Evaluation of Nitrogen Efficiency on Soil for Rice Plant by Using *A.caroliniana* Willds. as Biofertilizer

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Abstract

This research work is concerned with the studies for the evaluation of nitrogen efficiency by using green manure. *A.caroliniana* is used as a biofertilizer on soil for rice plant. *A.caroliniana,* green manure can fix substantial amount of atmospheric nitrogen. The green manure *A.caroliniana* plants were collected from Maubin Township. The characteristics of *A.caroliniana* plants were identified at the Department of Botany, Maubin University. The pot experiment was carried out with three types. The first one is using only soil, second one is using biofertilizer and the last one is using commercial NPK chemical fertilizer. The biofertilizer was prepared by mixing soil with *A.caroliniana*. The native rice plant (Ma-Naw-Thukha) was used for this experiment. The increase of nitrogen percentage in soil was measured every 20 days interval and the characteristics of rice plant (plant height, panicle length, total tiller, number of effective tillers, etc) were monitored. It was found that the characteristics of rice plant in *A.caroliniana* incorporated with soil are better than pure soil and nearly in soil mixed with NPK fertilizer.

Keywords: *A.caroliniana*, green manure, nitrogen efficiency, panicle length, effective tillers

Introduction

Azolla caroliniana

Scientific Classification

Family	:	Azollaceae
Genus	:	*Azolla*
Species	:	*caroliniana*
Scientific Name	:	*Azolla caroliniana*
English Name	:	Water Fern

Figure 1 ***Azolla caroliniana***

Rice growing areas have recently increased interest in the use of the symbiotic nitrogen fixing water fern *Azolla* either as an alternate nitrogen source or as a supplement to commercial nitrogen fertilizers (Armstrong, 1979). Use of biofertilizers is one of the important components of integrated nutrient managements, as they cost effective and renewable source of plant nutrients to supplement the chemical fertilizers for sustainable agriculture (FAO, 1984).

Azolla being incorporated into the soil as a green manure crop is one of the most effective ways of providing nitrogen source for rice (Watanabe and Liu, 1992).

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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. In tropical rice soils the applied *Azolla* mineralizes rapidly and its nitrogen is available to the rice crop in very short period (Martinez, 1984).

The utilization of biofertilizers has several advantages over chemical fertilizers. First, biofertilizers are inexpensive, making use of freely available solar energy, atmospheric nitrogen and water. Second, biofertilizers utilize renewable resources whereas the productions of chemical fertilizers depend on petroleum, a diminishing resource. Third, biofertilizers are nonpolluting. Fourth, besides supplying nitrogen to crops, biofertilizers also supply other nutrients such as vitamins and growth substances. Fifth, biofertilizers improve the general fertility of the soil by increasing the availability to crops of a number of nutrients, by increasing the organic matter in soil, and by improving soil structure (Kumarasinghe and Eskew, 1993).

Materials and Methods

Sample Collection and Identification of Collected Samples

A.caroliniana samples were collected from No. (2), Quarter in Maubin Township. Duration time is from 1st July to 31st October. The collected sample was identified by authorized botanists, in the Department of Botany, Maubin University. *A.caroliniana* was washed with water and dried in air.

Figure 2 *Azolla caroliniana* from Maubin Township

Characterization of *Azolla caroliniana*

Some characteristics of *Azolla caroliniana* were determined, such as moisture content by drying oven method and primary macronutrients (total nitrogen content by Kjeldahl digestion method, total K_2O content by flame spectrophotometric techniques and total P_2O_5 content by ammonium molybdate method).

Preparation of Soil Sample and Pots

Same sizes (25cm-diameters) of eighteen pots were collected and soil sample was taken from 1st July, 2011 to 31st October, 2011 on Maubin University Campus. The soil sample was broken up into small lumps. In each pot, the same amount of soil (3000 g) was mixed with water and puddled by hand and then kept under submerged condition for twenty days. The experiment was carried out in three treatments. There are six pots in one treatment. In three pots, rice plant was transplanted and in other three pots, no rice plant was transplanted. The samples for determinations were taken out from these three pots (no rice plant) and average values were calculated.

Preparation of Green Manure Fertilizer for Pot Experiment

The pot experiment was conducted to evaluate the nitrogen effect of the application of green manure fertilizer and NPK fertilizer on rice growth.

A.caroliniana is used as a green manure fertilizer. In six pots, the soil and *A.caroliniana* (500 g) were puddled by hand and in other six pots, the soil and NPK fertilizer (6.4 : 3.2 : 1.6) were mixed. In each of the three pots, rice plant was transplanted and in each of other three pots, no rice plant was transplanted. The samples for determinations were taken out from these three pots (no rice plant) and average values were calculated. And then, these twelve pots were kept under submerged condition for twenty days and made ready for seedlings transplanting.

Determination of the Physicochemical Properties of Untreated Soil and Chemical Composition of Fertilizer Treated Soil Samples from Experimental Pots (Before Transplanting)

The physicochemical properties of untreated soil samples were determined, such as moisture content by drying oven method, pH values by pH meter, soil texture by pipette method, nitrogen content by Kjeldahl's method, available nutrients (P_2O_5 content by Troug method and K_2O content by atomic absorption spectrophotometic method). After twenty days before transplanting, chemical composition of fertilizer treated soil samples were determined, such as moisture, texture, pH, total nitrogen, available nutrients (P_2O_5 and K_2O)

Cultivation of Rice Plant in the Pots

Pot experiment was conducted under natural daylight condition on Maubin University Campus, on 20th July, 2011, i.e, twenty days after preparation of fertilizer treated soil samples. The rice plant was transplanted in nine pots at twenty days after preparation of samples. First three pots were untreated soil pots. Second three pots were filled with soil incorporated with *A.caroliniana*. Third three pots were filled with soil mixed with NPK fertilizer. The native rice plant (Ma-Naw-Thukha) was used in this study (3 plant pot^{-1}). The maturity-age of this variety is 120 days. *A.caroliniana* green manure was fed every twenty days interval. The evaluation of nitrogen percent was studied at every twenty days interval for all pots. After that, the characteristics of rice plant (plant height, panicle length, total tillers, number of effective tillers, etc) were monitored.

Determination of the Evaluation of Nitrogen Efficiency on *Azolla caroliniana* Treated Soil Samples

In six pots, the soil and *A.caroliniana* (500g) were puddled by hand and then kept under submerged condition for twenty days. After twenty days, the rice plant was transplanted in three pots. 20g of soil samples were taken out from three pots (without rice plant) to determine the total nitrogen content of samples. Afterwards, *A.caroliniana* (500g) was added in six pots. The total nitrogen content of samples were determined by Kjeldahl's method and average values were calculated (Jones and Benton,1991). Taking out soil samples and adding *A.caroliniana* were treated repeatedly every twenty days up to five times.

Determination of the Increase of Total Nitrogen Percent at Twenty Days Interval

The increase of total nitrogen percent at twenty days interval were calculated from the soil incorporated with *A.caroliniana* (no rice plant) pots.

Determination of the Relative Characteristics of Rice Plant Procedure

The rice plant was cultivated in nine pots, on Maubin University Campus, on 20th July, 2011. The first three pots were untreated soil (without any fertilizers), the second three pots were soil incorporated with *A.caroliniana* (500 g) pots and third three pots were soil mixed with NPK fertilizer pots. The characteristics of rice plant (plant height, panicle length, total tillers, number of effective tillers, etc) in nine pots were determined and average values were calculated.

Results and Discussion

Identification of *Azolla caroliniana*

Azolla has seven species. They are *A.microphylla*, *A.filiculoides*, *A.pinnata*, *A.caroliniana*, *A.nilotica*, *A.rubra* and *A.mexicana*. *Azolla* was collected from No. (2), Quarter Maubin Township. Collected *Azolla* was identified both literally and botanically. Among them, collected *Azolla* was confirmed as *A.caroliniana*.

Determination of Moisture Content and Primary Macronutrients (N, P, K) in *Azolla caroliniana*

Moisture contents were found to be 15.74%. The total nitrogen contents, P_2O_5 contents and K_2O contents were 2.86%, 0.771% and 1.502% respectively. The amount of nitrogen % in *A.caroliniana* is larger than P_2O_5 and K_2O. Nitrogen is a part of the chlorophyll and is an essential constituent of all proteins. It is responsible for the dark green colour of stem and leaves, vigorous growth, tillering, leaf production, size enlargement and yield formation. Phosphorus is essential for growth, cell division, root lengthening, seed and fruit development and early ripening. Potassium improves utilization of light during cool and cloudy weather and enhances plant's ability to resist cold and other adverse conditions. Since *A.caroliniana* contains not only nitrogen but also P_2O_5 and K_2O, it can be used as fertilizer for cultivation of rice.

Table 1 Characterization of *Azolla caroliniana*

Sample	Moisture (%)	Total Nitrogen (%)	Total P_2O_5 (%)	Total K_2O (%)
A.caroliniana	15.74	2.86	0.771	1.502

Physicochemical Properties of Soil in Experimental Pots (Before Transplanting)

The moisture content was found to be 8.550%. From the agricultural view, it is somewhat adequate for cultivation. The soil contained 1.55% sand, 23.6% silt and 72.5% clay. Therefore, types of soil were observed to be clay. It holds moisture for long periods of time when wet and dries hard as a brick (Bear, 1965). The pH value of the soil was 6.93, neutral, and this value is suitable to cultivate many plants, such as rice, wheat, maize, bean, pea, cotton etc. The total nitrogen content, the available K_2O content and the available P_2O_5 content of soil samples were found to be 0.209%, 16.95 % and 0.835 %. According to literature value, the available nutritents K_2O and P_2O_5 of soil samples are medium and low content (Russell, 1967).

Table 2 Physicochemical Properties of Soil in Experimental Pots (Before Transplanting)

No.	Parameters	Results
1.	Sand (%)	1.55
2.	Silt (%)	23.6
3.	Clay (%)	72.5
4.	Moisture (%)	8.550
5.	Total N_2 (%)	0.209
6.	Available P_2O_5 (%)	0.835
7.	Available K_2O (%)	16.95
8.	pH	6.93

Soil type - Clay

Chemical composition of fertilizer treated soil samples (before transplanting)

In untreated soil and soil incorporated with *A.caroliniana* biofertilizer pots, the soil texture is clay type. In soil mixed with NPK fertilizer pots, the soil texture is silty clay. Clay soils are heavy, do not drain or dry easily and have good reserves of plant nutrients. Clay soils often have higher organic contents that persist longer than other soil types (Bear, 1965). The pH values of different samples are 6.93 (nearly neutral) in untreated soil, 6.97 (≈7) (neutral) in soil incorporated with *A.caroliniana* biofertilizer pots and 6.29 (slightly acid) in soil mixed with NPK fertilizer pots. So, *A.caroliniana* biofertilizer's pH value of soil does not change, i.e. neutral. Total nitrogen and available nutrients K_2O and P_2O_5 content in soil incorporated with *A.caroliniana* biofertilizer treatment are higher than untreated soil pots.

Table 3 Chemical Composition of Fertilizer Treated Soil Samples (Before Transplanting)

Samples	Moisture %	pH	Texture				Total nitrogen %	Available Nutrients	
			Sand %	Silt %	Clay %	Total %		P_2O_5 (%)	K_2O (%)
Untreated Soil	8.550	6.93	1.55	23.6	72.5	97.65	0.209	0.835	16.95
Soil+ *A.caroliniana*	8.518	6.97	2.10	40.5	55.3	97.90	0.211	1.538	23.47
Soil + NPK	8.125	6.29	1.45	34.5	61.1	97.05	0.247	6.080	53.47

N : P : K= (6.4 : 3.2 : 1.6) g/ pot, *A.caroliniana*= 500g/pot, soil = 3000g/pot

Evaluation of Nitrogen Efficiency on *Azolla caroliniana* Treated Soil Samples

Nitrogen efficiency on *A.caroliniana* treated soil samples were evaluated. The average total nitrogen percent of untreated soil was 0.209. The average total nitrogen percent of soil incorporated with *A.caroliniana* (500 g) treatment were 0.211%, 0.262%, 0.284%, 0.362% and 0.400%. By using integrated supplying of *A.caroliniana* biofertilizer, evaluation of nitrogen efficiency is significantly increased on soil for rice plant than the using of untreated soil and NPK fertilizer.

Table 4 Evaluation of Nitrogen Efficiency on *Azolla caroliniana* Treated Soil Samples

Samples	Period (days)	Total Nitrogen (%)			
		Pots (1)	Pots (2)	Pots (3)	Average
UntreatedSoil (control)	-	0.206	0.209	0.212	0.209
Soil + A.caroliniana	1st 20	0.211	0.213	0.210	0.211
Soil + A.caroliniana	2nd 20	0.265	0.262	0.259	0.262
Soil + A.caroliniana	3rd 20	0.287	0.281	0.283	0.284
Soil + A.caroliniana	4th 20	0.359	0.362	0.365	0.362
Soil + A.caroliniana	5th 20	0.397	0.400	0.403	0.400

Estimated *A.caroliniana* amount incorporated (g/pot) = 500 g
Soil = 3000 g/pot

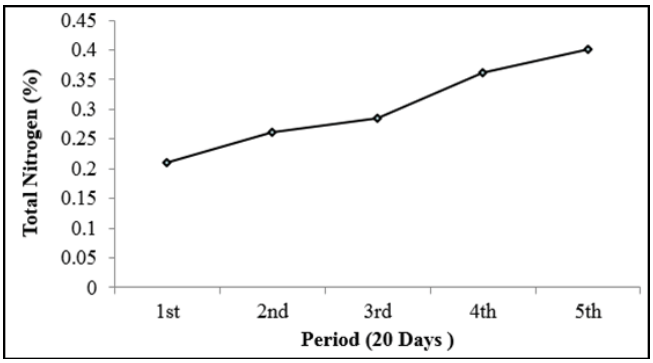

Figure 1 Evaluation of nitrogen efficiency at twenty days interval

The increase of Total Nitrogen Percent at Twenty Days Interval

After every twenty-day interval, the increase of total nitrogen percent were 1%, 25%, 36%, 73% and 91% respectively. Therefore, *A.caroliniana* biofertilizer produced the substantial amount of nitrogen on soil for rice plant.

Table 5 Increase of Total Nitrogen Percent at Twenty Days Interval

Period (Days)	Increase of Total Nitrogen (%)
20	1
40	25
60	36
80	73
100	91

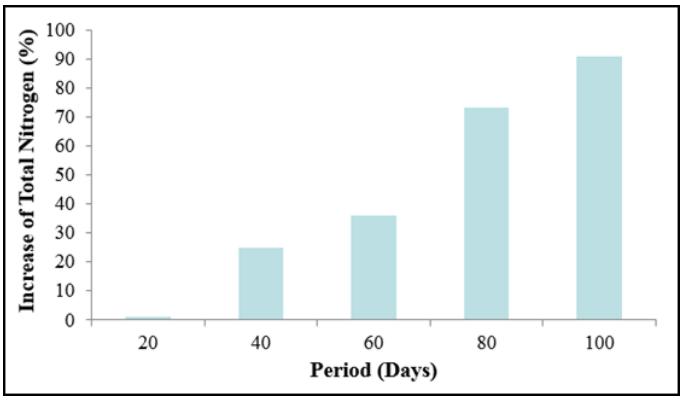

Figure 2 Increase of Total Nitrogen Percent at Twenty Days Interval

Relative Characteristics of Rice Plant

Among all treatments, (soil with NPK fertilizer and soil with *A.caroliniana* biofertilizer) treatments gave the good relative characteristics of rice plant. However, untreated soil (without any fertilizer) gave the lowest relative characteristics of rice plant. Therefore, *A.caroliniana* biofertilizer can substitute instead of chemical nitrogenous fertilizer.

Table 6 Related Characteristics of Rice Plant

Samples	Plant height[a] (cm)	Panicle length[a] (cm)	Total tillers[a]	No.of Effective tillers[a]	Total dry weight matter[b] (g)	Root weight[b] (g)	Panicle weight[b] (g)	No.of filled grains per panicle[b]
Untreated soil (control)	80.0	22	11	10	34	19	15	59
Soil + A.caroliniana	85.5	24	16	15	50	28	25	72
Soil + NPK	90.0	28	23	21	55	29	29	83

N:P:K = (6.4 : 3.2 : 1.6) g /pot, *A.caroliniana* = 500 g / pot, Soil = 3000 g/pot

a = before harvesting stage

b = after harvesting stage

1. Pure Soil 2. Soil + A.caroliniana 3. Soil + NPK

Figure 3 The growth of rice plant with prepared fertilizers (pot experiment)

Conclusion

The main aim of this research work was to study for the evaluation of nitrogen efficiency by using green manure *Azolla caroliniana* used as a biofertilizer on soil for rice plant. The green manure *Azolla caroliniana* plants were collected from Maubin Township and the plants were identified both literally and botanically. In *Azolla caroliniana* plants, the moisture content was found to be 15.74% and primary macronutrients (total nitrogen content was found to be 2.86%, total P_2O_5 content was found to be 0.771% and total K_2O content was found to be 1.502%) respectively. From these results, *Azolla caroliniana* was nitrogen rich plants.

The physicochemical properties of control soil was found to be moisture content 8.550%, pH value 6.93 (neutral), texture 97.65% (clay), total nitrogen content 0.209% and available nutrient (P_2O_5 content 0.835 % and K_2O content 16.95 %) respectively. According to these results, types of soil were observed to be clay. Clay soil retain nutrients and is very fertile.

The chemical composition of soil incorporated with *Azolla caroliniana* biofertilizer was found to be moisture content 8.518%, pH 6.97 (neutral), texture 97.90% (clay), total nitrogen content 0.211% and available nutrients (P_2O_5 content 1.538 % and K_2O content 23.47 %) respectively. By using of *Azolla caroliniana* biofertilizer, the soil texture does not change (i.e, clay), pH value also does not change (i.e, neutral) and the nitrogen content was increased in soil.

The average total nitrogen percent of every twenty-day interval *Azolla caroliniana* biofertilizer treated soil samples were found to be 0.211%, 0.262%, 0.284%, 0.362% and 0.400% respectively. The increase of total nitrogen percent was found to be 1%, 25%, 36%, 73% and 91% respectively. Moreover, the characteristics of rice plant in soil incorporated with *Azolla caroliniana* pots were found to be plant height 85.5 cm, panicle length 24 cm, total tillers 16, number of effective tillers 15, total dry weight matter 50 g, root weight 28 g, panicle weight 25 g and number of filled grain per panicle 72 respectively. In this research, the characteristics of rice plant are significantly increased in soil incorporated with *Azolla caroliniana* pots than untreated soil pots and nearly in soil mixed with NPK fertilizer.

It can be concluded that *Azolla caroliniana* is rich in nutrients as in chemical fertilizer, which has a good affinity for plant growth and results in a replacement of chemical nitrogenous fertilizer. Moreover, *Azolla caroliniana* biofertilizer can support the production of chemical free crops. The utilization of *Azolla caroliniana* green manure as biofertilizer may be an economical alternative.

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