INOCULATION EFFICIENCY OF RICE PLANTS WITH AZOLLA AS A BIOFERTILIZER IN THE PRESENCE OF DIFFERENT LEVELS OF PHOSPHORUS

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ABSTRACT

Inoculation efficiency of rice plants with proper species of *Azolla* was studied. In this research two species of *Azolla* namely *Azolla filiculoides* and *A. pinnata* were tested for growth efficiency and nitrogen fixation rate. Results showed that *Azolla filiculoides* gave greater fresh and dry yield of biomass compared with *A. pinnata*. Also, *A. filiculoides* gave higher percentage of total nitrogen and N_2-ase activity than *A. pinnata*. So, *A. filiculoides* was used in further study. When rice plants were inoculated with *Azolla* as a biofertilizer at a rate of 100 g and 150 g/m^2 combined with 30 Kg P_2O_5/fed, the highest densities of total bacteria and inorganic phosphate solubilizing bacteria were obtained, respectively.

Counts of ammonifiers and nitrifiers were increased with the increasing of *Azolla* inoculum rate. Ammoniacal nitrogen was the highest with *Azolla* application at a rate of 150 g/m^2 combined with 45 kg P_2O_5/fed. Highest values of plant height, fresh and dry weights of shoot system were obtained from *Azolla* inoculum rate of 150 g/m^2 combined with 30 kg P_2O_5/fed. Interaction effect between *Azolla* inoculum rates and P-levels was significant on total nitrogen, phosphorus and potassium in rice plants. Highest values of chlorophyll a, b and carotenoids as well as total carbohydrates were obtained in the treatment of *Azolla* inoculum at a rate of 150 g/m^2 combined *with 45 kg* P_2O_5/fed. The highest values of grains and straw yield and 1000-grain weight of rice were obtained in the treatment of *Azolla* inoculation at a rate of 150 g/m^2 combined with 30 kg P_2O_5/fed. in both seasons of cultivation.

Key words: Azolla, Biofertilizer, Rice, Inoculation, Phosphorus

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INTRODUCTION

Rice is one of the most important cereal crops in Egypt. Nitrogenous and phosphatic fertilization are important factors in increasing yield of rice. Inorganic nitrogenous fertilizers are added in large amounts as well as phosphatic and organic fertilizers, which represent the major cost in rice production. Many efforts were made to minimize the costs including using *Azolla* to substitute inorganic nitrogen fertilizer and organic matter addition. *Azolla* has been routinely used as either a green manure or a biofertilizer for rice in China and South East Asia.

Azolla-anabaena symbiosis can produce one ton of vegetative growth per hectare/day, containing 3 kg of fixed nitrogen which is equivalent to 15 kg of ammonium sulphate or 7 kg of urea. *Azolla* can double its weight in a nitrogen free substrate within three to five days and vigorously growing, *Azolla* contains from 3-5% nitrogen on dry weight basis **(Khan 1988).**

Singh *et al* (1988) found that using *Azolla* as a biofertilizer increased grain and straw yields of rice. **Krishnan *et al* (1994)** reported that *Azolla* application at a rate of 200 g/m^2 gave the highest grain yield. **Singh and Singh (1995)** found that grain and straw yhields of rice were increased by both *Azolla* inoculation and phosphorus fertilizer application during intercropping.

The aim of this research is to study the inoculation efficiency of rice plants with *Azolla* in the presence of different doses of phosphorus and its effect on rice growth and soil fertility.

MATERIAL AND METHODS

Growth and nitrogen fixation of *Azolla pinnata* and *Azolla filiculoides* as affected by different types of water

The effect of different types of water i.e., irrigation water, drainage water and mixed water (1:1) of Kafr El-Sheikh Governorate, on the growth and N_2 fixation of ***Azolla pinnata*** and ***Azolla filiculoides*** was studied. Water characteristics are presented in Table (1).

Table 1. Chemical analysis of different types of water (meq/l.) of Kafr El-Sheikh Governorate.

Parameters		E.C	Cations (meq/l.)			Anions (meq/l.)			
Types of water	pH	mmhos /cm	Na^+	Ca^{++}	Mg^{++}	Cl^-	HCO_3^-	$CO_3^=$	$SO_4^=$
Irrigation water	7.21	0.65	2.47	2.0	2.0	2.24	4.23	-	-
Drainage water	7.81	1.30	7.39	3.5	5.0	5.75	10.14	-	-
Mixed water	7.56	0.90	5.00	2.5	3.5	3.40	7.60	-	-

Azolla used

Azolla pinnata 7001 and *Azolla filiculoides* 1001 were kindly supplied by Soil, Water and Environment Res. Inst. ARC, Giza, Egypt.

Cultivation of *Azolla*

Cultivation of *Azolla* was undertaken in plastic dishes. Dishes were filled with 5 liter water of different types of water, inoculated with standard inoculum of the proper species (5 g/dish) and then incubated in a wire proof greenhouse under normal conditions. Four replicates of each treatment were done. *Azolla* cultures were kept at a constant volume of water throughout the experimental period by frequent irrigation with different types of water to compensate water losses due to evaporation process. Samples of *Azolla* were taken at intervals of 3, 6, 10, 21 and 28 days of incubation to determine fresh and dry weights, nitrogen content and nitrogenase activity of *Azolla* fronds. Ammoniacal and nitrate nitrogen were also estimated in the growth medium of *Azolla* at the same above-mentioned intervals.

Determinations

- Total nitrogen was determined according to **Black *et al* (1965).**
- Ammoniacal and nitrate nitrogen were estimated according to **Bermner and Keeny (1965).**
- Nitrogenase activity was determined according to **Hardy *et al* (1973).**

Availability and efficiency of nitrogen from *Azolla filiculoides* for rice growth

Two field experiments were conducted at the Research and Experimental Station of Moshtohor, Fac. of Agriculture, Qualubia Governorate during 1993/94 and 1994/95 seasons to study the inoculation efficiency with *Azolla* as N source for rice manuring (Giza 171 cultivar). The soil used in the experiment was clay loamy soil. Mechanical and chemical analyses of the soil are presented in Table (2). Chemical analyses was estimated in saturated soil paste according to **Black *et al* (1965).** Whereas, mechanical analysis was estimated according to **Piper (1950).** A randomized complete block design (RCBD) with three replicates was used in both seasons and plot area was 10.5 m^2.

Preparation of *Azolla* nursery

Azolla filiculoides 1001 was grown in a separate nursery in the field near the experimental soil in a 3 x 3 m plots. *Azolla* cultures were kept at a constant volume of water throughout the growth period by frequent irrigation till *Azolla* covered the entire surface of the water, then it was harvested.

Effect of rice inoculation with *A. filiculoides* and different levels of phosphorus application

In this experiment different levels of *Azolla* were used as a sole source of nitrogen for rice fertilization . *Azolla* *filiculoides* was applied 7 days after transplanting of rice plants as a single N-manure. Fresh *Azolla* was added to transplanted rice at three rates *i.e.* 100, 150 and 200 g fresh *Azolla*/m^2 using three levels of phosphatic fertilizer as triple super phosphate (TSP). Treatments of the designed combined different *Azolla* rates and different phosphorus levels were as follows :

1. *Azolla* 100 g/m^2 + 15 kg P/fed.
2. *Azolla* 100 g/m^2 + 30 kg P/fed.
3. *Azolla* 100 g m^2 + 45 kg P/fed.
4. *Azolla* 150 g/m^2 +15 kg P/fed.
5. *Azolla* 150 g/m^2 + 30 kg P/fed.
6. *Azolla* 150 g/m^2 + 45 kg P/fed.
7. *Azolla* 200 g/m^2 + 15 kg P/fed.
8. *Azolla* 200 g/m^2 + 30 kg P/fed.
9. *Azolla* 200 g/m^2 + 45 kg P/fed.

Phosphatic fertilizer was supplied at tillering stage of plant growth.

Soil sampling and determinations

Microbiological analyses of rhizosphere soil samples were periodically determined at tillering, heading and maturity stages of rice plant growth.

Microbiological determinations

1. Total count
2. Inorganic phosphate solubilizing bacteria.
3. Ammonifiers. 4. Nitrifiers
5. Denitrifiers

Table 2. Chemical and mechanical analysis of experimental soil of Moshtohor

a. Chemical analysis

Organic matter (%)	pH	T.N %	T.P. %	E.C. mmhos/cm
1.63	7.43	0.02	0.043	1.5

Cations (meq/l.)			Anions (meq/l.)			
K^+	Ca^{++}	Mg^{++}	Cl^-	CHO^-_3	$CO^=_3$	$SO^=_4$
0.85	4.8	3.0	1.5	3.7	--	3.45

b. Mechanical analysis

Coarse sand (%)	Fine sand (%)	Silt (%)	Clay (%)	Textural class
2.32	19.63	23.17	54.82	Clay

E.C: Electric conductivity T.N: Total nitrogen
T.P.: Total phosphorus

Chemical analyses

Ammoniacal and nitrate nitrogen were determined periodically in the water of the field under the developed plants at tillering, heading and maturity stages. Also, after crop harvesting, soil samples were taken and total nitrogen, phosphorus and potassium were estimated.

Chemical analyses of plant

1. Total nitrogen, phosphorus and potassium were periodically determined in the dry matter of shoot system at tillering, heading and maturity stages of rice growth.

2. Chlorophyll (a) and (b) as well as carotenoids were estimated in the 3rd leaf of the plant at heading stage.

3. Total nitrogen, phosphorus, potassium and crude protein were determined in dry grains.

4. Total carbohydrates content was determined in dry grains by the phenol sulphuric acid method and calculated as percentage.

Plant growth parameters estimated

1. Plant height (cm).
2. Fresh and dry weight of shoot system (g/plant).

Yield and its components

At the end of the experiment, rice plants were harvested, then straw yield ton/fed, grain yield ton/fed and weight of 1000-grains (g) were determined.

Microbiological analysis

1. Total count was estimated by using soil yeast extract agar medium according to **Allen (1953).**

2. Inorganic phosphate solubilizing bacteria were estimated by using **Bunt and Rovira medium (1955)** modified by **Abd El-Hafez (1966).**

3. Ammonifiers were determined according to **Allen (1953).**

4. Nitrifiers were determined according to **Black *et al* (1965).**

5. Denitrifiers were determined according to **Tiedje (1982).**

Ammonifiers, Nitrifiers and Denitrifiers were Counted by Using Most Probable Number (MPN) technique **(Cochran, 1950).**

Chemical analysis

1. Total nitrogen was determined by using (Kjeldahl digestion) method according to **Black *et al* (1965).**
2. Total phosphorus was colorimetrically determined according to **APHA (1989).**

3. Total potassium was estimated by flame photometer apparatus **(Brown and Lilliland 1946).**

4. Ammoniacal and nitrate nitrogen were determined according to **Bermner and Keeny (1965).**

5. Chlorophyll a,b and carotenoids were determined according to **Wettstein (1957).**

6. Total carbohydrates was determined according to **Michel *et al* (1956).**

7. Crude protein was calculated according to the following equation:

   Crude protein= % total nitrogen x 5.95 **(A.O.A.C., 1980).**

Statistical analysis

Analysis of variance (ANOVA) of data obtained from growth characters, yield and yield components and chemical analysis were carried out and significant differences among the means of various treatments were distinguished by L.S.D. **(Snedecor and Cochran, 1989).**

RESULTS AND DISCUSSION

Selection of the most efficient species of *Azolla* in growth and nitrogen fixation

A comparative sudy was performed using ***A. pinnata*** 7001 and ***A. filiculoides*** 1001 to select the most active species which exhibit the highest growth rate and nitrogen fixation in different types of water mentioned before to be used in the subsequent study. Data presented in Table (3) show that there was some differences between the two species of ***Azolla*** in growth yield and nitrogen content. ***A. filiculoides*** showed greater accumulation of fresh and dry yield of biomass compared with ***A. pinnata*** and this was true at all incubation periods. The highest yield of fresh and dry weights of biomass was obtained at the 21st day of incubation.

Results also show that the highest biomass yield was recorded in case of ***Azolla*** spp. grown in irrigation water. This result could be attributed to the low level of salts in the irrigation water which enhanced ***Azolla*** growth. Whereas, biomass yield slightly differed when ***Azolla*** was grown in both drainage and mixed water. **Rahoma (1985)** indicated that fresh and dry weights as well as total nitrogen content of the tested ***Azolla*** spp. increased with increasing the dilution of drainage water. He attributed this finding to the reduction in sodium chloride concentration. It must be stressed that ***A. filiculoides*** is relatively resistant to a high concentration of sodium chloride *i.e.* 2000 and 2500 ppm as compared with ***A. pinnata*** **(Neweigy *et al* 1992).** So, ***A. filiculoides*** exhibited higher yield of biomass than ***A. pinnata*** in different types of water.

Obtained data in Table (3) also indicate that the percentage of total nitrogen content of the two tested species of ***Azolla*** were not constant and exhibited fluctuations during the incubation period. These fluctuations were most probably due to temperature changes occurring in the greenhouse in which ***Azolla*** was grown. It could be also notice that ***A. filiculoides*** gave higher percentages of total nitrogen than *A. pinnata* under different irrigation waters and incubation periods. This result is in harmony with those obtained by **Neweigy *et al* (1992)** who found that *A. filiculoides* gave the highest nitrogen content compared with other species of *Azolla* i.e. *A. pinnata, A. microphylla* and *A. caroliniana*. Total nitrogen content % in both species of *A. filiculoides* and *A. pinnata* was the highest at the 21st day of incubation compared with the other incubation periods.

Nitrogenase acrivity of *Azolla* fronds, ammoniacal and nitrate nitrogen released from *Azolla* grown in different types of water

Data of nitrogenase activity of *Azolla*, ammoniacal and nitrate nitrogen released are presented in Table (4). Data show that these parameters differed in the different types of *Azolla* spp., type of water and incubation period. Results show that N_2-ase activity of the tested *Azolla* was not constant and exhibited fluctuations during the incubation period. Such fluctuation pattern of the N_2-ase activity during the growth period of *Azolla* was similar to that observed by **Manna and Singh (1991)** who reported that nitrogenase activity in the cultures is most probably controlled by repression and depression of nitrogenase biosynthesis. At high level of nitrogenase the cellular pool of ammonia increases, causing repression of enzyme synthesis. The following decline of nitrogenase activity is accompanied by a gradual reduction of ammonia pool, which at minimum level causes enhancing of nitrogenase biosynthesis.

Data recorded in Table (4) also, emphasize that nitrogenase activity values were the greatest in case of *Azolla* growing in irrigation water followed by mixed water, then drainage water. Both species of *Azolla* gave the highest nitrogenase activity values at the 21st day of incubation and decreased thereafter. Ammoniacal nitrogen in the growth medium increased with the increasing of incubation period while, nitrate nitrogen decreased with the increasing of incubation period. The same trend was observed in both *A. filiculoides* and *A.pinnata* as well as when the two *Azolla* species were grown in different types of water *A. filiculoides* exerted higher N_2 fixing capacity and N_2-ase activity than *A. pinnata*. These results are in accordance with those reported by **Rahoma (1985)** and **Neweigy *et al* (1992)** who reported that *A. filiculoides* gave the highest N_2-ase activity compared with the other species of *Azolla* especially under high level of salt concentration. Therefore, *A. filiculoides* was chosen for the further study.

Effect of different levels of *Azolla* and phosphorus on populations of some soil microorganisms in rhizosphere soil of rice plants:

1. **Changes in populations of total microbial flora and inorganic phosphate solubilizers**

Data in Table (5) show the effect of different levels of *Azolla* application as a biofertilizer combined with different

Table 3. Fresh and dry weight and total nitrogen content of *A. pinnata* and *A. filiculoides* grown in different types of Kafr El-Sheikh water.

Type of water	Irrigation water						Drainage water						Mixed water					
	A. pinnata			*A. filiculoides*			*A. pinnata*			*A. filiculoides*			*A. pinnata*			*A. filiculoides*		
Incubation period (days)	*Fr.wt. g/m²	Dr.wt. g/m²	T.N (%)	Fr.wt. g/m²	Dr.wt. g/m²	T.N (%)	Fr.wt. g/m²	Dr.wt. g/m²	T.N (%)	Fr.wt. g/m²	Dr.wt. g/m²	T.N (%)	Fr.wt. g/m²	Dr.wt. g/m²	T.N (%)	Fr.wt. g/m²	Dr.wt. g/m²	T.N (%)
3	375	9.42	3.51	451	8.31	3.75	451	10.68	2.75	560	18.00	3.50	426	8.55	3.30	488	13.20	4.01
7	425	12.42	3.86	521	13.71	4.50	484	12.00	2.90	600	19.92	3.18	447	12.87	3.20	598	18.42	3.86
14	506	16.42	3.69	589	20.79	4.30	562	19.71	3.00	676	21.00	4.04	566	18.60	4.70	743	19.20	4.90
21	917	41.34	4.35	1343	48.00	5.08	694	21.84	3.80	816	35.60	4.32	694	23.34	3.35	900	28.50	4.34
28	781	30.00	3.55	1107	35.10	4.16	586	16.05	3.21	710	30.00	3.60	634	18.60	2.98	764	24.00	3.22

• Fr.wt. = Fresh weight Dr.wt. = Dry weight T.N. = Total nitrogen

Table 4. Ammoniacal and nitrate nitrogen release (ppm) and nitrogenase activity (μ moles C_2H_4/hr./g dry weight of *Azolla*/hr.) of *A. pinnata* and *A. filiculoides* grown in different types of Kafr El-Sheikh water.

Type of water	Irrigation water						Drainage water						Mixed water					
	A. pinnata			*A. filiculoides*			*A. pinnata*			*A. filiculoides*			*A. pinnata*			*A. filiculoides*		
Incubation period (days)	*NH_4-N	NO_3-N	N_2-ase	*NH_4-N	NO_3-N	N_2-ase	*NH_4-N	NO_3-N	N_2-ase	*NH_4-N	NO_3-N	N_2-ase	*NH_4-N	NO_3-N	N_2-ase	*NH_4-N	NO_3-N	N_2-ase
3	10	20	4.34	14	30	6.40	20	30	3.16	24	10	4.34	16	18	3.92	20	--	5.59
7	15	10	7.75	18	18	9.90	22	22	5.46	28	16	6.43	25	15	6.00	30	25	7.80
14	18	15	8.57	25	15	12.25	31	16	6.16	36	10	8.32	34	10	7.37	38	15	9.12
21	30	8	12.96	32	15	15.16	40	10	9.24	48	8	11.66	30	7.5	10.30	34	10	12.50
28	33	10	8.10	40	12	10.9	46	12	4.26	52	10	7.22	40	6.4	5.70	45	12	8.6

*NH_4-N : Ammoniacal nitrogen (ppm) NO_3-N : Nitrate nitrogen (ppm) N_2-ase : Nitrogenase activity (μ moles C_2H_4/hr./g dry *Azolla*).

levels of phosphorus on bacterial counts. Obtained data clearly indicate that populations of total microbial flora were the highest when ***Azolla*** was inoculated at a rate of 100 g/m^2 combined with 30 kg P_2O_5 /fed. The counts of phosphate solubilizing bacteria (PSB) were the highest with inoculation of ***Azolla*** at a rate of 150 g/m^2 combined with 30 kg P_2O_5 /fed. This result was obtained in both seasons and all growth stages of rice plants.

It is clear also that the lowest total microbial counts were obtained in the treatment of the application of 150 g P_2O_5 /fed ***Azolla***/m^2 + 15 Kg, 100 g ***Azolla***/m^2 + 15 kg P_2O_5 /fed and 200 g ***Azolla***/m^2 + 15 kg P_2O_5 /fed at tillering, heading and maturity stages of rice growth, respectively. Whereas, the lowest count of (PSB) were obtained in the presence of 100 g ***Azolla***/m^2 + 45 kg P_2O_5 /fed, 150 g ***Azolla***/m^2 + 15 kg P_2O_5 /fed and 150 g ***Azolla***/m^2 + 45 kg P_2O_5 /fed at tillering, heading and maturity stages of rice growth, respectively.

Generally, it is worthy to notice that the increase of *Azolla* inoculum rate and phosphorus level didn's show increase in either total microbial counts or (PSB) and this was true in both seasons as well as at different growth stages.

2. Changes in populations of ammonifiers, nitrifiers and denitrifiers

Data recorded in Table (6) show that populations of ammonifiers increased with the increasing of ***Azolla*** inoculum and this trend was observed in both seasons and at various growth stages of rice plants. Populations of nitrifiers and denitrifiers did not increase with the increasing of inoculum rate of ***Azolla***. Increasing the level of phosphatic fertilizer did not show any increase in ammonifiers, nitrifiers or denitrifiers counts. The highest counts of ammonifiers were resulted in the treatment of application of 150 g ***Azolla***/m^2 + 30 kg P_2O_5 /fed, 200 g ***Azolla***/m^2 + 15 kg P_2O_5 /fed and 200 g ***Azolla***/m^2 + 30 kg P_2O_5 /fed at tillering, heading and maturity stages, respectively. Whereas, the highest counts of nitrifiers were resulted in case of the application of 150, 100 and 150 g ***Azolla***/m^2 combined with 45 kg P_2O_5 /fed at tillering, heading and maturity stages, respectively.

Data in Table (6) also show that the highest counts of denitrifiers were resulted from the application of ***Azolla*** at a rate of 150 g/m^2, 150 g/m^2 and 200 g/m^2 combined with 15 kg P_2O_5 /fed at tillering, heading and maturity stages, respectively.

On the other hand, the lowest counts of ammonifiers were obtained in the treatment of application of 100 g ***Azolla***/m^2 + 15 Kg P_2O_5 /fed, 100 g ***Azolla***/m^2 + 15 Kg P_2O_5 /fed and 100 g ***Azolla***/m^2 + 45 Kg P_2O_5 /fed at tillering, heading and maturity stages of rice growth, respectively. Also, the lowest counts of nitrifying bacteria were obtained in case of application of 100 g ***Azolla***/m^2 + 45 kg P_2O_5 /fed, 100 g ***Azolla***/m^2 + 15 kg P_2O_5 /fed and 100 g ***Azolla***/m^2 + 15 kg P_2O_5 /fed at tillering, heading and maturity stages, respectively. While, the lowest counts of different stages of growth in both seasons.

Plant growth stage / Treatments	Tillering				Heading				Maturity			
	T.C		P.S.B		T.C		P.S.B		T.C		P.S.B	
	S1	S2	S1	S2	S1	S2	S1	S2	S1	S2	S1	S2
***Azolla* addition as a biofertilizer**												
AZ.(100 g/m^2) + P (15 kg) P_2O_5/fed.	2820	3013	128.21	146.70	2775	2141	241.10	244.10	1560	1800	112.4	118.68
AZ.(100 g/m^2) + P (30 kg) P_2O_5/fed.	6060	4542	140.70	130.40	7384	6470	199.40	189.00	4840	4163	123.2	114.80
AZ.(100 g/m^2) + P (45 kg) P_2O_5/fed.	3650	3520	91.30	81.50	5112	3940	148.06	194.60	2320	3052	116.8	116.55
AZ.(150 g/m^2) + P (15 kg) P_2O_5/fed.	2490	2178	98.85	110.40	3349	3897	142.00	130.55	1760	2618	106.8	96.22
AZ.(150 g/m^2) + P (30 kg) P_2O_5/fed.	2990	2678	183.00	163.00	4970	3743	210.20	250.45	1840	2992	140.0	131.10
AZ.(150 g/m^2) + P (45 kg) P_2O_5/fed.	2747	3528	118.26	126.30	5921	4104	184.60	215.46	2160	2362	82.6	86.30
AZ.(200 g/m^2) + P (15 kg) P_2O_5/fed.	2630	2304	93.16	97.80	4889	2860	170.40	210.00	1120	1262	92.6	115.24
AZ.(200 g/m^2) + P (30 kg) P_2O_5/fed.	2830	2608	141.50	124.10	4236	3385	181.72	179.34	1480	1420	95.6	111.35
AZ.(200 g/m^2) + P (45 kg) P_2O_5/fed.	2660	2589	148.14	150.78	3337	3935	196.80	207.60	1880	1605	88.1	107.90

T.C : Total count P.S.B : Phosphate solubilizing bacteria S : Season P : Phosphorus AZ. : *Azolla*

Table 6. Ammonifiers, Nitrifiers and Denitrifiers counts (x 10^4 g dry weight of soil) in rhizosphere of rice plants at different stages of growth in both seasons.

Plant growth stage / Treatments	Tillering						Heading						Maturity					
	Am.	Am.	Ni.	Ni.	De.	De.	Am.	Am.	Ni.	Ni.	De.	De.	Am.	Am.	Ni.	Ni.	De.	De.
	S1	S2	S1	S2	S1	S2	S1	S2	S1	S2	S1	S2	S1	S2	S1	S2	S1	S2
***Azolla* addition as a biofertilizer**																		
AZ.(100 g/m^2) + P (15 kg) P_2O_5/fed.	39.09	33.06	26.52	25.87	46.01	52.06	60.5	62.80	14.26	12.03	33.37	24.22	18.4	24.25	8.20	10.74	14.2	16.49
AZ.(100 g/m^2) + P (30 kg) P_2O_5/fed.	49.88	45.09	26.73	20.03	48.16	54.45	68.9	66.20	27.10	24.40	44.02	27.60	19.2	22.75	16.80	16.02	18.0	19.65
AZ.(100 g/m^2) + P (45 kg) P_2O_5/fed.	51.46	49.17	13.24	11.56	41.46	58.06	64.0	68.30	29.23	32.32	27.04	33.09	16.2	19.35	13.64	11.47	19.6	22.20
AZ.(150 g/m^2) + P (15 kg) P_2O_5/fed.	52.37	36.45	19.88	16.87	55.73	63.35	70.7	76.08	18.52	18.44	44.50	50.14	18.8	25.44	14.60	15.48	11.6	14.39
AZ.(150 g/m^2) + P (30 kg) P_2O_5/fed.	76.01	72.77	30.46	32.23	51.46	50.53	81.7	84.80	28.90	24.67	36.92	30.09	21.2	18.62	11.20	12.34	10.2	12.65
AZ.(150 g/m^2) + P (45 kg) P_2O_5/fed.	65.73	62.06	38.46	36.02	44.54	40.97	76.9	82.80	23.91	21.04	34.02	23.78	24.8	20.84	26.80	24.32	18.4	20.26
AZ.(200 g/m^2) + P (15 kg) P_2O_5/fed.	62.92	58.88	36.56	32.77	36.56	32.16	92.7	95.70	25.75	19.75	22.01	24.40	29.6	26.61	11.20	15.68	28.8	24.80
AZ.(200 g/m^2) + P (30 kg) P_2O_5/fed.	67.39	60.21	31.46	23.03	40.92	35.86	89.2	80.10	27.10	23.79	31.24	28.89	34.8	32.50	20.80	14.70	16.4	15.50
AZ.(200 g/m^2) + P (45 kg) P_2O_5/fed.	69.01	64.61	37.32	29.91	38.60	36.97	82.2	88.14	24.91	19.03	27.04	25.82	32.8	28.90	16.60	14.66	24.8	18.80

Am. : Ammonifiers Ni : Nitrifiers De. : Denitrifiers S : Season P : Phosphorus AZ. : *Azolla* nitrifying bacteria were resulted in the treatment of application of 200 g Azolla/m^2 + 15 Kg P_2O_5 /fed, 200 g Azolla/m^2 + 15 Kg P_2O_5 /fed and 150 g Azolla/m^2 + 30 kg P_2O_5 /fed at tillering, heading and maturity stages of rice growth, respectively (Table, 6).

Effect of different levels of *Azolla* and phosphorus on ammoniacal and nitrate nitrogen in rhizosphere soil of rice plants

Data in Table (7) show that ammoniacal and nitrate nitrogen in rhizosphere soil of rice plants gradually increased in all treatments with the increasing of growth period. Ammoniacal and nitrate nitrogen slightly increased in the 1st season compared with the 2nd one. In addition, ammoniacal nitrogen level was the highest in case of *Azolla* application at a rate of 150 g/m^2 combined with 45 kg P_2O_5 /fed.

On the other hand, nitrate nitrogen was the highest in case of *Azolla* application at a rate of 200 g/m^2 combined with 45 kg P_2O_5 /fed. The same trend was observed at all growth stages of rice plants. In contrast, ammoniacal nitrogen concentrations were the lowest in case of *Azolla* application at a rate of 100 g/m^2 combined with 15 kg P_2O_5 /fed, while nitrate nitrogen concentrations were the lowest in case of *Azolla* application at a rate of 100 g/m^2 combined with 30 kg P_2O_5 /fed.

It could be concluded that ammoniacal and nitrate nitrogen increased in the rhizosphere with the increasing of *Azolla* inoculum rate (200 g/m^2) as well as phosphatic fertilization level (45 kg P_2O_5 /fed.). This may be due to the increase of nitrogen fixation by increasing *Azolla* inoculum rate and the suitable level of phosphorus for *Azolla* growth and nitrogenase activity.

Effect of different levels of *Azolla* and phosphorus on nitrogen, phosphorus and potassium in rhizosphere soil at the end of the experiment

Data in Table (8) emphasize that the highest values of total nitrogen, phosphorus and potassium were obtained in the *Azolla* treatments at a rate of 150 g/m^2 combined with 30 kg P_2O_5 /fed, 200 g ***Azolla***/m^2 + 30 kg P_2O_5 /fed and 200 g ***Azolla***/m^2 + 30 kg P_2O_5 /fed, respectively. Whereas, the lowest values of total nitrogen, phosphorus and potassium were obtained in the *Azolla* treatment at a rate of 100 g/m^2 combined with 15 kg P_2O_5 /fed and the same trend of results was obtained in both growing seasons.

Generally, total nitrogen in soil after harvesting was higher in the 1st season than the 2nd one. While, total phosphorus and potassium were lower in the 1st season than the 2nd one. The differences between the two seasons were obtained on NPK content in soil may be due to the difference in meterological factors. These results were observed in all treatments. Similar results of nitrogen in soil were obtained by many investigators.

As regard to the effect of *Azolla* application on soil fertility, **Main and Stewart (1985)** reported that inoculation of *Azolla* in rice fields resulted the

Table 7. Effect of *Azolla* treatments and P levels on ammoniacal and nitrate nitrogen (ppm) in rhizosphere of rice plants at different stages of growth in both seasons.

Plant growth stage	Tillering				Heading				Maturity			
	NH_4-N		NO_3-N		NH_4-N		NO_3-N		NH_4-N		NO_3-N	
Treatments	S1	S2	S1	S2	S1	S2	S1	S2	S1	S2	S1	S2
***Azolla* addition as a biofertilizer**												
AZ.(100 g/m²) + P (15 kg) P_2O_5/fed.	65.5	59.5	50.0	44.0	78	75	63	60	83	80	78	76
AZ.(100 g/m²) + P (30 kg) P_2O_5/fed.	71.5	74.5	45.5	40.5	80	74	55	53	90	86	75	72
AZ.(100 g/m²) + P (45 kg) P_2O_5/fed.	82.0	75.0	48.5	40.5	95	90	65	62	100	98	84	82
AZ.(150 g/m²) + P (15 kg) P_2O_5/fed.	72.0	69.2	47.0	44.5	115	110	75	65	120	118	82	80
AZ.(150 g/m²) + P (30 kg) P_2O_5/fed.	79.0	76.0	54.5	51.5	123	115	70	69	150	140	100	95
AZ.(150 g/m²) + P (45 kg) P_2O_5/fed.	110.0	105.0	67.0	47.0	128	122	75	70	153	150	90	85
AZ.(200 g/m²) + P (15 kg) P_2O_5/fed.	78.0	73.0	60.0	55.0	97	92	85	80	140	135	98	95
AZ.(200 g/m²) + P (30 kg) P_2O_5/fed.	80.0	78.8	65.5	58.8	108	103	95	90	120	115	99	94
AZ.(200 g/m²) + P (45 kg) P_2O_5/fed.	94.0	90.0	80.0	70.0	118	112	106	96	128	125	115	112

S : Season P : Phosphorus AZ. : *Azolla*

Table 8. Effect of different *Azolla* and P-levels on total nitrogen, phosphorus and potassium content (ppm) in rhizosphere of rice plants at the end of the experiment.

Parameters	Total nitrogen		Total phosphorus		Total potassium	
Treatments	Season I	Season II	Season I	Season II	Season I	Season II
***Azolla* addition as a biofertilizer**						
AZ.(100 g/m²) + P (15 kg) P_2O_5/fed.	435	416	366.6	445	380	403
AZ.(100 g/m²) + P (30 kg) P_2O_5/fed.	571	550	480.0	500	448	437
AZ.(100 g/m²) + P (45 kg) P_2O_5/fed.	483	470	520.0	563	385	396
AZ.(150 g/m²) + P (15 kg) P_2O_5/fed.	720	650	506.3	656	490	501
AZ.(150 g/m²) + P (30 kg) P_2O_5/fed.	800	750	553.0	696	470	493
AZ.(150 g/m²) + P (45 kg) P_2O_5/fed.	691	646	666.0	720	475	499
AZ.(200 g/m²) + P (15 kg) P_2O_5/fed.	683	616	626.0	810	445	512
AZ.(200 g/m²) + P (30 kg) P_2O_5/fed.	700	616	760.0	983	480	540
AZ.(200 g/m²) + P (45 kg) P_2O_5/fed.	783	666	680.0	730	445	520

AZ. : *Azolla* P : Phosphorus

highest organic carbon and total nitrogen content of the soil at harvesting time.

Interaction effect between *Azolla* inoculum rates as a biofertilizer and phosphorus levels on plant height, fresh and dry weights of rice plants in both seasons

Data in Table (9) indicate that plant heights were insignificantly affected by different inoculum rates and phosphorus levels added and this was true in both growing seasons.

Regarding the fresh and dry weights of rice plants, data show that fresh and dry weights of the plants significantly increased with the application of *Azolla* at a rate of 150 g/m^2 in the 1st season. Fresh and dry weights of rice plants were non-significantly affected by different phosphorus fertilization levels and this result was obtained in both seasons. Meanwhile, the interaction between *Azolla* inoculum rates and phosphorus levels was significant on the mean values of fresh weight in the 1st season. The highest value of fresh weight was obtained from using *Azolla* inoculum rate 150 g/m^2 combined with 30 kg P_2O_5 /fed (Table 9).

Subramani and Kannaiyan (1987) found that application of ***Azolla pinnata*** at 200 g/m^2 7 days after transplanting gave the greatest plant height, number of tillers and number of panicles.

Kalita and Sarma (1994) found that inoculation with ***Azolla pinnata*** increased plant height, number of panicles, number of grains/panicle, weight of panicle and number of panicles/plant of rice. On the other hand, **El-Shahat (1997)** found that ***A. filiculoides*** gave maximum records of the number of panicles/plant of rice variety Giza 171 compared with ***A. pinnata***.

Interaction effect between *Azolla* inoculum rates and phosphorus levels on nitrogen content of rice plants at different stages of growth

Data presented in Table (10) indicate that mean values of total nitrogen content were non significantly affected by either ***Azolla*** inoculum rates or phosphorus levels at all growth stages in the 1st season. On the other hand, the interaction between *Azolla* inoculum rates and phosphorus levels had a significant effect on total nitrogen in rice plants in both tillering and heading stages. At tillering stage, the highest value of total nitrogen content was obtained in the treatment of inoculation of ***Azolla*** at a rate of 150 g/m^2 combined with 15 kg P_2O_5 /fed while, the lowest value was obtained when ***Azolla*** inoculation was at a rate of 100 g/m^2 combined with 15 kg P_2O_5 /fed. At heading stage, data showed that the highest nitrogen content was obtained when ***Azolla*** inoculation was at a rate of 200 g/m^2 combined with 45 kg P_2O_5 /fed were used. While, the lowest value was obtained when 200 g/m^2 ***Azolla*** combined with 30 kg P_2O_5 /fed. Generally, the mean values of total nitrogen content of the plants were increased at tillering stage in all treatments and decreased thereafter.

In the 2nd season, ***Azolla*** inoculum rates as well as phosphorus levels had a

Table 9. Interaction effect between *Azolla* inoculum rates and phosphorus levels on plant height, fresh and dry weight of rice plants in both seasons.

First Season

	Plant height (cm)				Fresh weight (g/plant)				Dry weight (g/plant)			
P-levels (kg P_2O_5/fed.)	15	30	45	Mean	15	30	45	Mean	15	30	45	Mean
100 g *Azolla*/m²	101.67	101.83	98.17	100.56	11.61	10.85	13.33	11.93	3.12	2.90	3.50	3.18
150 g *Azolla*/m²	95.50	102.00	108.77	102.09	12.48	14.52	10.80	12.60	3.59	3.77	3.76	3.71
200 g *Azolla*/m²	94.77	98.40	91.50	94.89	9.85	9.63	13.79	11.09	3.09	3.13	2.49	2.90
Mean	97.31	100.74	99.48		11.31	11.67	12.64		3.27	3.27	3.25	
L.S.D. at 5%												
***Azolla* (A)**	N.S				1.02				0.47			
Phosphorus (P)	N.S				N.S				N.S			
(A) x (P)	N.S				2.09				N.S			

Second Season

	Plant height (cm)				Fresh weight (g/plant)				Dry weight (g/plant)			
P-levels (kg P_2O_5/fed.)	15	30	45	Mean	15	30	45	Mean	15	30	45	Mean
100 g *Azolla*/m²	100.27	100.07	93.30	97.88	9.26	8.32	8.94	8.84	3.19	2.13	3.29	2.87
150 g *Azolla*/m²	98.57	94.33	103.43	98.87	11.94	9.77	9.43	10.38	4.39	3.56	3.19	3.72
200 g *Azolla*/m²	100.77	91.40	96.23	96.13	10.38	9.73	12.29	10.80	3.51	3.17	3.87	3.52
Mean	99.87	95.27	97.66		10.53	9.27	10.22		3.70	2.95	3.45	
L.S.D. at 5%												
***Azolla* (A)**	N.S				N.S				N.S			
Phosphorus (P)	N.S				N.S				N.S			
(A) x (P)	N.S				N.S				N.S			

N.S. : Non significant

Table 10. Interaction effect between *Azolla* inoculum rates and phosphorus levels on nitrogen content (%) of rice plants at different stages of growth in both seasons.

First Season

P-levels (kg P_2O_5/fed.)	Tillering stage 15	30	45	Mean	Heading stage 15	30	45	Mean	Maturity stage 15	30	45	Mean
100 g *Azolla*/m²	1.66	2.29	2.41	2.12	1.09	1.14	0.95	1.06	0.65	0.68	0.64	0.66
150 g *Azolla*/m²	2.81	2.18	2.34	2.44	1.07	1.19	0.98	1.08	0.52	0.62	0.74	0.64
200 g *Azolla*/m²	2.36	2.68	1.82	2.29	0.88	0.86	1.26	0.99	0.56	0.83	0.87	0.75
Mean	2.28	2.38	2.19		1.01	1.06	1.06		0.58	0.71	0.76	
L.S.D. at 5%												
***Azolla* (A)**	N.S				N.S				N.S			
Phosphorus (P)	N.S				N.S				N.S			
(A) x (P)	0.29				0.27				N.S			

Second Season

P-levels (kg P_2O_5/fed.)	Tillering stage 15	30	45	Mean	Heading stage 15	30	45	Mean	Maturity stage 15	30	45	Mean
100 g *Azolla*/m²	1.38	0.84	0.91	1.07	0.92	1.02	0.93	0.96	0.60	0.72	0.78	0.69
150 g *Azolla*/m²	1.45	0.69	0.99	1.02	0.98	1.07	1.03	1.03	0.45	0.73	0.86	0.68
200 g *Azolla*/m²	0.90	0.82	1.07	0.93	1.31	1.24	1.33	1.29	0.80	0.84	0.63	0.76
Mean	1.25	0.79	0.99		1.07	1.11	1.10		0.62	0.76	0.76	
L.S.D. at 5%												
***Azolla* (A)**	0.11				0.11				N.S			
Phosphorus (P)	0.11				N.S				N.S			
(A) x (P)	0.19				N.S				0.27			

N.S. : Non significant

significant effect on the mean values of total nitrogen at tillering stage. Whereas, total nitrogen content was significantly affected by *Azolla* inoculum rates only at heading stage and non significantly affected by either *Azolla* inoculum rates or phosphorus levels at maturity stage. On the other hand, the interaction effect between *Azolla* inoculum rates and phosphorus levels was significant on total nitrogen content at tillering and maturity states. The highest total nitrogen was observed in case of *Azolla* application at a rate of 150 g/m^2 combined with 15 kg P_2O_5 /fed, 200 g/m^2 combined with 30 kg P_2O_5 /fed at tillering and maturity stages, respectively. Generally, the mean values of total nitrogen were higher at tillering and heading stages than maturity stage in the 2nd season under all treatments. Total nitrogen content was higher in the 1st season than the 2nd one at tillering stage in all treatments. This difference between the two seasons was obtained on total nitrogen content may be due to the change in meterological factors (Table, 10).

Interaction effect between *Azolla* inoculum rates and phosphorus levels on phosphorus content % of rice plants at different stages of growth

Results in Table (11) show that the mean values of total phosphorus in the 1st season in rice plants were non significantly affected by different *Azolla* inoculum rates at tillering and heading stages but were significantly affected at maturity stage. The highest total phosphorus at maturity stage was observed when *Azolla* was inoculated at a rate of 150 g/m^2. Whereas, levels of phosphorus added had a significant effect on total P content % in the 1st season and this was true in all growth stages. The percentage of phosphorus was increased by increasing phosphorus level and this result observed at all growth stages.

Data also show that the interaction effect was non significant on phosphorus content at tillering stage while, it was significant at heading and maturity stages. The highest value of P content was observed when *Azolla* inoculum was 150 g/m^2 combined with 30 kg P_2O_5 /fed at heading and maturity stages, whereas, the lowest phosphorus % was obtained when *Azolla* inoculum was 150 g/m^2 combined with 15 kg P_2O_5 /fed and 100 g/m^2 combined with 15 Kg P_2O_5 /fed at heading and maturity stages,respectively.

In the 2nd season, total-P-content of rice plants non significantly affected by either *Azolla* inoculum rates or phosphorus levels. The interaction between *Azolla* and phosphorus addition didn't show any significant effect on P content of rice plants and this was true at all growth stages. Generally, total phosphorus content % was higher at tillering stage than heading and maturity stages in both growing seasons. Also, the mean values of total phosphorus content % were higher at the 1st season than the 2nd one at all growth stages in all treatments.

Kadu *et al* (1991) reported that phosphorus content in rice plants was higher with the highest phosphorus fertilization level. **Mahajan *et al* (1994)** found that phosphorus uptake increased with the

Table 11. Interaction effect between *Azolla* inoculum rates and phosphorus levels on phosphorus content (%) of rice plants at different stages of growth in both seasons.

First Season

P-levels (kg P_2O_5/fed.)	Tillering stage				Heading stage				Maturity stage			
	15	30	45	Mean	15	30	45	Mean	15	30	45	Mean
100 g *Azolla*/m^2	0.39	0.47	0.48	0.45	0.29	0.30	0.26	0.28	0.08	0.13	0.16	0.13
150 g *Azolla*/m^2	0.37	0.46	0.46	0.43	0.15	0.32	0.19	0.23	0.13	0.22	0.14	0.16
200 g *Azolla*/m^2	0.41	0.41	0.57	0.46	0.29	0.26	0.21	0.26	0.12	0.19	0.11	0.13
Mean	0.39	0.45	0.50		0.26	0.29	0.32		0.11	0.13	0.18	
L.S.D. at 5%												
***Azolla* (A)**	N.S				N.S				0.02			
Phosphorus (P)	0.06				0.04				0.02			
(A) x (P)	N.S				0.07				0.04			

Second Season

P-levels (kg P_2O_5/fed.)	Tillering stage				Heading stage				Maturity stage			
	15	30	45	Mean	15	30	45	Mean	15	30	45	Mean
100 g *Azolla*/m^2	0.25	0.19	0.40	0.28	0.08	0.12	0.14	0.11	0.08	0.08	0.12	0.09
150 g *Azolla*/m^2	0.25	0.19	0.20	0.21	0.13	0.16	0.16	0.15	0.09	0.08	0.09	0.09
200 g *Azolla*/m^2	0.22	0.21	0.29	0.24	0.12	0.15	0.11	0.13	0.13	0.06	0.14	0.11
Mean	0.24	0.20	0.30		0.11	0.14	0.14		0.09	0.7	0.11	
L.S.D. at 5%												
***Azolla* (A)**	N.S				N.S				N.S			
Phosphorus (P)	N.S				N.S				N.S			
(A) x (P)	N.S				N.S				N.S			

increasing of phosphorus fertilization level. **Solaiman *et al* (1990)** indicated that total phosphorus significantly increased due to *Azolla* manuring and urea application but *Azolla* manuring gave better results than urea application.

Interaction effect between *Azolla* inoculum rates and phosphorus levels on potassium content % of rice plants at different stages of growth

Data in Table (12) show that *Azolla* inoculum rates had a significant effect on total potassium content of rice plants in all growth stages in the 1st season. The highest percentages of total potassium were obtained from *Azolla* inoculum at rates of 200, 100 and 200 g/m^2 at tillering, heading and maturity stages, respectively. On the other hand, the lowest values of total potassium content % were obtained when *Azolla* inoculum rates were 100, 150 and 150 g/m^2 at tillering, heading and maturity stages, respectively. Data also emphasize that total potassium significantly affected by phosphorus levels at heading and maturity stages. The highest K content was obtained when 15 kg P_2O_5 /fed was applied, while, the lowest ones were observed in case of 45 and 30 kg P_2O_5 /fed phosphorus were applied at heading and maturity stages, respectively.

Total potassium content was significantly affected by the interaction between *Azolla* and phosphorus additions and this result was observed at all growth stages. The highest percentages of K were obtained in the treatments of 150 g *Azolla*/m^2 combined with 45 kg P_2O_5 /fed, 100 g *Azolla*/m^2 + 15 kg P_2O_5 /fed and 100 g *Azolla*/m^2 + 15 kg P_2O_5 /fed at tillering, heading and maturity stages, respectively. While the lowest K content % were obtained in the treatments of 100 g *Azolla*/m^2 + 45 kg P_2O_5 /fed, 150 *Azolla* + 45 Kg P_2O_5/fed and 100 *Azolla*/m^2 + 45 kg P_2O_5 /fed, at tillerin heading and maturity stages, respe tively.

In the 2nd season, data show th total potassium was significantly affect by *Azolla* inoculum rate at tillering ar maturity stages but non significantly a fected at heading stage. The highest pe centages of K were obtained when *Azol* inoculum rate was 200 g/m^2 at tillerin and maturity stages while, the lowe ones were obtained when *Azolla* inoc lum rate was 100 g/m^2. Also, total po tassium was significantly affected b phosphorus levels at all growth stage The highest values of total potassiu content % were observed in case of ap plication of 45, 30 and 30 kg P_2O_5 /fed tillering, heading and maturity stage respectively, whereas, the lowest one were observed in case of application 15 kg P_2O_5 /fed at all growth stages.

Data also showed that the intera tion effect between *Azolla* and phospho rus on potassium content % was signif cant at tillering and maturity stages, b was not significant at heading stage. Th highest percentages of K were obtaine in the treatments of 200 g *Azolla*/m^2 + 4 kg P_2O_5 /fed, 100 g *Azolla*/m^2 + 15 k P_2O_5 /fed and 200 g *Azolla*/m^2 + 30 k P_2O_5 /fed at tillering, heading and ma turity stages, respectively, while, th lowest ones were in case of 150 *Azolla*/m^2 + 15 kg P_2O_5 /fed, 100 *Azolla*/m^2 + 30 kg P_2O_5 /fed and 100

Table 12. Interaction effect between *Azolla* inoculum rates and phosphorus levels on potassium content (%) of rice plants at different stages of growth in both seasons.

	Tillering stage				Heading stage				Maturity stage			
P-levels (kg P_2O_5/fed.)	15	30	45	Mean	15	30	45	Mean	15	30	45	Mean
First Season												
100 g *Azolla*/m²	2.89	2.62	1.63	2.38	2.05	1.40	1.45	1.63	2.80	1.87	1.58	2.08
150 g *Azolla*/m²	2.25	2.13	2.93	2.44	1.47	1.50	1.32	1.43	1.80	1.85	2.28	1.98
200 g *Azolla*/m²	2.90	2.80	2.74	2.83	1.73	1.55	1.40	1.56	2.35	1.85	2.09	2.09
Mean	2.69	2.52	2.48		1.75	1.48	1.39		2.32	1.86	1.99	
L.S.D. at 5%												
***Azolla* (A)**	0.23				0.14				0.09			
Phosphorus (P)	N.S				0.14				0.09			
(A) x (P)	0.40				0.25				0.16			
Second Season												
100 g *Azolla*/m²	2.39	2.56	2.20	2.38	2.08	1.53	1.64	1.75	1.46	1.62	1.65	1.54
150 g *Azolla*/m²	1.76	2.10	2.52	2.13	1.81	1.63	1.63	1.69	1.62	1.50	1.51	1.58
200 g *Azolla*/m²	2.61	2.20	2.65	2.49	1.76	1.67	1.69	1.71	1.59	1.97	1.78	1.78
Mean	2.25	2.29	2.46		1.61	1.88	1.66		1.55	1.69	1.65	
L.S.D. at 5%												
***Azolla* (A)**	0.08				N.S				0.07			
Phosphorus (P)	0.08				0.17				0.07			
(A) x (P)	0.13				N.S				0.13			

Azolla/m^2 + 15 kg P_2O_5 /fed at tillering, heading and maturity stages, respectively.

Kadu ***et al*** **(1991)** found that potassium percentage in rice plants was higher with the highest phosphorus fertilization level. Also, **Prakassii and Bardinatii (1995)** found that phosphorus application and liming increased the uptake of N, P and K.

Interaction effect between *Azolla* inoculum rates and phosphorus levels on N, P, K and crude protein percentages in rice grains in both seasons

Data in Table (13) indicate that *Azolla* inoculum rates had non significant effect on total nitrogen, crude protein, total phosphorus and total potassium in rice grains in the 1st season. Different levels of phosphorus had a significant effect on total nitrogen and potassium, but its effect was non significant on total phosphorus and crude protein. The highest percentages of total nitrogen and potassium were obtained when phosphorus application was 30 kg P_2O_5 /fed while, the lowest ones were obtained when phosphorus application was 15 kg P_2O_5 /fed.

Regarding the interaction effect of P and *Azolla* additions, data reveal that total nitrogen and potassium were significantly affected by the interaction between *Azolla* and phosphorus, while, crude protein and total phosphorus were non significantly affected. The highest values of total nitrogen and potassium were obtained when ***Azolla*** inoculum was 200 g/m^2 combined with 30 kg P_2O_5 /fed, whereas, the lowest ones were observed when ***Azolla*** inoculum was 100 g/m^2 combined with 15 kg P_2O_5 /fed.

In the 2nd season, data showed that total nitrogen and crude protein were non significantly affected by either different ***Azolla*** rates or phosphorus levels and interaction between them. On the other hand, total phosphorus was significantly affected by the interaction between ***Azolla*** inoculum rates and phosphorus levels. The treatment of ***Azolla*** inoculum rate of 100 g/m^2 combined with 30 kg P_2O_5 /fed gave higher total phosphorus % than all other treatments while, the lowest value was obtained in the treatment of ***Azolla*** inoculum rate of 100 g/m^2 combined with 15 kg P_2O_5 /fed. Meanwhile, ***Azolla*** rates, phosphorus levels additions and the interaction between them had a significant effect on total potassium in rice grains. The highest percentage of total potassium was observed in the treatment in which ***Azolla*** was used at a rate of 200 g/m^2 + phosphorus application at a level of 30 kg P_2O_5 /fed in the 1st season. While, ***Azolla*** inoculum rate of 150 g/m^2 combined with 30 kg P_2O_5 /fed gave the highest percentage of total potassium in the 2nd season. In addition, the lowest percentage of K was resulted from the application of ***Azolla*** at a rate of 100 g/m^2 + phosphorus application at a rate of 15 kg P_2O_5 /fed in the 1st season. While, ***Azolla*** inoculum 100 g/m^2 combined with 30 kg P_2O_5 /fed gave the lowest percentage of K in rice grains in the 2nd season.

Total nitrogen, crude protein and total phosphorus percentages in rice grains were higher in the 1st season than in the 2nd one in all treatments, while, total potassium content % was higher

Table 13. Interaction effect between *Azolla* inoculum rates and phosphorus levels on nitrogen, crude protein, phosphorus and potassium in rice grains in both seasons.

First Season

	Total nitrogen (%)				Crude protein (%)				Total phosphate (%)				Total potassium (%)			
P-levels (kg P_2O_5/fed.)	15	30	45	Mean	15	30	45	Mean	15	30	45	Mean	15	30	45	Mean
100 g *Azolla*/m²	1.08	1.14	1.32	1.18	7.54	7.50	8.17	7.74	0.63	0.57	0.42	0.54	0.24	0.30	0.35	0.40
150 g *Azolla*/m²	1.37	1.32	1.17	1.28	7.42	7.50	8.23	7.72	0.54	0.56	0.54	0.55	0.34	0.33	0.36	0.34
200 g *Azolla*/m²	1.53	1.58	1.20	1.11	6.22	7.53	7.51	7.09	0.55	0.49	0.44	0.49	0.47	0.62	0.39	0.39
Mean	0.99	1.34	1.23		7.06	7.51	7.97		0.58	0.54	0.47		0.29	0.48	0.37	
L.S.D. at 5%																
***Azolla* (A)**	N.S				N.S				N.S				N.S			
Phosphorus (P)	0.23				N.S				N.S				0.08			
(A) x (P)	0.24				N.S				N.S				0.13			

Second Season

	Total nitrogen (%)				Crude protein (%)				Total phosphate (%)				Total potassium (%)			
100 g *Azolla*/m²	0.95	0.90	0.95	0.93	7.84	7.15	7.97	7.65	0.22	0.34	0.29	0.29	0.42	0.36	0.44	0.45
150 g *Azolla*/m²	0.95	0.73	0.90	0.86	7.27	7.46	7.50	7.41	0.28	0.26	0.29	0.28	0.49	0.58	0.44	0.50
200 g *Azolla*/m²	0.88	1.00	0.87	0.91	5.74	6.59	6.03	6.12	0.32	0.24	0.26	0.27	0.47	0.39	0.53	0.53
Mean	0.93	0.88	0.91		6.95	7.06	7.17		0.28	0.27	0.28		0.39	0.62	0.47	
L.S.D. at 5%																
***Azolla* (A)**	N.S				N.S				N.S				0.04			
Phosphorus (P)	N.S				N.S				N.S				0.04			
(A) x (P)	N.S				N.S				0.07				0.08			

in the 2nd season than in the 1st one (Table 13). These differences between the two seasons are likely to be due to the difference in climatic conditions.

Interaction effect between *Azolla* inoculum rates and phosphorus levels on chlorophylls, carotenoids and total carbohydrates of rice plants in both seasons

Data in Table (14) show that the highest values of chlorophyll a,b and carotenoids were obtained in the treatment of ***Azolla*** inoculum rate at 150 g/m^2 combined with 45 kg P_2O_5 /fed in both seasons. On the other hand, the lowest values of chlorophyll a,b and carotenoids were observed in case of using ***Azolla*** inoculum at a rate of 100 g/m^2 combined with 15 kg P_2O_5 /fed in both seasons.

Data also show that the highest percentages of total carbohydrates were obtained in case of using ***Azolla*** inoculum at a rate of 150 g/m^2 combined with 45 kg P_2O_5 /fed. While, the lowest percentages were obtained when ***Azolla*** inoculum rate was 100 g/m^2 combined with 15 kg P_2O_5 /fed.

From the above-mentioned data it could be generally concluded that carbohydrate content was proportional to chlorophyll levels in various investigated treatments since chlorophylls are responsible of photosynthesis and consequently carbohydrate formation.

Interaction effect between *Azolla* inoculum rates and phosphorus levels on grains and straw yields and 1000-grain weight of rice in both seasons

Data in Table (15) indicate that grains and straw yields as well as 1000-grain weight of rice were significantly affected by different inoculum rates of ***Azolla*** and phosphorus levels added in both growing seasons. Also, the interaction effect was significant on the above-mentioned characters. The highest grain and straw yields as well as 1000-grain weight were obtained from ***Azolla*** application at a rate of 150, 200 and 200 g/m^2, respectively, whereas, the lowest ones were obtained from 200, 100 and 200 g/m^2, respectively. The same trend of results was observed in both seasons.

In addition, the highest values of grain and straw yield and 1000-grain weight were obtained when phosphorus was applied at a rate of 30 kg P_2O_5 /fed, while the lowest ones were obtained when phosphorus was applied at a rate of 15 kg P_2O_5 /fed. The same trend of results was observed in both seasons.

Regarding the interaction effect between ***Azolla*** and P additions, data in Table (15) reveal that grain yield, straw yield and 1000-grain weight were the highest in case of using ***Azolla*** inoculum rate at 150 g/m^2 combined with 30 kg P_2O_5 /fed in both seasons. On the other hand, grains yield, straw yield and 1000-grain weight were the lowest in case of application of 100 g ***Azolla***/m^2 + 15 kg P_2O_5 /fed, 100 g ***Azolla***/m^2 + 45 kg P_2O_5 /fed and 200 g ***Azolla***/m^2 + 30 kg P_2O_5

Table 14. Interaction effect between *Azolla* inoculum rates and phosphorus levels on chlorophyll, carotenoids and total carbohydrates of rice plants in both seasons.

Treatments	Chlorophyll a mg/g fresh matter		Chlorophyll b mg/g fresh matter		Carotenoids mg/g fresh matter		Total carbohydrates (%)	
	Season I	Season II	Season I	Season II	Season I	Season II	Season I	Season II
***Azolla* addition as a biofertilizer**								
AZ.(100 g/m²) + P (15 kg) P_2O_5/fed.	0.97	1.05	1.11	1.13	0.81	1.16	32.00	34.0
AZ.(100 g/m²) + P (30 kg) P_2O_5/fed.	3.13	2.55	1.85	1.42	1.96	1.78	42.00	45.5
AZ.(100 g/m²) + P (45 kg) P_2O_5/fed.	1.80	1.28	1.68	2.20	2.43	1.84	37.50	39.0
AZ.(150 g/m²) + P (15 kg) P_2O_5/fed.	1.26	1.25	1.10	1.30	1.04	1.23	36.25	41.8
AZ.(150 g/m²) + P (30 kg) P_2O_5/fed.	2.23	3.40	1.73	1.96	1.12	1.72	43.00	45.0
AZ.(150 g/m²) + P (45 kg) P_2O_5/fed.	3.44	3.87	1.85	3.11	2.50	1.90	60.30	64.0
AZ.(200 g/m²) + P (15 kg) P_2O_5/fed.	1.78	1.99	1.92	1.94	1.94	1.48	45.00	48.0
AZ.(200 g/m²) + P (30 kg) P_2O_5/fed.	2.01	3.34	1.74	2.29	1.20	1.46	51.00	56.0
AZ.(200 g/m²) + P (45 kg) P_2O_5/fed.	1.48	2.16	1.45	2.43	1.21	1.79	48.00	52.0

AZ. : *Azolla* P : Phosphorus

Table 15. Interaction effect between *Azolla* inoculum rates and phosphorus levels on grains and straw yields and 1000-grain weight of rice.

First Season

P-levels (kg P_2O_5/fed.)	Grain yield (ton/fed.) 15	30	45	Mean	Straw yield (ton/fed.) 15	30	45	Mean	1000-grain weight (g) 15	30	45	Mean
100 g *Azolla*/m²	3.33	3.97	5.17	4.10	5.33	5.07	4.80	5.07	21.65	21.11	21.91	21.56
150 g *Azolla*/m²	4.40	5.60	4.63	4.33	5.73	6.86	6.50	6.36	21.85	23.19	17.93	20.99
200 g *Azolla*/m²	3.67	4.40	3.90	3.99	5.60	6.40	6.43	6.81	19.74	17.78	18.20	18.57
Mean	3.80	4.66	4.57		5.56	6.77	5.91		19.35	21.69	21.41	
L.S.D. at 5%												
***Azolla* (A)**	0.34				0.65				1.25			
Phosphorus (P)	0.34				0.65				1.25			
(A) x (P)	0.59				1.12				2.16			

Second Season

P-levels (kg P_2O_5/fed.)	Grain yield (ton/fed.) 15	30	45	Mean	Straw yield (ton/fed.) 15	30	45	Mean	1000-grain weight (g) 15	30	45	Mean
100 g *Azolla*/m²	3.53	4.17	5.40	4.68	6.23	5.33	5.20	5.59	24.07	24.60	23.83	24.17
150 g *Azolla*/m²	4.70	5.90	5.10	4.86	6.10	7.23	6.93	6.76	22.63	24.79	22.33	23.25
200 g *Azolla*/m²	3.92	4.74	4.13	4.26	5.97	6.67	6.70	7.11	23.90	21.83	24.37	23.00
Mean	4.05	4.94	4.91		6.10	7.08	6.28		23.07	24.18	23.57	
L.S.D. at 5%												
***Azolla* (A)**	0.28				0.82				0.45			
Phosphorus (P)	0.28				0.82				0.83			
(A) x (P)	0.49				1.43				1.44			

/fed, respectively. The same trend of results was observed in both growing seasons.

Singh ***et al*** **(1988)** found that inoculation with *Azolla* (500 kg fresh weight/ha) 10 days after rice transplanting as a biofertilizer, grain yield was 4.37 - 4.59 ton/ha. **Subramani and Kannaiyan (1987)** found that *Azolla pinnata* inoculated at a rate of 200 g/m^2 gave the highest grain yield and 1000-grain weight. Also, **Ventura** ***et al*** **(1992)** found that *Azolla* application increased rice grain yield and grain weight that were generally increased by application of Zn and P with *Azolla* inoculation. **Krishnan** ***et al*** **(1994)** found that grain yield of rice was the highest in case of 200 g/m^2. **Singh and Singh (1995)** found that grain and straw yields of rice increased both by phosphorus enrichment of *Azolla* inoculation and by phosphorus fertilizer application (40 or 60 kg P_2O_5 /ha) during intercropping.

CONCLUSION

Obtained results generally indicate that, using fresh or living *Azolla* in inoculating rice fields as a biofertilizer combined with inorganic phosphorus fertilizer is economically beneficial and very effective. The small amounts of *Azolla* used substitute inorganic nitrogen fertilization in rice fields.

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كفاءة تلقيح نباتات الأرز بالآزولا فى وجود مستويات مختلفة من الفوسفور

[٤]

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فى هذا البحث تم إختبار نوعين من الأزولاهما *A. pinnata, Azolla filiculoides* من حيث كفاءة النمو ومعدل تثبيت النيتروجين لإستخدامهما فى دراسة كفاءة تلقيح نباتات الأرز بالآزولا وذلك مع استخدام مستويات مختلفة من التسميد الفوسفاتى .

وقد أسفرت أهم نتائج الدراسة عن الآتى

أعطى النوع *A. filiculoides* كمية أعلى من النمو الخضرى والجاف ونسبة أعلى من النيتروجين الكلى وكذلك أظهر نشاط أعلى لإنزيم النيتروجينيز مقارنة بالنوع *A. pinnata* لذلك استخدم النوع *A. filiculoides* فى بقية الدراسة . وعند تلقيح نباتات الأرز بالآزولا من النوع *Azolla filiculoides* كسماد حيوى بمعدل ١٠٠ جم ، ١٥٠ جم/م^{2} مع إضافة ٣٠ كجم فو$_{2}$أ$_{5}$/فدان أعطى ذلك أعلى أعداد للميكروبات الكلية والبكتريا المذيبة للفوسفات الغير عضوية على التوالى . ولقد زادت أعداد بكتيريا النشدرة والتأزت بزيادة معدل لقاح الآزولا .

لوحظت أعلى قيمة للنيتروجين الأمونيومى مع التلقيح بالآزولا بمعدل ١٥٠ جم/م^{2} مع إضافة ٤٥ كجم فو$_{2}$أ$_{5}$/فدان وعند تلقيح الآزولا بمعدل ١٥٠ جم/م^{2} مع إضافة الفوسفور بمعدل ٣٠ كجم فو$_{2}$أ$_{5}$/فدان أعطى ذلك أعلى أطوال لنباتات الأرز وكذلك أدت نفس المعاملة إلى زيادة الوزن الطازج والجاف للمجموع الخضرى لنباتات الأرز .

كان تأثير التفاعل بين معدل لقاح الأزولا ومستوى التسميد الفوسفاتى معنويا على محتوى نباتات الأرز من النيتروجين والفوسفور والبوتاسيوم . أعطت المعاملة ١٥٠ جم لقاح آزولا/م^{2} مع إضافة ٤٥ كجم فو$_{2}$أ$_{5}$/فدان أعلى مستوى من كلوروفيل أ ، ب ، الكاروتينيدات وكذلك الكربوهيدرات الكلية .

عند تلقيح الأزولا كسماد حيوى بمعدل ١٥٠ جم/م٢ مع إضافة ٣٠ كجم فو$_{2}$أ$_{5}$/فدان أدى ذلك إلى الحصول على أعلى محصول من الحبوب والقش وكذلك وزن الـ ١٠٠٠ حبة خلال موسمى الدراسة .

من نتائج هذه الدراسة يتضح أن استخدام كميات بسيطة من الآزولا كلقاح (سماد حيوى) للأرز مع الفوسفات المعدنية، تمد النباتات بالنيتروجين اللازم لها طول فترة النمو ويغنى ذلك عن استخدام الأسمدة النيتروجينية المعدنية .