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Azolla (*Azolla pinnata*) Response to Different Phosphorus and Radiation Treatments at Two Cultivation Dates

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ABSTRACT

In order to investigate the effect of different phosphorus and radiation treatments on Azolla growth and water use efficiency (WUE), an experiment was carried out at the Agricultural Research Greenhouse of Razi University, Kermanshah, Iran. The experiment was a factorial with three factors based on a completely randomized design with three replications. The first factor was cultivation dates (4 May and 4 June), the second was radiation levels (0, 50 and 100% of full sunlight) and the third was phosphorus treatments including control (without fertilizer), chemical fertilizer and chicken manure. Results indicated that the highest dry matter produced and water use efficiency occurred at the 100% level of full sunlight. At the higher radiation levels (50 and 100% of full sunlight) chicken manure performed better as compared with the other phosphorus treatments. Moreover, at the 100% of full sunlight, there was no significant difference between the two cultivation dates in terms of WUE.

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1. Introduction

Farmers in Iran heavily depend on synthetic chemical fertilizers to provide nutrients for their cropping systems. It has been observed that continuous use of synthetic chemical fertilizers in an imbalanced form deteriorates soil physical properties (Biswas *et al*., 1971; Prasad *et al*., 1983). Detrimental effects of chemical fertilizers even in a balanced form on soil physical properties are also being observed. For example, a decline in soil organic carbon and associated decline in system productivity under a rice-wheat system with the long-term use of recommended NPK was observed in some field studies (Nambiar, 1994; Abroal *et al*., 2000; Yadav *et al*., 2000).

In sustainable agriculture, an alternative method for chemical fertilization is the use of green manure. Green manure is a crop used primarily as a soil amendment and a nutrient source for subsequent crops (Cherr *et al*., 2006). Application of green manures to soil is considered a good management practice in any agricultural production system because it can increase cropping system sustainability by reducing soil erosion and ameliorating soil physical properties (MacRae and Mehuys, 1985; Smith *et al*., 1987), increasing soil organic matter and fertility levels (Doran and Smith, 1987; Power, 1990), enhancing nutrient retention (Drinkwater *et al*., 1998; Dinnes *et al*., 2002), and reducing global warming potential (Robertson *et al*., 2000).

Azolla (*Anabaena azollae*) is a free-floating, eco-friendly, fast-growing water fern having symbiotic, heterocyst-forming and N_2-fixing cyanobiont, *A. azollae* in their leaf cavities, which fulfills the N requirement of the symbiotic system through N_2-fixation (Singh *et al*., 2010). It is distributed worldwide in tropic and temperate fresh water ecosystems. Due to N_2-fixation, organic carbon and available phosphorus, it is mostly used in agriculture to improve the fertility of the soil and to replace the use of chemical fertilizers.

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Besides N biofertilizer, Azolla has multiple uses like biological herbicide, animal feed and water purifier.

Azolla is also known for accumulating nutrients like P, K and minerals from the water and making them available to plants as it is decomposed. Research is being done to put Azolla to agricultural use under dryland conditions also in nutrient-poor soils (Kiguli, 2000). The possibility of using Azolla as a slow-release organic fertilizer for essential elements like Fe for the nutrition of vegetable crops under deficient conditions is also seen. Though Azolla grows well in water habitats with relatively low nitrogen and phosphorus conditions, it has greater efficiency to accumulate nutrients than its requirement and thus soil or crops benefit after the decomposition of Azolla.

Phosphorus is an essential and least available plant nutrient (Passarinho *et al*., 2000) in water-logged and deep-water rice fields. In absence of inorganic phosphate, plants use organic phosphate either from soil organic matter or from synthetic fertilizers (Tadano *et al*., 1993; Hallmann, 1999) through phosphatase (acid or alkaline) enzyme only to contribute phosphate nutrition to the plants (Julie *et al*., 2000).

Soils in many regions of Iran are very poor in terms of organic matter. Organic fertilizers including green manure can play an important role in order to enhance soil organic matter and consequently its fertility. Seaweeds such as Azolla may be grown as valuable green manures to increase soil organic matter. However, water limitation is one of the most important concerns in arid and semi-arid regions of the world like Iran. Therefore, the application of the practices which can reduce water consumption and increase the water use efficiency of plants is very important. Both sun radiation and fertilization are important factors that notably influence growth and water use efficiency. The present study was conducted to evaluate the effects of different phosphorus and radiation treatments on Azolla growth and water use efficiency under two cultivation dates.

2. Materials and methods

The study was carried out in 2011 at the Agricultural Research Greenhouse of Razi University, Kermanshah Province, western Iran. One species of *Azolla* viz. *A. pinnata* was prepared from the Rice Research Institute of Iran, Guilan Province, Northern Iran. The experiment was a factorial with three factors based on a completely randomized design with three replications. The first factor was the cultivation dates (4 May and 4 June), the second was the radiation levels (0, 50 and 100% of full sunlight) and the third was the phosphorus treatments including control (without fertilizer), chemical fertilizer and chicken manure. Daily minimum and maximum temperatures during two experimental periods and the analysis result of the chicken manure are shown in Table 1 and Table 2, respectively.

Table 1. Daily minimum and maximum temperatures during the experimental periods.

Cultivation date		Daily temperatures (°C)										Mean
		1	2	3	4	5	6	7	8	9	10	
May	Minimum	9	10.6	12.2	11.5	5.3	13.9	9.3	9.3	10.8	13.4	10.5
	Maximum	23.5	22.4	22.6	24.3	24.1	26	29.6	28.8	27.6	26.1	25.5
June	Minimum	13.3	13.7	12.5	18	15.7	17.2	17	15.6	12.1	10.8	14.6
	Maximum	33	25.9	36	36.4	35.5	33.3	32.1	32.8	30.4	31.5	33.7

Table 2. The analysis result of the chicken manure used in the study.

Constituents (%)											pH
N	P	K	Ca	Mn	S	Mg	Fe	Na	Zn	B	
5	4	4	4.5	1.1	0.5	0.045	0.13	0.45	0.03	0.0045	6.5-7.5

The fronds of Azolla were transferred into the flat wash-tubs with 30 cm diameter containing 10 cm height of soil and 4000 cm^3 of water at the before mentioned cultivation dates. 0 and 50% of full sunlight treatments were achieved by using perfect and striped cardboards. For the 100% of full sunlight treatment, no cardboard was used. Light intensity was measured in each treatment by a lux meter (model TES 1339) to ensure the light treatments were achieved carefully.

For fertilizer treatments, chemical phosphate fertilizer and chicken manure were applied at 0.36 and 4.05 g, respectively. These quantities were equivalent

with 0.162 g of pure phosphorus. The duration of each experiment was 10 days. On the 10th day, the fronds of Azolla were collected from each flat wash tub, dried at 60° C for 48 h, weighed and the dry matter produced by Azolla was determined. Water use efficiency (WUE) was calculated by the following equation:

WUE= (Dry matter produced by Azolla (g)/ Water consumed by Azolla (lit)) × 100

Data analyses were carried out by using SAS software (SAS Institute 2003).

3. Results and discussion

Analysis of variance (data not shown) revealed that Azolla dry matter (DM) and water use efficiency (WUE) were significantly influenced by radiation level, phosphorus treatment and cultivation date. Moreover, significant two-way interactions (radiation level × cultivation date and radiation level × phosphorus treatment) were found for the traits under study. However, there was no significant three-way interaction (radiation level × phosphorus treatment × cultivation date) for the Azolla traits.

The highest produced dry matter and water use efficiency were obtained at the 100% of full sunlight (Table 3). Although, there was no significant difference between the 50 and 100% of full sunlight in terms of WUE (Table 3). At the 100% of full sunlight, Azolla dry matter was increased by 22.2 and 188.8% as compared with the 50 and 0% of full sunlight, respectively (Table 3).

At the 0% of full sunlight, there were no significant differences between the phosphorus treatments in terms of Azolla dry matter, but at the higher radiation levels (50 and 100% of full sunlight) fertilizer application significantly improved the dry matter produced by Azolla (Fig. 1).

Table 3. The effects of radiation level, phosphorus treatment and cultivation date on Azolla dry matter and water use efficiency.

Factor		Dry matter (g)	Water use efficiency (g. lit^{-1})
Radiation level	0	0.54444c	0.23756b
(% of full sunlight)	50	1.28667b	0.38717a
	100	1.57222a	0.39228a
Phosphorus treatment	Control (without fertilizer)	0.99667b	0.29856c
	Chemical fertilizer	1.02222b	0.31117b
	Chicken manure	1.38444a	0.40728a
Cultivation date	May	0.93593b	0.38981a
	June	1.33296a	0.28819b

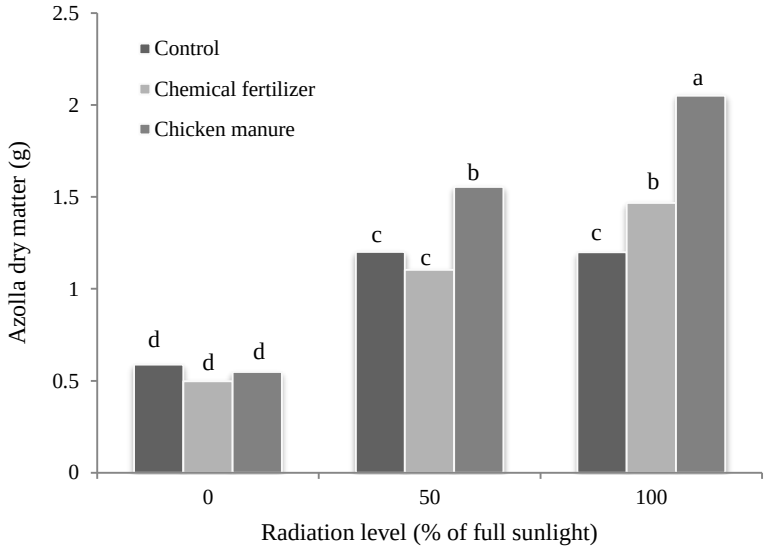

Figure 1. The produced dry matter by Azolla under different phosphorus and radiation treatments.

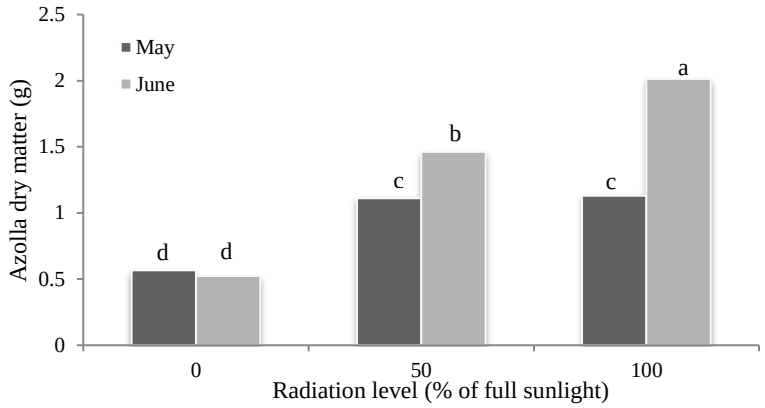

Figure 2. The produced dry matter by Azolla under different radiation levels and cultivation dates.

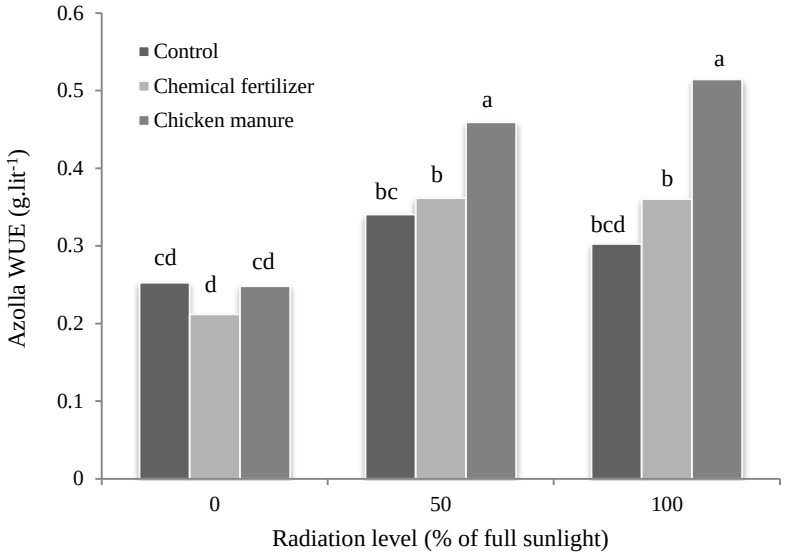

Figure 3. Azolla water use efficiency (WUE) under different phosphorus and radiation treatments.

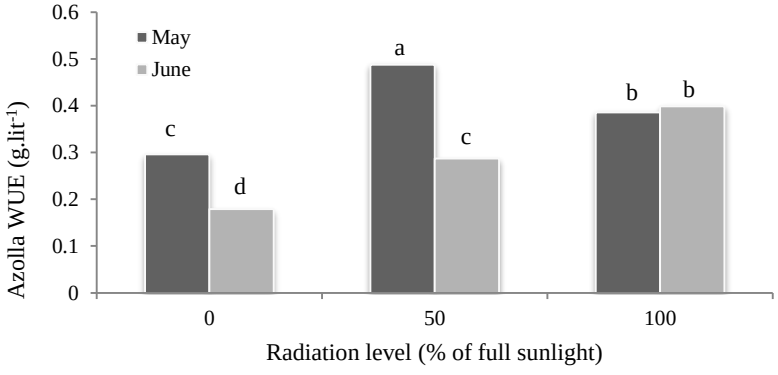

Figure 4. Azolla water use efficiency (WUE) under different radiation levels and cultivation dates.

Although, at the 0% of full sunlight, there was no significant difference between the two cultivation dates in terms of Azolla dry matter produced, at the other radiation levels, the second cultivation date (June) led to the higher dry matter produced by Azolla (Fig. 2). As this cultivation date increased Azolla dry matter by 78 and 31.6% at the 100 and 50% of full sunlight, respectively as compared with the first cultivation date (May).

As observed for dry matter, WUE was not significantly different between the phosphorus treatments at the 0% of full sunlight, but at the higher radiation levels (50 and 100% of full sunlight) the WUE was notably improved when chicken manure was used (Fig. 3).

For both 0 and 50% of full sunlight, WUE was notably lower at the second cultivation date (June) compared to May (Fig. 4). However, at the 100% of full sunlight, there was no significant difference between the two cultivation dates in terms of WUE (Fig. 4).

Lower levels of sunlight (0 and 50%), significantly reduced Azolla dry matter as compared with full sunlight (Table 3). According to Lumpkin (1987a), low radiation intensities cause Azolla to suffer or die. Cary and Weerts (1992) also reported that biomass produced by two species of Azolla exposed to 30% sunlight was less than one-third of those exposed to full sunlight.

However, improving Azolla WUE in response to increased sunlight level was relatively lower when compared to Azolla dry matter (Table 3). It seems that, although increasing radiation levels from the 50 to 100% of full sunlight notably increased the dry matter produced by Azolla, but could not improve the WUE equally (Table 3). This can be attributed to a higher evapotranspiration rate when sunlight is at its highest level (100%). In other words, at the 100% of full sunlight, the water consumed by Azolla to produce 1 gram of dry matter was increased significantly, probably due to higher temperatures at this radiation level.

At the 0% of full sunlight, there were no significant differences between the phosphorus treatments in terms of Azolla dry matter, but at the higher radiation levels (50 and 100% of full sunlight) fertilizer application significantly improved the dry matter produced by Azolla (Fig. 1). It can be concluded that phosphorus can improve Azolla growth and biomass production only at the presence of enough radiation. In general, phosphorus is the most important and often limiting nutrient for the growth of aquatic plants such as Azolla (Lumpkin 1987a, 1987b). In the present study, chicken manure performed better compared with the chemical fertilizer (Fig. 1). In fact, water-soluble phosphate in synthetic chemical fertilizers (such as superphosphate) is subject to absorption into the soil (especially alkaline soils similar to that used in the present study) probably through contact of fertilizer granules with the soil surface and adsorption of soluble phosphorus onto suspended soil particles in the floodwater (Watanabe *et al*., 1980).

At the 0% of full sunlight, there was no significant difference between the two cultivation dates in terms of Azolla dry matter produced, but at the other radiation levels, the second cultivation date (June) led to the higher dry matter produced by Azolla (Fig. 2). This can be explained by more appropriate temperatures for Azolla growth during June. According to Tuan and Thuyet (1979), the optimum temperature for Azolla growth is between 18 and 28°C which is approximately similar to the mean temperatures during the second cultivation date (June) in our study (Table 1). Watanabe (1982) also found that the optimum temperature for *A. pinnata* growth is about 30° C.

WUE was not significantly different between the phosphorus treatments at the 0% of full sunlight, but at the higher radiation levels (50 and 100% of full sunlight) the WUE was notably improved when chicken manure was used (Fig. 3). Chicken manure contains different nutrient elements (Table 2) which can effectively support the Azolla growth and dry matter production. Moreover, organic fertilizers such as chicken manure have a more water holding capacity which consequently can lead to a less water evaporation rate. According to Singh et al. (2010) acid phosphatase activity (cellular or extracellular), located in the cell wall of different Azolla species is a kind of adaptive change that has the ultimate aim of acquiring or utilizing maximum inorganic phosphate from the organic materials of the surroundings. The available phosphate through acid phosphatase activity supported the growth and physiological processes in P-deficient conditions as observed in different plant species (Lefebvre *et al*., 1990; Duff *et al*., 1994).

For both 0 and 50% of full sunlight, WUE was notably lower at the second cultivation date (June) compared to May. This may be attributed to a higher evapotranspiration rate caused by warmer air temperatures during June (Table 1). However, at the 100% of full sunlight, there was no significant difference between the two cultivation dates in terms of WUE. This may be due to a notable higher dry matter produced by Azolla under this radiation level (Fig. 2), which could compensate for the higher evapotranspiration rate in June.

4. Conclusion

In general, this study revealed that the highest Azolla growth occurred under the highest radiation level (100% of full sunlight) and chicken manure application. However, decreasing radiation levels until 50% of full sunlight did not significantly reduce the WUE for all phosphorus treatments. The second cultivation date (June) led to a higher dry matter production by Azolla. Although, under the lower radiation levels, the higher WUE was observed when Azolla was cultivated in May. At the 0% of full sunlight, there was no significant difference between the two cultivation dates in terms of Azolla dry matter produced. Moreover, under this radiation level, WUE was not significantly different from the phosphorus treatments indicating that phosphorus can improve Azolla WUE and biomass production only under suitable radiation conditions.

Conflict of interests

All authors declare no conflict of interest.

Ethics approval and consent to participate

No human or animals were used in the present research.

Consent for publications

All authors read and approved the final manuscript for publication.

Availability of data and material

All the data are embedded in the manuscript.

Authors' contributions

All authors had an equal role in study design, work, statistical analysis and manuscript writing.

Informed consent

The authors declare not to use any patients in this research.

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