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Usage of *Azolla* spp. as a Biofertilizer on the Environmental-Friendly Agriculture

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Abstract - The aquatic fern *Azolla* spp. is of value as a bio-fertilizer for wetland paddy. It is popular and cultivated widely in other countries like China, Vietnam, and the Philippines, but has yet to be taken up in Korea, in a big way. It fixes nitrogen as high as 3-5kg N per day, because it contains nitrogen fixing blue-green algae, *Anabaena azollae*. Azolla's ability to create a light-proof mat that suppresses other weeds has been used for centuries in rice production. *Azolla* spp. has also the capacity to take up the heavy metals such as Mercury and Chromium (75~100%) and may be used as a bioaccumulator in the phytoremediation. Azolla meal also can be used as an unconventional feed resource has a potential as a feedstuff for livestock.

Key words - *Anabaena azollae*, Blue-Green Algae, Biofertilizer, Nitrogen fixing

Introduction

According to the World Health Organization (WHO, 2002), around one million chemical compounds are used worldwide and millions of tons of these chemicals are threatened on human health and in our environments. In 2005 from World Economic Forum, the Environmental Sustainability Index (ESI) of Korea was rated at 143th and at 138th out of 146 countries for the quantity consumed of synthetic pesticide and chemical fertilizer, respectively. At this point, Korea government has set a goal of forty percent decrease in both of them untill 2013. However, it is still high amounts compare to the world's average.

Bio-organic farming with Blue-Green Algae (BGA) and *Azolla* spp. offers the potental solution to the various problems facing the current agricultural practices.

Azolla plant, a small floating aquatic fern, is found in rice fields, ponds, and wetlands of warm-temperature and tropical regions. It is called "Man-Kang-Hong" in Korea because azolla's leaves turns red under nutrient deficient and strong light conditions during hot summer or cold winter.

Azolla has a seven species grouped in two sections. The two sections are distinguished by characteristics of the megasporcarps (Table 1) (Svenson, 1944).

Azolla plant is a triangular in shape and exceeds 1~5cm. 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 (van Hove, 2002). It fixes nitrogen as high as 3~5kg N per day (Gevrek, 2000) 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.

Azolla is sensitive to desiccation, and optimum temperature for the growth is at about 25℃. Azolla can tolerate up to -5℃ (Uheda *et al.,* 1999). Under nutrient deficient and strong light conditions, the color of azolla leaves become red. During hot summer or cold winter, it also turns red or brownish red. Under shade conditions or nutrient-rich conditions, it remains green. Azolla absorbs nutrient from water. The growth of azolla is very dependent upon phosphorus fertilizer. The application of phosphorus as superphosphate can singnificantly increase nitrogen fixation. Azolla prefers slightly acidic pH 4~7. Lepidoptera, Pyralidae larva are most harmful insect pests to azolla (Gevrek, 2001).

Azolla has yet to be taken up in Korea, in a big way, in spite of it's great advantage.

According to the Kwon *et al.* (2007), the subtropical region is only limited in Jeju-do and the farmost southern coastal area of South Korea at the moment. The future projected climate for the period of 2071~2100, subtropical climate region extended to most of stations except for the Taeback and Sobaek Mountain range. For this reason, it is being worth noticing to make the best use of azolla.

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Table 1. Synopsis of the classification of *Azolla* spp.

Family	Azollaceae		
Genus	*Azolla*		
Subgenera	*Azolla*		*Tetrasporocarpia*
Sections	*Azolla*	*Rhizosperma*	
Species	*A. caroliniana*	*A. pinnata*	*A. nilotica*
	A. filiculoides		
	A. mexicana		
	A. microphylla		
	A. rubra		
Subspecies		*A. pinnata subsp. africana*	
		A. pinnata subsp. asiatica	
		A. pinnata subsp. pinnata	

* Proposed by Saunders and Fowler (1993).

Azolla as a bio-fertilizer

Azolla has been used as a green manure crop provides an excellent sources of organic matter and nitrogen for crops in Vietnam and China for centuries (Ferentinos *et al.,* 2002; Kang *et al.,* 2004). Azolla as a green manure can be grown as a monocrop, intercrop and both as a monocrop and an intercrop (Lumpkin and Plueknett, 1985). It is also reported to improve soil physical properties by increasing the porosity (3.7～4.2%), and decreasing the specific gravity of soils (Anonymous, 1975), and the bulk density of soil decreases with continued Azolla use (Ventura and Watanabe, 1993).

The effect of azolla on rice yields has been reported (Talley *et al.,* 1977; Lumpkin and Plucknett, 1980; Keith and Janiya, 1992; Kang *et al.,* 2004). One crop of azolla produces 10 to 20 tons of fresh biomass and supplies 20 to 40kg N/ha (Singh, 2002). It fixes nitrogen as high as 3 to 5kg N per day under the optimum condition (Eyini, 1999; Gevrek, 2000). About 70% of nitrogen contents in azolla were mineralized to NH4-N after 20 to 30 days of incorporation (van Hove, 1989). About 50% of the urea-N can be lost when urea is applied to floodwater 2 weeks after transplanting rice. N losses due to ammonia volatilization increase with high temperature, high floodwater pH, high ammonium concentration in floodwater and high wind speed. Several approaches, such as use of urea super-granules and urease inhibitors, have been conducted to control ammonia volatilization (Cai *et al.,* 1987; Freney *et al.,* 1993). According to Villlegas (1985), the presence of an azolla cover reduced ammonia volatilization by 20 to 50% of that without azolla. Apart from a direct nutritional effect, azolla can act as a physical barrier to the escaping NH_3, has a cooling effect on the floodwater, and acts as a windbreak (De Macale and Vlek, 2004).

Singh *et al.* (1981) reported that the incorporation of azolla in to rice fields also increases P availability in soils on the decomposition of azolla's biomass may be reduction and chelation. Increased availability of P in soils on the incorporation of azolla ultimately increases the uptake of P by rice plants and their P concentrations (Singh and Singh, 1987).

Table 2. Chemical composition of the *Azolla* spp.

	A. pinnata	*A. mexicana*	*A. caroliniana*
	----------	% on dry matter	----------
Nitrogen	4-5	3.92	5.0
Phosphorus	0.5-0.9	0.52	0.5
Potassium	2-4.5	1.25	2.0-4.5
Calcium	0.4-4.0	4.3	0.1-1.0
Magnesium	0.5-0.65	1.1	0.65

Chemical content of azolla differs in relation to the ecology, genotype and vegetation period (Table 2). On dry weight basis, nitrogen content was 3.92~5.0% (Singh, 1987; Gevrek and Yagmur, 1997) than the compost (0.5~0.9%). Also it has N-content close to the soybean cake (4.2%), which has the highest value (Khan, 1983). The phosphorus rate was less (0.52~3.9%) as well as the other organic sources. Potassium rate was reported between 1.25~4.5%. These rates were higher than those of the organic sources (Khan, 1983). Another factor in azolla effectiveness as a green manure is its low carbon to nitrogen ratio of between 10:1~18:1, which allows it to decompose rapidly (Gevrek and Yagmur, 1997).

Control of weed, diseases and insect by using azolla

The cost of weed control in rice fields constitutes as much as 20 ~25% of the total cost of the cultivation of rice (De Datta, 1981). The ability of azolla to create a light-proof mat that suppresses other weeds has been mentioned (Kathiresan, 2007) (Table 3). An azolla mat covering the floodwater surface of rice fields reduces weed's photosynthetic activity by intercepting light and thus significantly depresses their growth (Mandal *et al.,* 1999). Ngo (1973), while recording the suppressive effect of different mat densities of *A. pinnata* on the quantity of *Echinochloa crusgalli*, observed that after a 6-weeks the 50% azolla cover plot had a 70% reduction, and the 100% azolla cover plot a 93% reduction in the *Echinochloa crusgalli* population compared to the control. Rains and Tally (1977) reported that early development of *A. filiculoides* eliminated *Cyperus difformis* and *Polygonum* species, but not *Echinochloa crusgalli.* According to Mandal *et al.* (1999), the suppressive effect of azolla on weeds is influenced by the density or thickness of their mat, and mats of azolla have to be developed prior to the re-emergence of weeds after pudding the rice fields.

The insects which attack azolla do not attack rice plants. Insect attacks increase during the summer, when the temperature goes above 28℃. Larvae of *Chironomus, Pyralis,* and *Nymphula* species are pests. Reduction in water temperature by azolla may help in the prevention of root diseases like *Pythium* spp. that are more prevalent as temperatures increase (Lumpkin and Plucknett, 1980).

Ability of azolla to remove heavy metals from water and soil

Heavy metals are among the most dangerous substances in the environment, because of their high level of durability and harmfulness to live organisms. There are many plants that can bind heavy metals and they are called hyperaccumulators. Phytoremediation is one of the way to solve the problem of heavy metal pollution using plants (hyperaccumulators) (Henry, 2000; Bennicelli *et al.,* 2004).

Bennicelli *et al.* (2004) reported that azolla (*A. caroliniana*) has the capacity to accumulate large quantities (70~964 mg kg^{-1} dm) of heavy metals such as mercury and chromium in spite of its growth was inhibited 20~31% in the presence of heavy metals (Table 4). The maximum zinc uptake by azolla in batch systems at an optimum pH of 6.0 was found to be 46.2mg/g (Zhao and Duncan, 1997). Benaroya *et al.* (2004), lead content in azolla whole plant increase by 200% after 2 day of growth when compared to azolla apoplast.

Azolla as livestock feed

Azolla is very rich in proteins, essential amino acids, vitamins (A and B12), growth promoter intermediaries and ninerals. The carbohydrate and fat content of azolla is very low. Livestock easily digest it, owing to its high protein and low lignin content, and they quickly grow accustomed to it (Kamalasanana Pillai *et al.,* 2005). Chemical analysis indicated that azolla meal contained (% DM) 21.4 total protein, 12.7 total fibre, 2.7 ether extract, 16.2 ash and

Table 3. Influence of farming elements on rice weed dry matter (kg ha^{-1})

Treatment	Weed dry matter (kg ha^{-1})
Rice unweeded	1091.4
Rice + Fish (unweeded)	873.8
Rice + Poultry (unweeded)	900.2
Rice + Fish + Poultry (unweeded)	657.8
Rice + Fish + Poultry - twice hand weeded	315.1
Rice + Fish + Poultry - Azolla at 1 t ha^{-1}	336.8

* modified from Kathiresan, R. M. (2007).

Table 4. *A. caroliniana* biomass and concentration of heavy metals after 12 days of cultivation

Treatment	Conc. (mg dm^{-3})	Fresh Mass (g)	Conc. in solution (mg dm^{-3})	Conc. in biomass (mg kg^{-3} dm)
Control		57.0	-	-
Hg (II)	0.1	43.9	0.02	70.8
	0.5	39.3	0.04	306
	1.0	40.8	0.07	578
Cr (III)	0.1	52.0	0.02	83.5
	0.5	58.7	0.06	412
	1.0	37.4	0.25	964
Cr (VI)	0.1	45.3	0.00	91.1
	0.5	45.4	0.08	157
	1.0	41.6	0.12	356

* modified from Bennicelli *et al.* (2004).

47.0 carbohydrate, and a gross energy value of 2,039 kcal kg^{-1} (Alalade and Iyayi, 2006).

Basak *et al.* (2002) concluded that: 1) azolla is a good source of protein and may be used up to 5% level in the broiler diet for better performance, 2) azolla meal had no deleterious effect on palatability of the diets, 3) aAzolla meal is an unconventional feed ingredients at low price and may be used as a poultry feed to reduce feed cost.

When introducing azolla as feed, the fresh azolla should be mixed with commercial feed in 1:1 ratio to feed livestock. After a fortnight of feeding on azolla mixed with concentrate, livestock may be fed with azolla without added concentrate.

Other value of azolla

Near 49 million year, a major occurance of the azolla and accompanying abundant freshwater organic and siliceous microfossils indicates an episodic freshening of artic surface waters with cooler temperatures of about 10℃ during an 800,000-year interval. The presence of freshwater in the arctic may have triggered the initiation of sea-ice formation that increased albedo and contributed to global cooling (Moran *et al.*, 2006).

According to Joo *et al.* (1985), azolla mixed with rice straw gave a high biogas production per unit volatile solid compared with manure-rice straw combination. It may its high biodegradability and high nitrogen content.

Sisworo *et al.* (1990), Singh and Singh *et al.* (1987) reported a significant increase in the organic C content in soils due to successive azolla cropping with rice plants. However, wide variation value of organic C (0.5～43 t ha-1) was reported (Reynaud and Roger, 1981).

Conclusions

Azolla has been of interest to botanists and agriculturists for years because of its symbiotic relationship with a nitrogen-fixing, blue-green alga, Anabaena. It provides an excellent source of organic matter and nitrogen for crops. As azolla grows, it forms a floating, light-proof mat of living plants that suppresses weed growth. In addition, azolla can be fed to a variety of farm animals, it is an effective water purifier, it help to reduce ammonia volatilization from fertilizers.

In spite of its big utility value, azolla has yet to be taken up in Korea, in a big way. Azolla has been turned away the while because it can not be passed the winter in Korea. However, the climate condition around the Korean Peninsula is being changed rapidly to the sub-tropical climate according to the global warming. For this reason, study for the agricultural revaluation of azolla should be started in Korea as soon as possible.

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