0°C, growth is retarded and plants may even die if temperature rise much above 40.0°C for more than a few hours. Azolla species and strains have different levels of tolerance to temperature extremes, and as a result some can survive within a very wide temperature range while others can survive only within a very narrow temperature range. A. filiculoides and A. rubra are quite tolerant to low temperatures, whereas A. mexicana, A. microphyla, A. caroliana and certain strains A. pinnata possess considerable tolerance to high summer temperatures (Thomas et. al., 1982).

The maximum air temperature in June exceeds, 30.0°C (Table 1). Since the water temperature is often 3.0-5.0°C higher than the air temperature, the plant stops growing, yield decreases and eventually the plant dies. The highest air and water temperatures were observed at Aydın followed by Antalya and İzmir (Table 1 and 2)

A two-fold fresh weight increase was observed at 6.32 days averaged over years and locations similar to the findings of Hove (1989) and Thomas et al. (1982). Significant differences were observed among locations. The shortest time to reach a two-fold increase was

		April	May	October	Mean		
Total N (%)	İzmir	2.99	2.93	3.09	3.00		(LSD 0.05)
	Aydın	2.94	3.05	3.09	3.03	Location (L)	0.08
	Antalya	3.15	3.21	3.31	3.22	Time (T)	0.07
	Mean	3.03	3.06	3.16	3.08	LxT	ns
Total P (%)	lzmir	0.43	0.43	0.44	0.43		(LSD 0.05)
	Aydın	0.43	0.43	0.43	0.43	Location (L)	0.04
	Antalya	0.50	0.47	0.54	0.50	Time (T)	ns
	Mean	0.45	0.44	0.47	0.46	LxT	ns
Total K (%)	İzmir	1.33	1.37	1.18	1.29		(LSD 0.05)
	Aydın	1.16	1.25	1.35	1.25	Location (L)	ns
	Antalya	1.10	1.22	1.17	1.16	Time (T)	ns
	Mean	1.20	1.28	1.23	1.24	LxT	ns
Total Ca (%)	lzmir	3.07	3.53	3.77	3.46		(LSD 0.05)
	Aydın	2.88	3.16	3.21	3.08	Location (L)	0.22
	Antalya	3.10	2.97	3.02	3.03	Time (T)	0.16
	Mean	3.02	3.22	3.33	3.19	LxT	0.23
Total Mg (%)	lzmir	0.95	0.93	0.88	0.92		(LSD 0.05)
	Aydın	0.79	0.71	0.64	0.71	Location (L)	0.07
	Antalya	0.70	0.65	0.63	0.66	Time (T)	0.08
	Mean	0.81	0.76	0.72	0.76	LxT	ns
Total Fe (%)	lzmir	0.37	0.32	0.41	0.37		(LSD 0.05)
	Aydın	0.37	0.32	0.34	0.34	Location (L)	0.02
	Antalya	0.30	0.32	0.32	0.31	Time (T)	0.03
	Mean	0.35	0.32	0.36	0.34	LxT	ns
Total Na (%)	Izmir	1.12	1.29	1.24	1.22		(LSD 0.05)
	Aydın	0.97	1.29	1.26	1.17	Location (L)	0.26
	Antalya	0.40	0.57	0.51	0.49	Time (T)	ns
	Mean	0.83	1.05	1.00	0.96	LxT	ns
Total Cu ppm	Izmir	14.77	16.40	18.23	16.47		(LSD 0.05)
	Aydın	17.17	17.23	20.07	18.16	Location (L)	, ns
	Antalya	15.00	18.93	16.67	16.87	Time (T)	ns
	Mean	15.65	17.52	18.32	17.16	LxT	ns
Total Zn ppm	Izmir	673.33	689.33	508.33	623.66		(LSD 0.05)
	Aydın	738.33	725.67	643.00	702.33	Location (L)	ns
	Antalya	480.00	701.33	688.33	623.22	Time (T)	ns
	Mean	630.55	705.44	613.22	649.74	LxT	ns
Total Mn ppm	İzmir	1100.00	1083.33	1267.67	1150.00		(LSD 0.05)
	Aydın	1083.33	963.33	1166.67	1071.11	Location (L)	ns
	Antalya	970.00	996.67	1150.00	1038.89	Time (T)	111.02
	Mean	1051.11	1014.44	1194.45	1086.67		ns

Table 5. Macro and micro nutrient elements of *A. mexiacana*-2026 grown at Izmir, Aydın and Antalya in 1999, 2000 and 2001.

ns refers to not significant.

observed at İzmir (4.34 days).

Locations and times were significantly different in terms of dry matter. The highest dry matter (95.58 g m²) corresponding 7.30 % increase rate was obtained at İzmir. Dry matter accumulation per square meter was low at Aydın (60.80 g m²) and Antalya (69.45 g m²) because of a low growth rate as compared to Izmir (Table 4). Peters et al. (1980) reported that the pH for optimum growth of azolla in culture was within the range 4.5 to 7.0. Soils at Aydın and Antalya were highly calcareous with pH values of 7.6 and 7.9, respectively (Table 3). These results showed that *A. mexicana*-2026 strain is well adapted to environmental conditions of Izmir so the highest fresh weight, dry matter and shortest time to a two-fold increase could be obtained.

The highest organic matter (58.66 %) was obtained at Antalya and organic matter ranged between 52.13 % and 60.50 % among locations averaged over years (Table 4). Organic matter (%) was significantly affected by locations but not by time and their interaction were non-significant.

C/N ratio affects the decomposition rate of soilincorporated azolla (Watanabe et al., 1977). C/N ratio in this experiment was 10.59 which indicates that decomposition and mineralization of azolla occurs in a very short time. Gevrek and Yağmur (1996), reported that *A. mexicana* increases soil organic matter to 30.0-38.8 % and total nitrogen ratio to 38 -56 % in 2-3 weeks.

The water holding capacity of dry azolla plants was

determined as 400-500 % and could be an alternative to turf used as a growing media. There were no significant differences with the location and time.

The total protein ratio in azolla plants dry weight was determined between 16.72 and 18.85 %. Since azolla doubles its weight in 3 to 6 days when grown in optimum conditions, It would be well considered as a protein resource in animal feed rations.

The nitrogen content of azolla is strongly influenced by its environment. The total N content was 2.99-3.31 % averaged over years (Table 5). The highest nitrogen content (3.22 %) was obtained at Antalya. Although the dry weight nitrogen content of azolla has been reported to be as high as 6.50 % (Peters et al., 1980), it is probably closer to 3.05-4.00 %.

Azolla inoculated into a medium poor in phosphorus florished by using its reserves. The total P content in dry matter was between 0.43 and 0.54% averaged over years. No significant differences were observed with the inoculation time. However, the highest P content (0.50 %) in dry matter was obtained at Antalya. Azolla can accumulate P, the most common element limiting the growth of azolla and its content is up to 1 or 2 % of dry matter (Hove, 1989).

The total K content (%) in dry weight was between 1.10 and 1.37 % averaged over years. No significant differences were observed with the inoculation time and location. The K content of dry matter of azolla has been reported to be between 0.31 and 5.97 % (Thomas et al., 1982). Ca, Mg, Fe, Na, Cu, Zn and Mn which are necessary for nitrogen fixation were also found in different amounts (Table 5).

Conclusion

A. mexicana-2026 strain showed high performance with respect to vegetative development and fresh weight increase rate in the Agean and the Mediterranean climate and can be grown successfully in the Mediterranean countries. Especially early inoculation (April) gave better results in terms of fresh weight increase rate and total organic matter when the air temperatures were between 10.0 and 30.0°C and relative humidity at 50.0 to 60.0 %. Since its growth was strictly restricted in June, July, August, and September due to high air and water temperatures, azolla should be grown in April, May, and October in the regions where Mediterranean climate is dominant.

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