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The performances of Azolla Mexicana in Turkish rice field

Mithat Nuri Gevrek

Ege University, Faculty of Agriculture, Department of Crop Science (Turkey)

I – Azolla and its use

1. What is Azolla

Azolla (*Azolla anabaenae*) is an aquatic fern. It lives on water surface of rice fields, small ponds, canals and rivers. It quickly covers the surface of water. It lives harmoniously under the canopy of rice plants, but does not affect its growing. Generally it multiplies vegetatively, and often sexually. Its size is 1-5 cm. There are a lot of lops on the Azolla's leaves. The blue-green algae *Anabaena Azollae* lives upon lops and in symbiosis with Azolla plant. It fixes nitrogen as high as 3-5 kg N per day under the optimum condition. Because it contains nitrogen fixing blue-green algae, Azolla plant develops at a fast rate and can double its weight within 3 to 5 days (1-2-3).

2. Conditions for growth

Azolla is sensitive to desiccation. When water depth over soil is a few cm, it grows well. Azolla multiplies at the daily mean temperature of 15-30°C. Optimum temperature is about 25°C. Azolla can tolerate (survive) up to -5°C. Under nutrient deficient and strong light conditions, Azolla becomes red. During hot summer or cold winter, it also turns red or brownish red. Under shaded conditions or nutrient-rich conditions, it remains green. Azolla absorbs nutrient from water. Azolla is very dependent upon phosphorus fertilizer. The application of phosphorus as superphosphate can significantly increase nitrogen fixation. Azolla prefers slightly acidic pH 4-7. Lepidoptera, Pyralidae larva are most harmful insect pests to Azolla (4-5-6).

3. Use of Azolla

Because of symbiotic nitrogen fixation, and consequently, high nitrogen content, and Azolla has been used as a green manure and organic matter sources for wetland rice in northern Vietnam, and central to southern China for centuries (6). About 70 % of Azolla N contents were mineralized to NH₄+H after 20 days of incorporation. Rice plant can use easily NH₄+H form (7-8). When in excess, it is also converted into compost to be used for dry land crops and vegetables. At present, Azolla is used in more than a million hectares of rice land in China. Similarly, 400.000 hectares are under this crop in Vietnam. The use of Azolla in rice areas in the Philippines is increasing. It has been estimated that 44% of rice land will be fertilized with Azolla in near future (6). Azolla has a high protein content of approximately 23-37 percent (dry-weight basis). Azolla has been used as feed for pig, duck, and fish (6). Fresh Azolla can also be used in salads and sandwiches, just as alfalfa and bean sprouts are used (9).

II – Research results on Azolla grown under Egean ecological conditions

The Ege region on the west coast of Turkey has a distinct climate-temperature ranges from 15-35°C from the beginning of April to the first week of September, rainfall average 500-600 mm, and relative humidity is 57 %. Summer is warm and dry while winter is like mild and rainy.

Four different Azolla strains obtained (*A. microphylla*4030 (IRRI), *A. microphylla*4089 (China) and *A. filiculoides*1001, *A. mexicana*2026 (Latin America)), *A. mexicana*2026 was found adaptable to Izmir ecological conditions. In 1995, a field experiment was conducted to determine the suitable inoculation time for *A. mexicana*2026. *A. Mexicana*2026 was first grown at Izmir and then transferred to rice experimental fields at Menemen (sandy loam soil, 0.9 % organic matter and pH 7). Rice variety TOAG-92, was used in the experiment (10-11).

Four different inoculation times were tested one after the other on the same plots. A Randomized complete block design with three replications was used in the rice fields. For each application time, 300 g of fresh Azolla was inoculated per square meter and allowed to grow for 14 days. When the surface was covered with Azolla (7-10 days after inoculation), 0.2 g P₂O₅ m⁻² was applied. Azolla biomass was collected and fresh weight, dry weight, and total N were measured (Table 1). The first inoculation of Azolla was done when the rice plants were at the 3-leaf stage.

The inoculated amount of fresh weight (300 g/m⁻²) reached to 1122 g/m⁻² after 14 days (274 %). Statistical analysis showed that the first inoculation dates resulted in comparatively better yield performance than any other inoculation dates based on Duncan's multiple range test. the best time for Azolla inoculation was the first week of July (1200 g/m⁻²) (see table 1). However, inoculating Azolla at the end of August also gave reasonable amount of Azolla. N was 0.33 % in fresh Azolla, and mean N gain was 2.7 g m⁻².

Table 1. Average growth and nitrogen accumulations in the Azolla-inoculated field plot (m⁻²), Izmir, Türkiye, 1995

Treatments	Inoculation date	Mean temperature °C	Amount harvested (g m ⁻²) fresh	% increase in fresh wt.	N increase (g m ⁻²) ¹
1	10 Jul	30.0	1200 a ²	300	2.97a ²
2	24 Jul	30.6	1090 bc	263	2.61 bc
3	7 Aug	27.4	1060 c	253	2.51 c
4	21Aug	27.4	1140 ab	280	2.77ab
Av		28.9	1123	274	2.72
SD				60	0.10

¹ after 14 days inoculation date at 105°C

² Duncan (%5)

III – The effect of a Mexicana combined with inorganic fertilizer on rice yield in rice field under Aegean condition

The best combination of Azolla and inorganic N fertilizer and its effects on the organic structure of the soil under Menemen/Izmir ecological conditions were determined in 1996 and 1997. The study was supported by TUBITAK (The Scientific and Technical Research Council of Turkey. Project No: TOGTAG-1590) (12).

The experiment was conducted during the second crop production in 1996 and 1997 using a split-plot design with three replications. Two rice varieties, Baldo (110 d) and Toag92 (95 d) were tested with four Azolla and inorganic nitrogen (NH_4^+ -N) combinations. Toag92 matures 2 weeks earlier than Baldo. Azolla and NH_4^+ -N were applied at flowering period and tillering periods (Table 2). The Azolla was grown in a separate plot and harvested at the appropriate period. Azolla was applied at the rate of 300 g-fresh weight m^{-2} into the plots 7 days before the tillering and flowering stages. One third of the Azolla mat was incorporated into the soil at each stage. Total N content of Azolla % was 0.03 on a fresh weight basis. NH_4^+ -N rate was 180 kg/ha in ammonium sulphate form. At sowing period, 60 kg/ha NH_4^+ -N and P_2O_5 was applied per treatment including control. Plots of water were drained 3-5 days after Azolla application. The soil was sandy loam with low nitrogen, medium phosphorus, high potassium, high salinity and pH 7.0.

Table 2. Grain yield at different treatment combinations in the experiment conducted at Menemen/Izmir, Türkiye, 1996-1997

	Treatment Combinations			Grain yield ($\text{t/h}_{\text{a}}^{-1}$)			
	Beysis	Tillering	Flowering	1996		1997	
				Baldo	Toag92	Baldo	Toag92
1	60 kg N ²	+ ¹	+ ¹	3.1 bc ³	3.6 c	3.0 c	3.6 bc
2	60 kg N ²	60 kg N ²	+ ¹	3.0 c	3.7 c	2.8 c	3.4 c
3	60 kg N ²	60 kg N ²	60 kg N ²	3.5 a	4.4 a	3.3 a	4.2a
4	60 kg N ²	+ ¹	60 kg N ²	3.3 b	3.9 b	3.1 ab	3.8 b
5	Control	-	-	1.0 d	1.2 d	0.8 d	1.1 d

¹

(+) indicates Azolla was applied at 200 g fresh wt./ m^2 (60 kg Azolla's nitrogen per hectare)

² Applied as ammonium sulphate per hectare.

³ Duncan (%5)

In this study, interaction was found statistically important according to grain yield among years, combinations and varieties (Table 2). The highest yields were found (Toag92 1996: 4.4 t ha^{-1} and 1997: 4.2 ha^{-1}) at 3rd treatment in each years. The significant differences were recorded among the Azolla treatments (1st and 2nd, 4th) where 4th treatment always followed the 3rd treatment in two years (Table 2). The results of study showed that Azolla-mineral N fertilizer treatments (4th) has given higher rice yield than other Azolla and mineral N treatments under Menemen/Izmir second crop conditions. Thus, it was concluded that applying Azolla with 200 g-fresh weight/ m^2 in rice crop might save about 1/3 of mineral nutrient in this study conditions. This result is important for organic farming (12).

As a result, all the treatments in this study, were statistically behind treatment 3, but future research with new strains in the Mediterranean Area has the potential to give better result than treatment 3. I hope joint-project will be conducted in the future.

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