RESULTS AND DISCUSSION

4. RESULTS AND DISCUSSION

4.1. Effect of cyanobacteria and *Azolla* inoculation on rice growth, rice yield components, nitrogen and phosphorus uptake and soil chemical properties:

Previous reports indicated the susceptibility of rice to inoculation with either cyanobacteria (SBI) or *Azolla* at different levels either alone or in combination with different levels of nitrogen fertilizer **(Lumpkin and Plucknett, 1982; Ashoub et al., 1993 and Mussa et al., 2002)**. Besides free living cyanobacterial either free living (SBI) or symbiotic candidates are successfully ameliorate rice seed germination, and their effect extended to modify root and shoot development. The objective of the current study was to investigate the possible contribution of cyanobacteria and *Azolla* as nitrogen biofertilizers in rice production process. Rice growth, rice yield components, nitrogen and phosphorus uptake and soil chemical properties as affected by (SBI) and *Azolla* inoculation at different levels either alone or in combination with different levels of nitrogen were investigated.

4.1.1. Effect of cyanobacteria inoculation:

Data in Table (3) and Figure (1) show the effect of cyanobacteria inoculation (SBI) and urea fertilization either alone or in combination on rice yield components as well as on total nitrogen and phosphorus uptake amounts by rice plants.

4.1.1.1. Effect on Plants

A: Straw and grain yields:

Data of Table (3) and Fig (1) reveal that using SBI-1 alone slightly increased both straw and grain yields, while using SBI-2 alone increased yields more without reaching the level of significance. However, using the third level of cyanobacteria (SBI-3) alone significantly increased both rice straw and grain yields. The treatment of SBI-3 produced 42.90 g pot^{-1} (straw yield) and 28.90 g pot^{-1} (grain yield). These yields were not significantly different from those resulted from urea-2 (60 kg N fed^{-1}) or urea-1 (30 kg N fed^{-1}). Concerning the effect of urea alone, using the first rate (30 kg N fed^{-1}) increased dry matter yield of straw and grain. Increasing N rate from rate 1 to rate 2 was accompanied by a significant increase of dry matter yield (straw and grain). In case of straw, dry matter yield increased by 119 % as compared with control. The corresponding increase in grain was 105 %.

Using urea at rate 2 (60 kg N fed^{-1}) either alone or combined with cyanobacteria inoculation at any level significantly increased both straw and grain yields. Straw yields were 55.60, 43.46, 48.74 and 55.75 g pot^{-1} for urea-2, (SBI-1 + urea-2), (SBI-2 + urea-2) and (SBI-3+ urea-2), respectively. The corresponding grain yields were 29.40, 32.04,37.49 and 33.28 g pot^{-1} with urea-2, (SBI-1 + urea-2), (SBI-2 + urea-2) and (SBI-3+ urea-2), respectively. Applying urea nitrogen at a rate of 30 kg N fed^{-1} (urea-1) either alone or combined with SBI-1 or SBI-2 did not significantly increase either straw and grain yield. However the use of (SBI-3 + urea-1) significantly increased both

Table (3): Rice yield components as affected by cyanobacteria (SBI) inoculation and Urea-N fertilization.

Treatment	Yield (g pot^{-1})		Plant height (cm)	1000- grain Weight (g)	Protein (%) in grains	Total-N uptake (mg pot^{-1})	Total-P uptake (mg pot^{-1})
	Straw	Grain					
Control	25.44	14.34	83.98	18.16	3.30	130	61
200 g fed^{-1} cyanobacteria (SBI-1)	25.47	14.35	84.77	18.93	5.56	245	89
1.5 kg fed^{-1} cyanobacteria (SBI-2)	37.80	22.58	95.05	20.23	5.71	255	127
3 kg fed-1 cyanobacteria (SBI-3)	42.90	28.90	92.75	21.50	6.56	493	195
30 kg-N fed^{-1} (Urea-1)	37.28	21.44	88.27	20.87	5.80	344	149
60 kg-N fed^{-1} (Urea-2)	55.60	29.40	87.49	22.23	6.86	554	285
SBI-1 + Urea-1	37.99	20.74	85.97	21.37	5.59	339	188
SBI-1 + Urea2	43.46	32.04	92.28	22.27	6.81	316	194
SBI-2 + Urea-1	32.13	26.66	91.60	20.40	4.56	298	178
SBI-2 + Urea2	48.74	37.49	85.83	21.49	6.46	306	166
SBI-3 + Urea-1	66.40	39.49	102.68	24.67	7.24	634	296
SBI-3 + Urea2	55.75	33.28	98.63	21.53	6.27	503	227
L. S. D. < 0.05	16.8	13.05	13.30	3.32	2.58	158	68

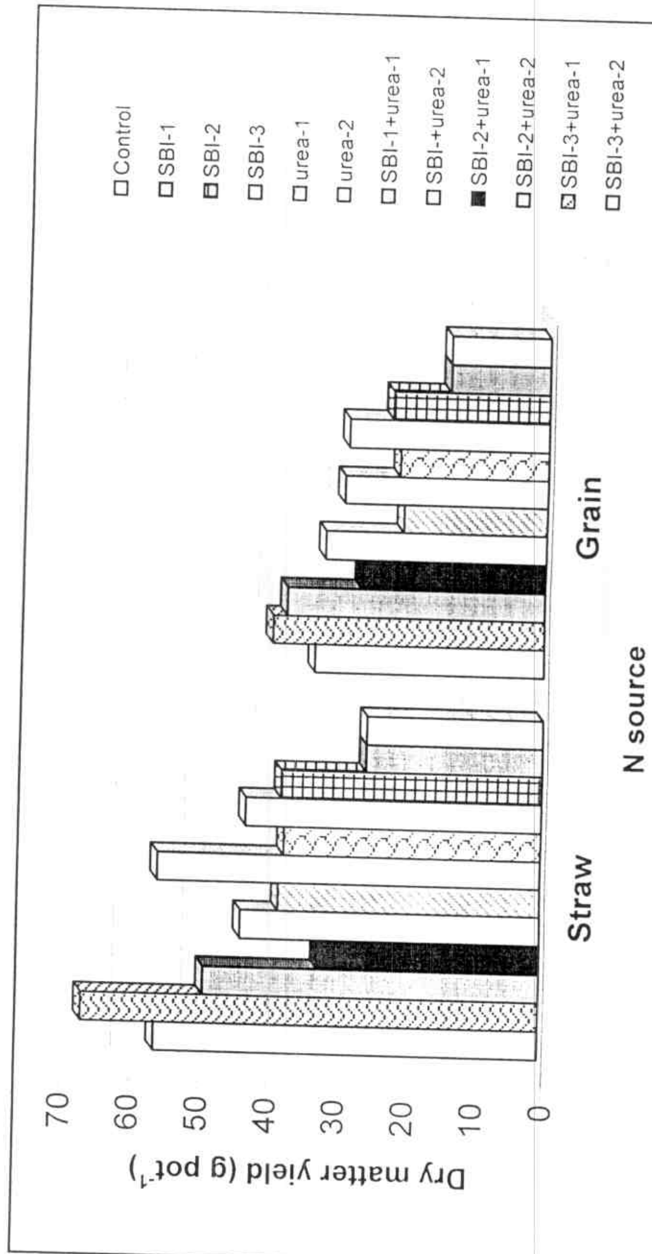

Fig. (1): Straw and grain yields of rice crop as affected by SBI and urea-N straw and grain yields to be 66.40 and 39.49 g pot^{-1}, respectively. These two values were not significantly higher than those resulted from the treatment of urea-2 either applied alone or in combination with different levels of SBI inoculum. The most effective treatment on straw and grain yields was SBI-3 + urea-1 as it increased straw yield by 161.01 % and grain yield by 175.40 % followed by (SBI-3 + urea-2) "for straw"and (SBI-2 + urea-2) "for grain".

These results are in agreement with those of **Mahaparta et al. (1987)** and **El-Mancy et *a*l. (1997)** in which SBI inoculation increased rice grain production by 9.4 and 2.07 %, respectively.

It is worthy to note that using 3 kg SBI plus 30 kg N urea (SBI-3 + urea-1) gave the highest yield as compared with 3 kg SBI + urea-2 (60 kg N). The inferiority of the last treatment may be attributed to a possible inhibition of N_2-fixation by SBI resulting from increasing mineral nitrogen from 30 to 60 kg fed^{-1}. This result is in harmony with those of **Alimagno and Yoshida (1975) Ghazal (1987), Aref (2001) and Tantawy (2002)** in which high nitrogen rate combined with SBI inoculation decreased the nitrogen fixation by cyanobacteria.

B: <u>Plant height</u>:

Results presented in Table (3) and illustrated in Fig. (2) show that application of SBI alone at first level did not increase plant height, while using either the second or third rates of SBI increased plant height. The increase tended to be significant with the second level of SBI. Using urea at rates of 30 and 60 kg N fed^{-1} did not increase plant height significantly with no significant difference between each other. Combining SBI and urea increased plant height. The increase was significant with (SBI-3 +urea-1). This treatment increased plant height by 22.3 %. A significant increase in plant height was noticed with using (SBI-3 + urea-2). Again, the most effective treatment was SBI-3 + urea-1. These results confirm the results of **Ghazal (1987), Herzalla et al. (2002) and Mussa et al. (2002)** which revealed that SBI inoculation combined with supplementation of mineral nitrogen increased rice plant height.

C: <u>1000-grain weight</u>:

Using the first rate of SBI did not significantly increase the weight of 1000 grains. However, the third rate (3 kg SBI fed^{-1}) significantly increased the weight of 1000 grains (Table 3 and Fig. 3). The two rates of urea (urea-1 and urea-2) were almost similar in their effects with SBI-2 and SBI-3. Significant responses of rice plants were observed with the highest rate of urea (urea-2) combined with either SBI-1, SBI-2 or SBI-3. However the combination of the lowest rate of urea (urea-1) with any rate of SBI did not affect 1000-grain weight significantly, but when combined with SBI-3 there was a marked significant increases.

It is valuable to ensure that the most effective treatment among the studied treatments on the 1000-grain weight was (SBI-3 + urea –1).

This result goes along with the results obtained by **Ghazal (1987)** and **Hammad et al. (1997)** who found that inoculation with cyanobacteria significantly increased the 1000-grain weight .

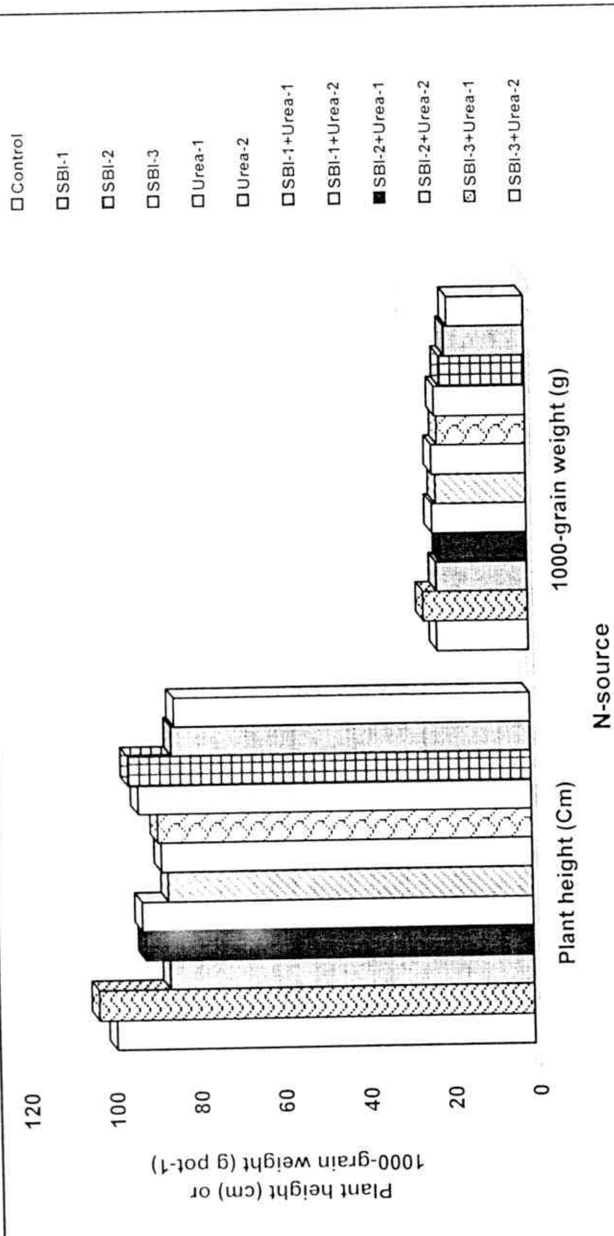

Fig. (2): Plant height and 1000-grain weight of rice plant as affected by SBI and urea-N.

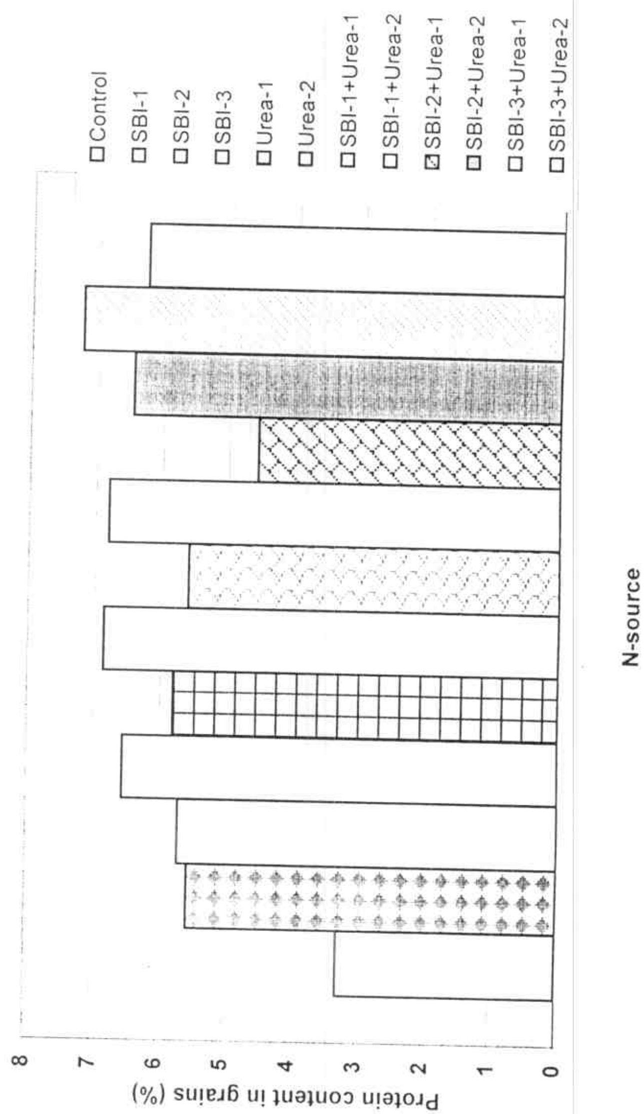

Fig. (3): Protein (%) in grains of rice plant as affected by SBI and urea-N.

D: <u>Protein percentage in grains</u>:

Some treatments increased significantly the protein percentage (Table 3 and Fig.3) of rice grains. Application of SBI alone increased protein content significantly in particular when applied at its third rate. Applying urea alone at its second rate increased protein content significantly. Application of urea-2 alone or combined with any cyanobacteria inoculum level significantly increased the protein percentage in rice grains. The inoculation of rice with 3 kg cyanobacteria inoculum (SBI-3) gave 6.56 % of protein in grains which was significantly higher than that of the control treatment (3.30 %). The highest protein parentage value (7.24) was for the treatment of (SBI-3 + urea-1). These results are comparable with those of **Ashoub (1993) and Mussa et al. (2002)** who showed that protein concentration in rice grains increased with increasing either nitrogen fertilization or cyanobacteria inoculation .

E: <u>Total nitrogen uptake</u>:

All treatments significantly increased total nitrogen uptake (Table 3 and Fig. 4) except those of SBI-1 and SBI-2 treatments. The highest N-uptake amounts were achieved with the treatments of (SBI-3 + urea-1) "634 mg N pot^{-1}", urea-2 "554 mg N pot^{-1}", (SBI-3 + urea-2) "503 mg N pot^{-1}"and SBI-3 "493 mg N pot^{-1}". However, it is clear in Table (3) and Fig.(4) that the inoculum (SBI-3) along with urea-1 gave the highest total N-uptake amounts by rice plants which was comparable to that achieved with using urea-2.

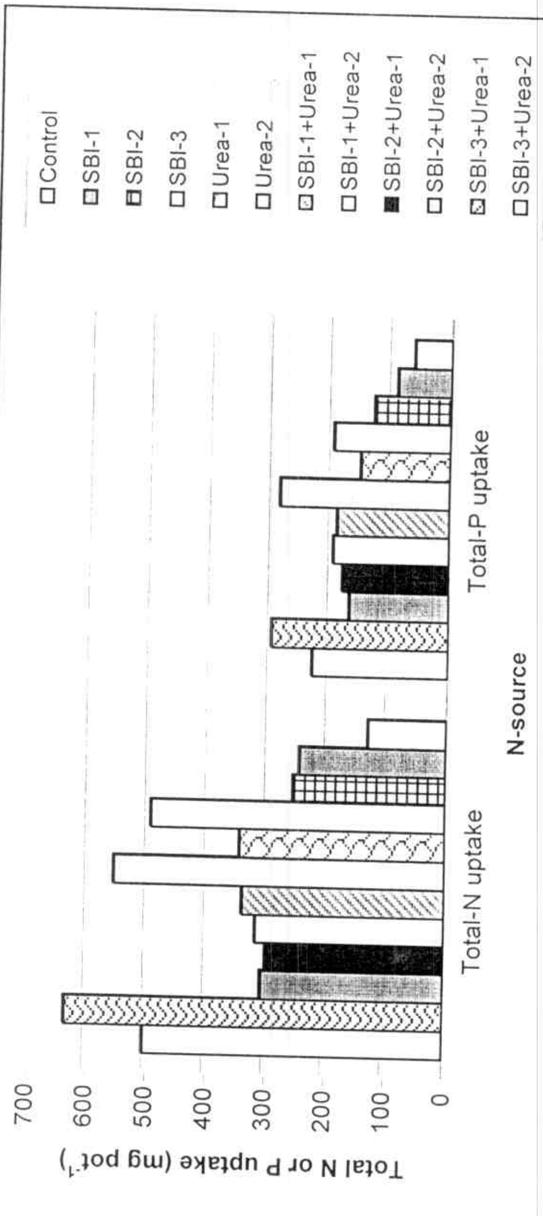

Fig. (4): Total-N and P uptake by rice crop as affected by SBI and urea-N.

Using both SBI and urea either alone or in combination resulted in a significant increase in N-uptake. This parameter (N-uptake) was the most affected parameter by the treatments among the studied ones. The results are similar to those obtained by **Hammad et al. (1997), El-Mancy et al. (1997) and Mussa et al. (2002).**

F: <u>Total phosphorus uptake</u>:

As shown with total N-uptake, application of SBI alone increased total P-uptake by straw and grains and the increase was particularly significant with the highest rate of SBI. Inoculation with cyanobacteria SBI-3 was better than the other two SBI levels either alone or combined with urea. Both urea-1 and urea-2 significantly increased P uptake by rice plants. Increasing the first N rate of urea to the second one resulted in one of the highest P-uptake value (285 mg P pot^{-1}). Combining SBI with urea resulted in the following:

(i) P-uptake was significantly increased when SBI-1 was combined with either urea-1 or urea-2. This trend indicates that urea could enhance the positive effect of SBI-1.

(ii) With SBI-2, P-uptake increased with using urea-1 and urea-2 and the increase with urea-1 was higher than the corresponding one with urea-2.

(iii) Concerning SBI-3, a positive and significant effect of urea was noticed with the low rate of urea and the highest rate of SBI (SBI-3 + urea-1) the total P-uptake increased to be 296 mg P pot^{-1}, while with the highest rate of urea, P-uptake recorded 227 mg P pot^{-1}.

Comparing the influence of urea on P uptake with inoculation of SBI, the results obtained reveal that the positive impact of urea (in particular) when added at 60 kg N fed^{-1} (urea-2) was decreased as compared to urea alone. On the other hand, the influence of the low rate of urea was enhanced with increasing SBI rate

The highest amounts of P uptake were recorded with treatments of SBI-3 + urea-1 (296 mg P pot^{-1}), urea-2 (285 mg P pot^{-1}) and SBI-3 + urea-2 (227 mg P pot^{-1}). It is also noticed that the total P uptake was 296 mg P pot^{-1} which was given by (SBI + urea-1) treatment was not significantly different from that of 285 mg P pot^{-1} due to (urea-2) treatment, while it was significantly higher than the 194 mg P pot^{-1} due to (SBI-1 + urea-2) treatment.

G: <u>Overall effect on plant</u>:

The aforementioned results reveal that the treatment of (SBI-3 + Urea-1) gave the highest values in all measured parameters. Also, the use of SBI alone at the level of 3kg SBI fed^{-1} was superior to the other two SBI levels and comparable with 60 kg N fed^{-1}. The highest grain yield (39. 49 g pot^{-1}), protein percentage (7.24), N uptake (634 mg N pot^{-1}) and P uptake (296 mg P pot^{-1}) due to the treatment (SBI-3 + Urea-1) could be attributed to the inoculated cyanobacteria fix nitrogen, increase available P in soil, improve some microbial, biochemical and physical soil properties, increase the soil fertility and the nitrogen uptake by rice plants which in turn increase the grain yield **(Mandal et al., 1999)**. The results obtained are in agreement with those of **El-Mancy et al. (1997)** who reported that SBI inoculation significantly increased rice grain and straw yields by 2.07 and 17.06 %, respectively, they also found that mineral fertilizer combined with SBI inoculation produced the highest grain yield.

Satapathy (1999) concluded that cyanobacteria (*Aulosira*) inoculated to rice in the dry season significantly produced higher rice grain yield than farmyard manure.

4.1.1.2. Effect of SBI and urea on some chemical properties of soil after harvest:

Data in Table (4) show the effect of different rates of SBI inoculation alone or in combination with different levels of urea nitrogen on some chemical properties of soil after rice harvesting such as total nitrogen, total phosphorus, available phosphorus and organic matter percentage.

A: Total nitrogen:

Cyanobacteria inoculation significantly increased the contents of total nitrogen (Table 4 and Fig. 5). Total N content in soil after rice harvesting significantly increased with using urea-2 rate. However, urea-1 rate did not significantly affect this parameter. Application of urea and SBI inoculation significantly increased the total nitrogen concentration treatment except with treatments of (urea-1) and (SBI-2 + urea-1) and (SBI-2 + urea-2) where the increases were not statistically significant. The highest total nitrogen amount (1100 mg N kg^{-1}) was accompanied with the (SBI-3 + urea-1) treatment. However, the treatment that received urea-1 or urea-2 alone gave total nitrogen values less than those attained due to the same treatments plus any rate of the cyanobacteria inoculation. For instance the treatment of

Table (4) Effect of cyanobacteria (SBI) inoculation and urea-N fertilization on soil N,P and organic matter (O.M) after rice harvesting.

Treatment	Total N (mg kg^{-1})	Total P (mg kg^{-1})	Available P (mg kg^{-1})	O. M. %
control	500	1500	6.32	2042
200 g fed^{-1} cyanobacteria inoculum (SBI-1)	800	1900	9.55	2059
1.5 kg fed^{-1} cyanobacteria (SBI-2)	860	1900	8.42	2.76
3 kg fed^{-1} cyanobacteria (SBI-3)	900	2200	7.54	2.61
30 kg N fed^{-1} (urea-1)	650	2300	8.06	2.78
60 kg N fed^{-1} (urea-2)	860	2000	7.53	2.91
SBI-1 +urea-1	900	2100	8.11	2.50
SBI-1 +urea-2	910	2100	6.86	2.89
SBI-2 +urea-1	690	1900	7.87	2.49
SBI-2 +urea-2	740	1900	8.05	2.42
SBI-3 +urea-1	960	2000	7.71	3.03
SBI-3 +urea-2	1100	1800	6.47	2.88
L. S. D. < 0.05	300	500	0.14	0.49

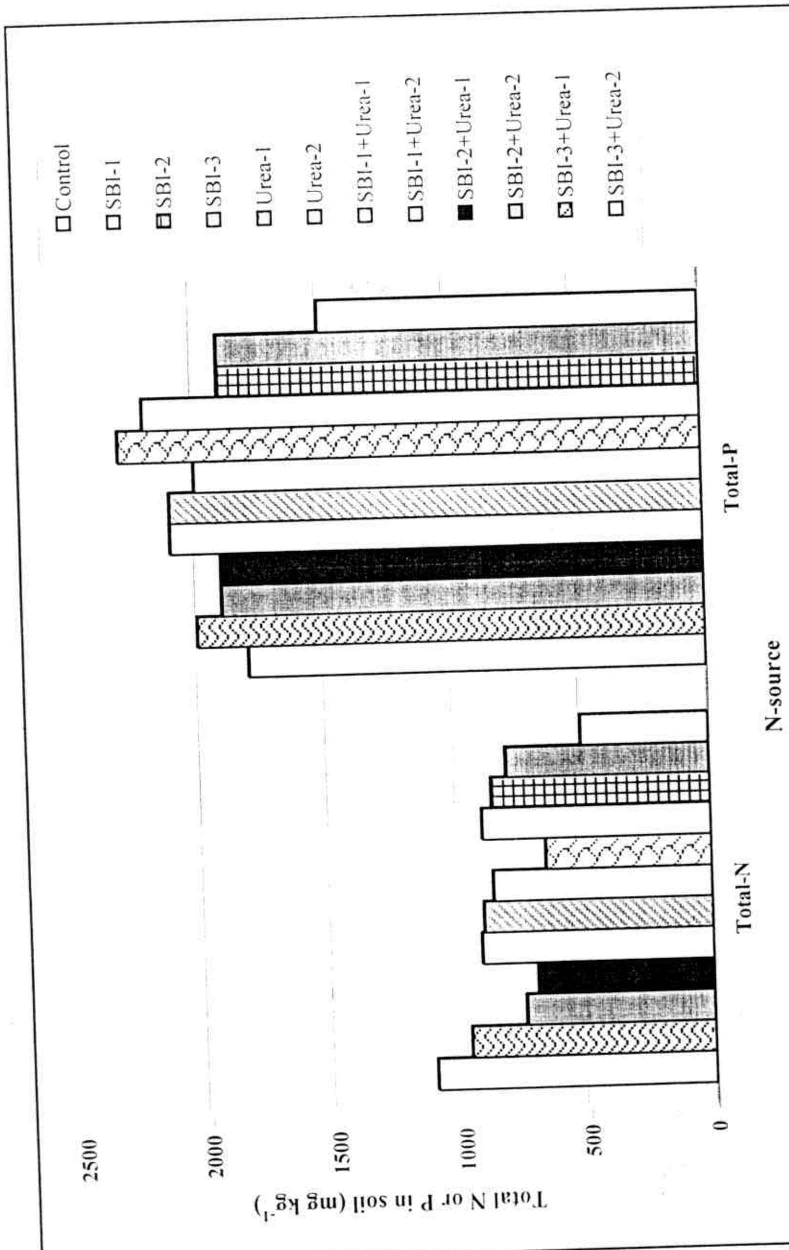

Fig. (5): Total-N and P amounts in soil as affected by SBI and urea-N after rice harvesting.

(urea-1) gave 860 mg N kg^{-1} against 910 mg N kg^{-1} when inoculated with SBI-1.

B: Total phosphorus:

Total phosphorus was increased over the control treatment when rice plants were fed with urea and / or SBI either alone or in combination (Table 4 and Fig. 5). Phosphorus content in soil increased with increasing SBI rate and significant increase was noticed with the highest SBI rate. The highest total phosphorus values were recorded with the treatments of urea-1 and SBI-3. The corresponding values were 2300 and 2200 mg P kg^{-1}. Application of urea alone at 30 and 60 kg N fed^{-1} significantly increased total P content in soil. Combining SBI and urea did not increase P content significantly except with the treatment of SBI-3 + urea-1.

The obtained results are in parallel with those of **Mandal et al. (1999)**.

C: Available Phosphorus:

Data in Table (4 and Fig. 6) reveal that all treatments increased available phosphorus. It is also noticed that the amount of available P decreased with increasing the rate of cyanobacteria inoculation when they used alone. The highest available P value (9.55 mg P kg^{-1}) was due to the inoculation (SBI-1) treatment. The lowest one (6.32 mg P kg^{-1}) was due to the control treatment.

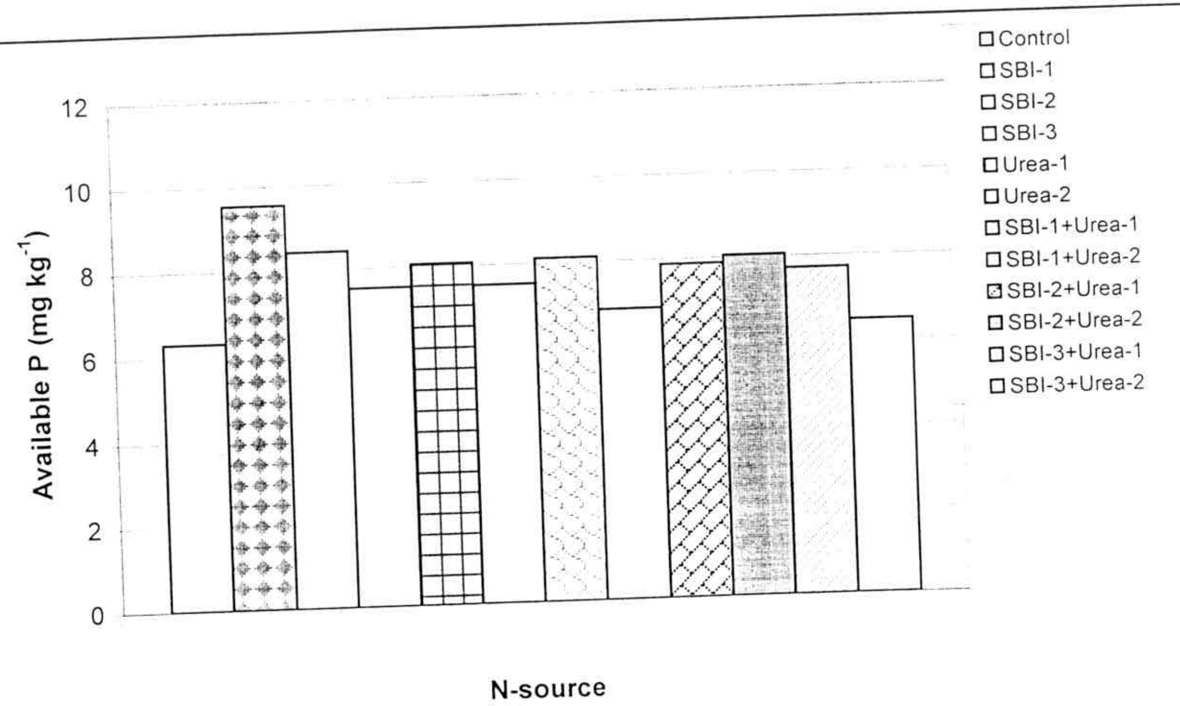

Fig. (6): Available P in soil as affected by SBI and urea-N after rice harvesting.

D: Organic Matter Percentage:

All treatments slightly and non-significantly increased the soil organic matter content (Table 4 and Fig. 7) except for the treatments of urea-2 and (SBI-3 + urea-1) that increased organic matter content significantly. The highest organic matter percentage (3.03) occurred with the treatment of (SBI-3 + urea-1). The treatment of urea-2 gave the second highest increase in organic matter content (2.91 %). This may be attributed to the high yield of rice, which increases plant residues, thus increasing organic matter in soil.

The polysaccharide sheath present around the cyanobacteria trichomes connects the soil particles and increases aeration and water holding capacity of soil **(Roychondhury et al., 1979)**. The use in cyanobacteria for soil improves the organic matter, soil texture, ammonium nitrogen content, available P and available N in soil **(Singh et al. 1988 b; Suri et al. 1995 and Stapathy, 1999)**.

Mandal et al. (1999) reported that cyanobacteria (SBI) act like P-solubilising bacteria and have the ability to mobilize the bound phosphate in insoluble Ca $(PO_4)_3$, Fe PO_4, Al PO_4 an hydroxyapatite ($Ca_5 (PO_4)_3.OH$) in soils, sediments or in pure cultures. They explained enhanced solubility of P to a possible synthesis of a chelator for Ca^{2+} , H_2CO_3 and other organic acids released during their growth which, could solubilize P from Ca sources and increased consequently the available-P amounts in soils.

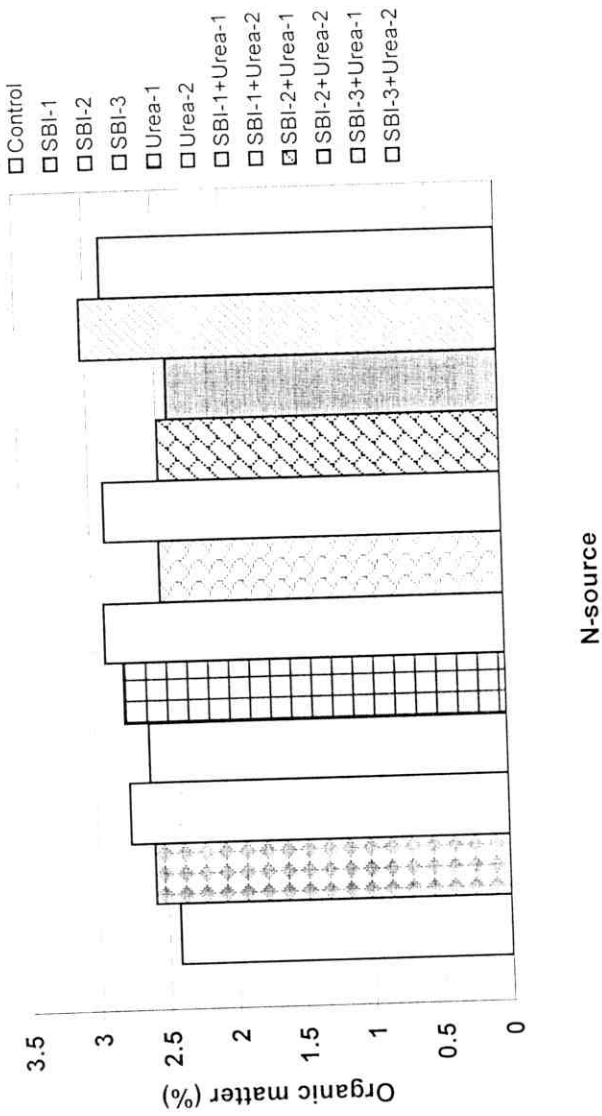

Fig. (7): Soil organic matter content (O.M.) after rice harvesting as affected by SBI and urea-N.

However, **(Mule et al., 1999)** obtained results and indicating that the soil inoculation with cyanobacteria (alone or combined with urea) exerted no effect on soil available-P.

4.1.2. Effect of *Azolla* inoculation:

Table (5) indicates the response of rice crop to *Azolla* inoculation and urea application either alone or in combination. The evaluated parameters were rice yield components and total nitrogen and phosphorus uptake in rice plants.

4.1.2.1. Effect of *Azolla* on plant:

A: Straw and grain yields:

Both urea and *Azolla* applied alone or in combination at different levels significantly increased rice straw and grain yields (Table 5 and Fig. 8). Straw and grain yields were significantly increased with using *Azolla* when applied at two rates Azol-1 and 2. Straw yield was increased by 163, and 119 % with the first and second rates of *Azolla*, respectively. Application of urea-1 and urea-2 rates increased straw yield by 111 and 194 %, respectively. With respect to grain yield, it was increased by 125 and 251 % with urea-1 and urea-2, respectively as compared with control. In the case of straw, application of urea at the first rate combined with *Azolla* at its highest rate produced straw yield which exceeded that obtained with using the second rate of urea alone. The positive effect of the second rate of urea on straw yield was decreased slightly when the combined with *Azolla*. The highest straw and grain yields of 77.35 and 55.70 g pot^{-1} , respectively were due to the treatment of (Azol-1 + Urea-1). The straw and grain yields obtained with the Urea-2 treatment were higher than those attained by the

Table (5): Rice yield components as affected by *Azolla* inoculation and Urea-N fertilization.

Treatment	Yield (g pot^{-1})		Plant height (cm)	1000-grain Weight (g)	Protein (%) in grains	Total-N uptake (mg pot^{-1})	Total-P uptake (mg pot^{-1})
	Straw	Grain					
Control	25.44	15.90	83.98	17.07	3.30	140	65
30 kg N *Azolla* fed-1 (Azol –1)	55.67	40.18	102.36	21.87	5.62	650	220
60 kg N *Azolla* fed-1 (Azol –2)	66.90	51.62	109.88	23.83	6.31	760	310
30 kg-N fed^{-1} (Urea-1)	53.67	35.70	104.17	23.12	4.88	480	270
60 kg-N fed^{-1} (Urea-2)	74.67	55.87	112.63	23.92	5.44	800	360
Azol –1 + Urea-1	77.35	55.70	115.64	24.93	6.52	840	510
Azol –1 + Urea-2	68.70	50.30	108.30	22.98	4.98	700	353
Azol –2 + Urea-1	67.60	49.71	109.20	23.67	5.22	610	350
Azol –2+ Urea-2	65.80	48.93	106.13	23.57	5.36	640	420
L. S. D. < 0.05	17.88	12.46	11.03	1.62	3.36	200	120

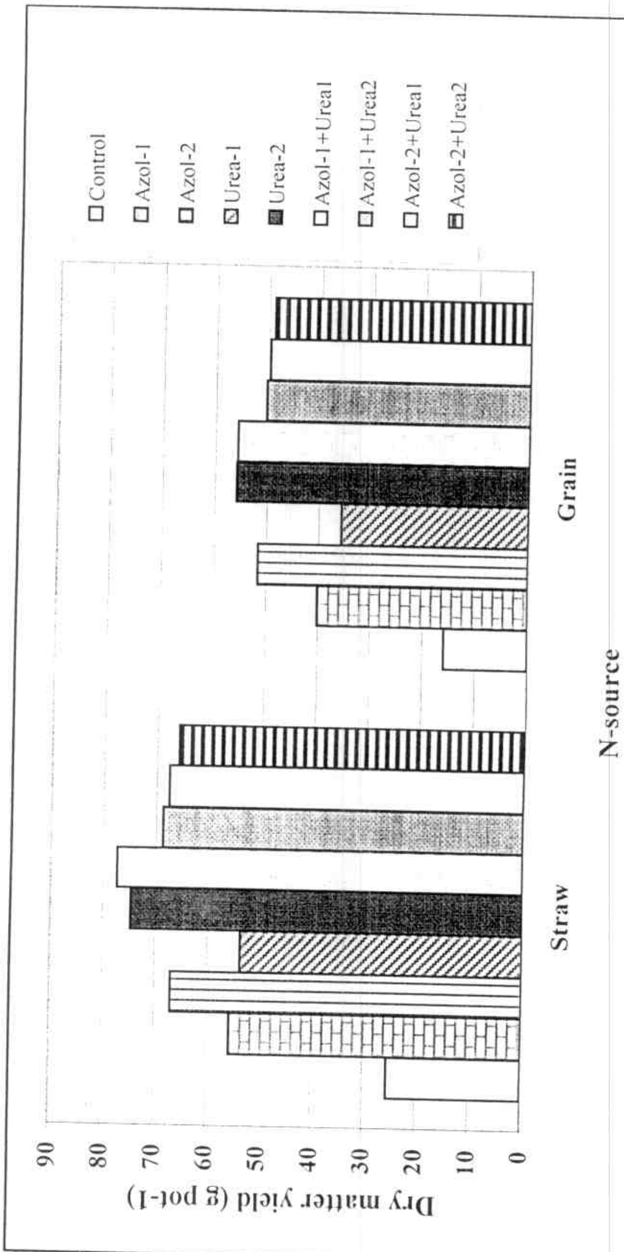

Fig. (8): Straw and grain yields of rice plants as affected by *Azolla* and urea-N .

(Azol-2) treatment. The highest straw yield was obtained with Azol-1 and urea-1 treatments.

B: Plant height:

All treatments significantly increased the plant height over the control (Table 5 and Fig. 9). The highest plant height of 115.64 cm was accompanied with the treatment of Azol-1 + Urea-1. This high value was significantly higher only than the 102.36 cm given by (Azol-2) or the 104.17 cm given by (Urea-1). The plant height of 112.63 which recorded with urea-2 was not significantly different from that obtained by Azol-2 treatment (109.88). *Azolla* nitrogen when combined with urea nitrogen gave significant increase in plant height. The most effective treatment on plant height was (Azol-1 + urea-1).

C: 1000-grain weight:

Fertilization of rice with *Azolla* or urea at different levels, either alone or together significantly increased the 1000- grain weight (Table 5 and Fig. 9) to be 24.93 g for (Azol-1+ Urea-1) treatment. This value was significantly greater than those recorded with the treatments of Azol-1, Urea-1 and (Azol-2 + Urea-1). The corresponding 1000- grain weights of 23.83, 23.92 and 23.67 g which were given by the treatments of Azol-2, Urea-2 and Azol-2 + Urea-2 were not significantly different from each other.

D: Protein Percentage:

The application of both *Azolla* and urea either alone or in combination in rice cultivation did not significantly increase the protein percentage. (Table 5 and Fig. 10). The highest protein percentage (6.52) was due to the treatment of (Azol-1 + urea-1)

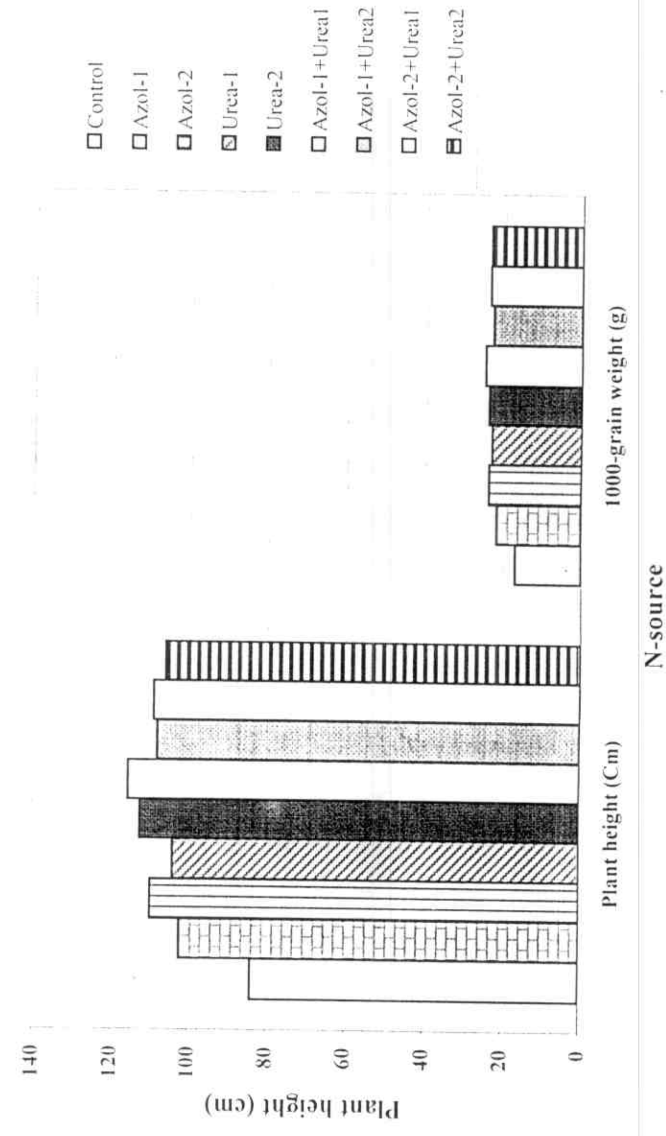

Fig. (9): Plant height and 1000-grain weight of rice plants as affected by *Azolla* and urea-N .

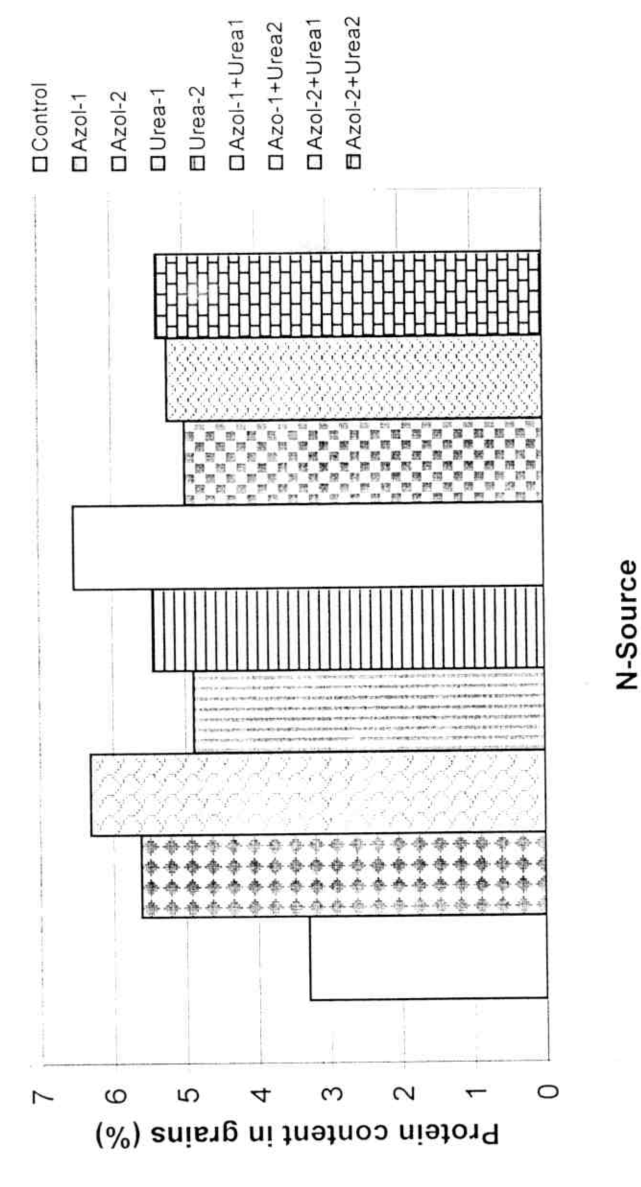

Fig. (10): Protein (%) in rice grains as affected by *Azolla* and urea-N .

followed with 5.62 % (Azol-1), 5.44 % (Urea-2), 5.36 % (Azol-2 +Urea-2), 5.22 % (Azol-2 + Urea-1) and 4.98 (Azol-1 + Urea-2).

E: Total nitrogen uptake:

Urea fertilization and *Azolla* inoculation applied to rice cultivation either alone or in combination raised significantly N-uptake by straw and grains (Table 5 and Fig. 11) of rice crop. The highest N-uptake amount of 840 mg N pot^{-1} was attained by the treatment of (Azol-1 + urea-1). This high N-uptake amount was not significantly different from any of those of 800 mg N pot^{-1}, 760 and 700 mg N pot^{-1} due to urea-2, Azol-2, (Azol-1 + urea-2) treatments, respectively. Mixing *Azolla* at 60 kg N fed^{-1} with 60 kg N fed^{-1} as urea (Azol-2 + urea-2) or (Azol-2 + urea-1) resulted in significant increases and the obtained N-uptake amounts were 640 and 610 mg N pot^{-1}, respectively. The most effective treatment on total N content in rice was (Azol-1 + urea-1). This treatment was slightly and insignificantly superior to the treatment of urea-2, it means that, the positive effect of *Azolla* could enhance the growth and plant quality as well as decreases the harmful effect of mineral nitrogen on the environment of soil.

F: Total phosphorus uptake:

The uptake of P by rice plants was significantly enhanced by the application of *Azolla* or urea alone or in combination as compared with control (Table 5 and Fig. 11). Application of (Azol-1 + urea-1) treatment *Azolla* increased P uptake to be 510 mg P pot^{-1}. The effectiveness of the studied treatments on total P uptake by straw and grain of rice could be arranged in the flowing order:

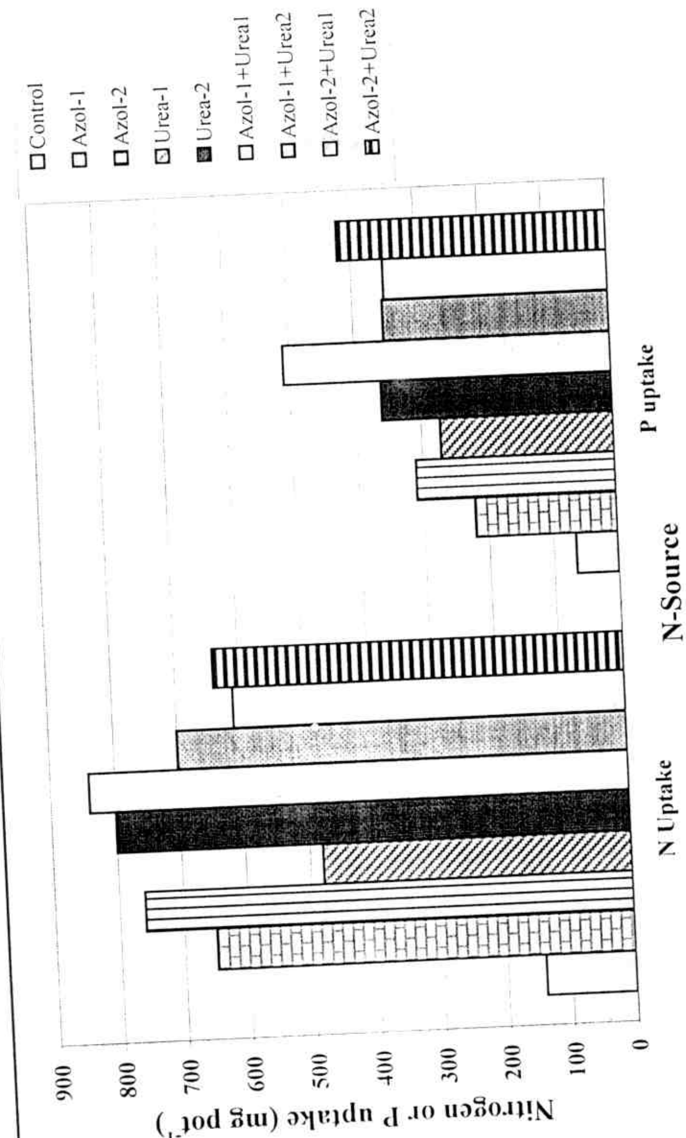

Fig. (11): Total N and total P uptake of rice crop as affected by *Azolla* and urea-N .

(Azol-1 + urea-1) > (Azol-2 + urea-2) > urea-2 > (Azol-1 + urea-2) > (Azol-2 + urea-1) >Azol-2 > urea-1 > Azol-1.

G: Overall effect on plant:

Azolla possess the ability to utilize atmospheric nitrogen due to a symbiosis with the cyanobiont *Anabaena Azollae*, which grows in the cavities of *Azolla* leaflets; and was used extensively and effectively for green manure in rice fields instead of chemical fertilizer especially in Asia (**Uheda et al., 1999**). The obtained results due to the use of *Azolla* inoculation as partial or full replacement for fertilizer nitrogen are in accordance with following studies:

Moore (1969) reported that *Azolla* assimilate 100 to 160 kg N ha^{-1} in three or four months. The utilization of 30 or 60 kg N ha^{-1}, 5 or 10 t *Azolla* ha^{-1}, alone and in combination with 30 kg N led to a conclusion that 5 t *Azolla* + 30 kg N ha^{-1} was similar to that of 60 kg N ha^{-1} in increasing paddy yields (**Vats *et al.* 1989**). The increase in the rice yield components obtained in the current study due to mixing 30 kg N ha^{-1} as *Azolla* + 30 kg N ha^{-1} as urea are in a reasonable agreement with the results of **Mandal et al. (1993); Dubey and Sharma (1995); Statapathy (1996); and El-Shahat et al. (2002)** whose results indicate that *Azolla* had the same effect on rice yield as fertilization with urea.

4.1.2.2. Effect of *Azolla* and urea on soil chemical properties:

The chemical properties of the soil after rice harvest are exhibited in Table (6).

Table (6): Effect of *Azolla* and Urea on soil N, P and O.M. after rice harvesting.

Treatment	Total N ($mg\ kg^{-1}$)	Total P ($mg\ kg^{-1}$)	Available P ($mg\ kg^{-1}$)	O. M (%)
Control	500	1500	63.23	2.42
30 kg N *Azolla* fed-1 (Azol -1)	800	1800	70.92	3.11
60 kg N *Azolla* fed-1 (Azol -2)	900	2100	80.06	3.25
30 kg-N fed^{-1} (Urea-1)	900	1600	72.30	2.95
60 kg-N fed^{-1} (Urea-2)	1100	1900	82.53	2.92
Azol -1+ Urea-1	1600	3500	87.27	3.49
Azol -1+ Urea-2	1400	2200	71.99	3.30
Azol -2 + Urea-1	1300	1800	79.29	3.15
Azol -2+ Urea-2	1500	2200	83.39	3.22
L. S. D. < 0.05	400	900	8.35	0.58

A: **Total nitrogen**:

Results indicate that the residues of all the previous rice treatments raised the total soil nitrogen after harvesting (Table 6 and Fig. 12). All the treatments that which contain *Azolla* and urea significantly increased total nitrogen amounts than those which contained urea or *Azolla* alone. For example, the treatments of Azol-2 and urea-2 gave corresponding total soil nitrogen content of 900 mg kg^{-1} and 1100 mg N kg^{-1} against 1500 mg N kg^{-1} for the treatments of (Azol-1 + urea-1). The highest total nitrogen amount (1600 mg N kg^{-1}) was achieved with the treatment of (Azol-1 + urea-1). This high total-N amount was not significantly different from the amounts recorded with the treatments contain both *Azolla* and urea.

On other hand, this high amount was significantly higher than those received either urea or *Azolla* alone. It is also worthy to note that the total nitrogen amounts attained with Azol-2, Azol-1, urea-2 and urea-1 were 900, 800, 1100 and 900 mg N kg^{-1}, respectively.

B: Total phosphorus:

No significant increase was observed in contents of total soil phosphorus due to using urea and *Azolla* either alone or in combination (Table 6 and Fig. 12). This trend was not true with the treatment of (Azol-1+ urea-1), which recorded the highest total phosphorus amount of 3200 mg P kg $^{-1}$. This high total P amount significantly exceeded all the treatments. Despite of the treatment of (Azol-2 + urea-2) which gave 2200 mg P kg^{-1} was higher than both of urea-2 (1900 mg P kg^{-1}) and Azol-2 (2100 mg kg^{-1}), the differences were not significant. Mixing 30 kg N

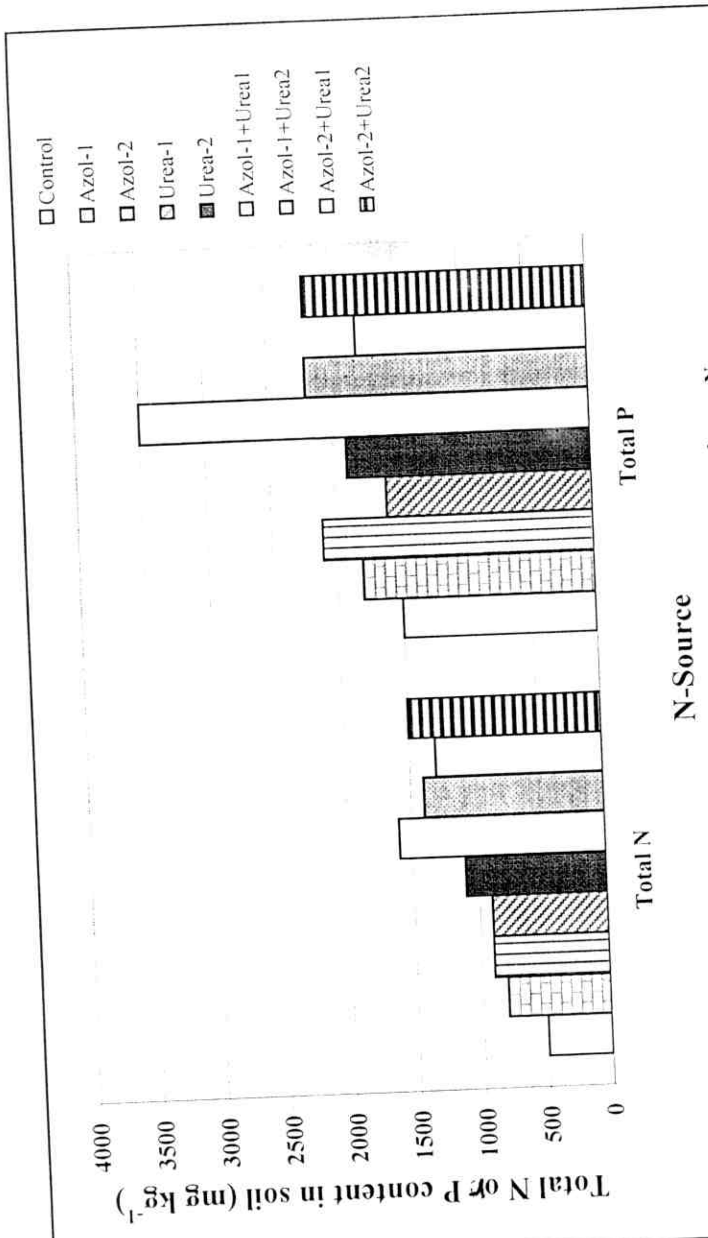

Fig. (12): Total N and P in soil as affected by Azolla and urea-N after rice harvesting.

fed^{-1} as *Azolla* (Azol-2) with both 30 and 60 kg N fed^{-1} as urea resulted in total phosphorus amounts of 2200 and 3500 mg P kg^{-1}.

C: <u>Available phosphorus</u>:

All the treatments significantly increased the amounts of available-P. The highest available P content of (8. 73 mg P kg^{-1}) was attained with (Azol-1 + urea-1) treatment (Table 6 and Fig. 13). This high available P content was significantly higher than those recorded with urea-2, urea-1 and (Azol-1 + urea-2.) Their corresponding phosphorus contents were 7.59, 7.23, and 7.20 mg P kg^{-1}. The highest value was not significantly different from those of urea-2 (8.25 mg P kg^{-1}), Azol-2 (8.01 mg P kg^{-1}), Azol-2 + urea-2 (8.34 mg P kg^{-1}) and Azol-2 + urea-1 (7.93 mg P kg^{-1}).

D: <u>Organic matter percentage</u>:

The treatments which received *Azolla* either alone or combined with different levels of urea –N significantly increased the organic matter percentage (Table 6 and Fig. 14) of the remained soil, while the treatments with only urea did not. The highest organic matter percentage (3.49%) was due to (Azol-1+ urea-1) followed by 3.30, 3.25, 3.22, 3.15, 3.11, 2.95 and 2.92 % for the treatments of Azol-1 + urea-2, Azol-2, (Azol-2 + urea-2), (Azol-2 + urea-1), Azol-1, urea-1 and urea-2, respectively. However, the highest organic matter percentage was not significantly different from those given by the other treatments. As it is obvious, these results revealed that *Azolla* inoculated to rice increased the organic matter percentage in the remained soil.

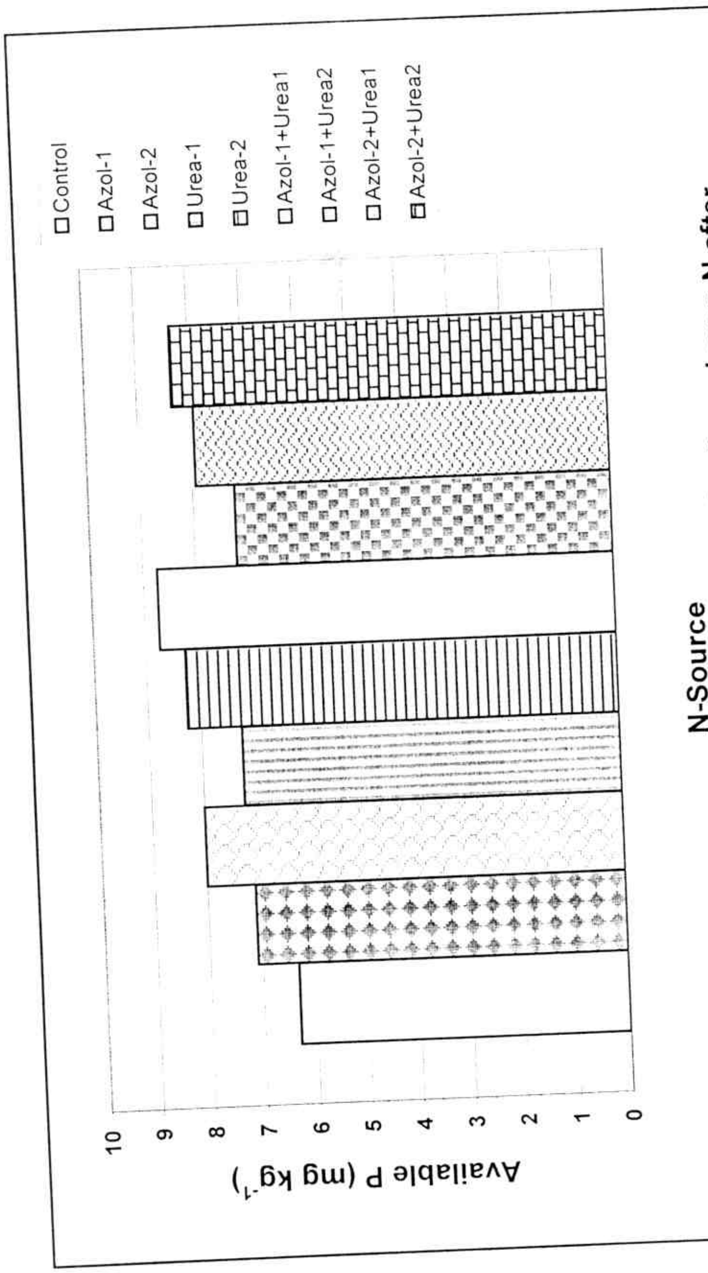

Fig. (13): Available P in soil as affected by Azolla and urea-N after rice harvesting

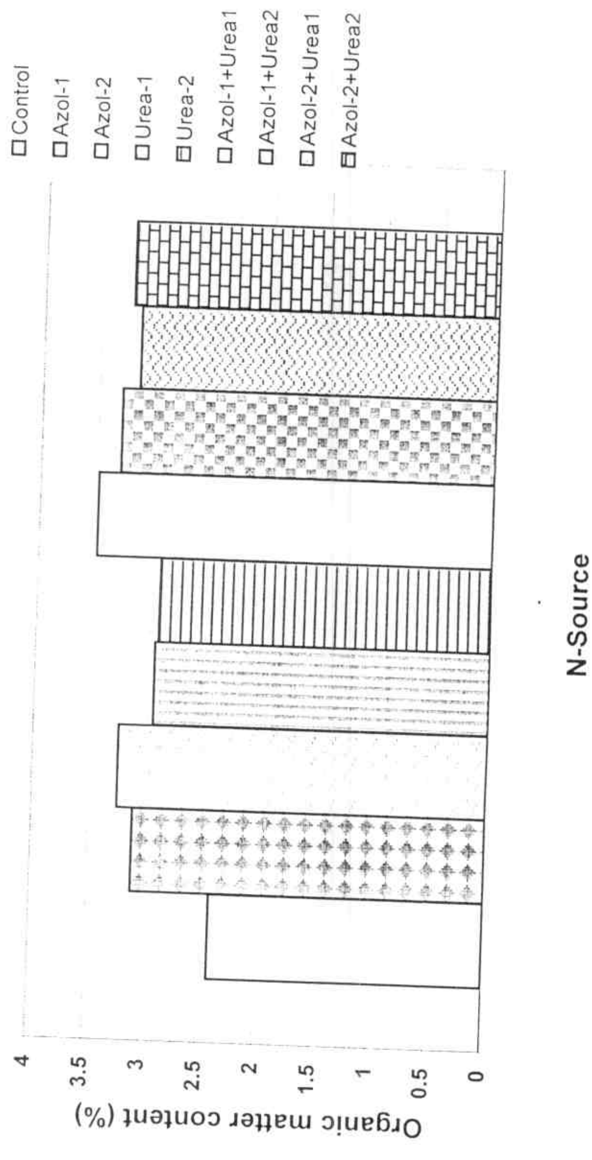

Fig. (14): Soil Organic matter content(O.M.) as affected by Azolla and urea-N after rice harvesting

E: **Overall effect on plant:**

The results obtained are in harmony with those recorded by many authors i.e **Nazeer and Prassad (1984)** found that due to *Azolla* application in rice soil an increase in nitrogen and organic matter content of the soil was noticed. **Staden (1990)** reported that *Azolla* use builds up organic matter content and can cycle mineral elements into forms more readily available to rice plants. As a cover crop, *Azolla* reduces evaporation and N volatilization from rice soil. **Tanagaraju and Kannaiyan (1993)** reported that *Azolla* application improved soil fertility by increasing total nitrogen, organic carbon and available phosphorus in the soil. They also pointed out that *Azolla* improved soil structure when incorporated because of its high productivity, which supplies large quantities of organic matter. Some reports emphasized that *Azolla* as a green manure improved the soil physical properties by increasing the porosity and decreasing the specific gravity of soils which decreased the bulk density of the soil. These changes in soil properties are important, since they reduce the amount of energy required for soil tillage and improve water infiltration, aeration and soil temperature **(Ventura and Watanabe 1993 and Mandal et al., 1999)**. Incorporation of *Azolla* as green manure also influences positively the transformation and availability of Fe, Mn, Zn and Cu in flooded rice soils **(Mandal et al., 1997)**. Sixty kg N as *Azolla* or urea utilized in rice field increased the soil organic carbon by 27.6 % and 42.5 % for urea and *Azolla* combination treatment while the organic carbon did not change with urea alone **(Herzallah et al., 2002).**

4.2. Residual effect of cyanobacteria (SBI) and / or *Azolla* on wheat plant and some soil chemical properties:

Data in Tables (7 and 8) show the residual effect of cyanobacteria inoculation (SBI) and urea fertilization each alone at different levels or in combination on wheat yield components, total nitrogen and phosphorus uptake by wheat crop as well as on some chemical properties of soil.

4.2.1. Residual effect of (SBI) and urea on plant:

A: Straw and grain yields:

Inoculation with SBI alone at the tested three rates failed to increase straw yield significantly (Table 7 and Fig. 15). However, using urea at the two tested rates succeeded to increase straw yield significantly. Therefore, The residual effect of urea-2, urea-1, (SBI-2 + urea-2) and (SBI-3 +urea-1) treatments on wheat straw yield exhibited significant increases, but increases caused by other treatments were not significant. The straw yields of these treatments were 20.70, 20.47, 19.64 and 21.70 g pot^{-1} against 12.87 g pot^{-1} for the control treatment. Although, the (SBI-3 + urea-1) treatment gave the highest straw weight of 21.70 g pot^{-1} among the treatments, it was not significantly different from them. Inoculation with SBI alone resulted in insignificant residual effect on wheat straw yield.

Concerning the grain yield, all the treatments receiving SBI and / or urea significantly increased the grain yield. Using SBI alone at the studied rates although increased grain yield significantly they were all similar in effect. Also using urea alone at two different rates increased grain yield significantly.

Table (7): Residual effect of cyanobacteria (SBI) inoculation and urea fertilization (to preceding rice crop) on wheat yield components.

Treatment	Yield (g/pot)		Plant height (cm)	1000- grain Weight (g)	Protein (%) in grains	Total-N uptake (mg pot^{-1})	Total-P uptake (mg pot^{-1})
	Straw	Grain					
Control	12.87	11.40	62.77	40.60	7.13	160	49
200 g fed^{-1} cyanobacteria (SBI-1)	16.93	15.40	72.37	41.60	9.23	240	103
1.5 kg fed^{-1} cyanobacteria (SBI-2)	16.70	15.50	70.57	44.37	8.73	275	112
3 kg fed-1 cyanobacteria (SBI-3)	17.10	16.00	72.77	45.70	9.30	303	127
30 kg-N fed^{-1} (Urea-1)	20.47	16.87	75.47	46.00	8.62	298	100
60 kg-N fed^{-1} (Urea-2)	20.70	18.78	76.67	46.23	9.70	335	98
SBI-1 + Urea-1	16.97	16.90	65.33	42.60	8.60	272	106
SBI-1 + Urea-2	16.63	18.80	67.43	45.27	9.60	276	127
SBI-2+ Urea-1	15.73	17.20	73.13	45.57	8.37	297	119
SBI-2 + Urea-2	19.64	16.47	62.00	43.30	8.37	289	115
SBI-3 + Urea-1	21.70	20.90	76.77	50.77	9.53	350	171
SBI-3 + Urea-2	18.50	15.63	73.33	45.23	9.47	310	150
L. S. D. < 0.05	5.78	2.06	8.81	4.78	1.35	68	36

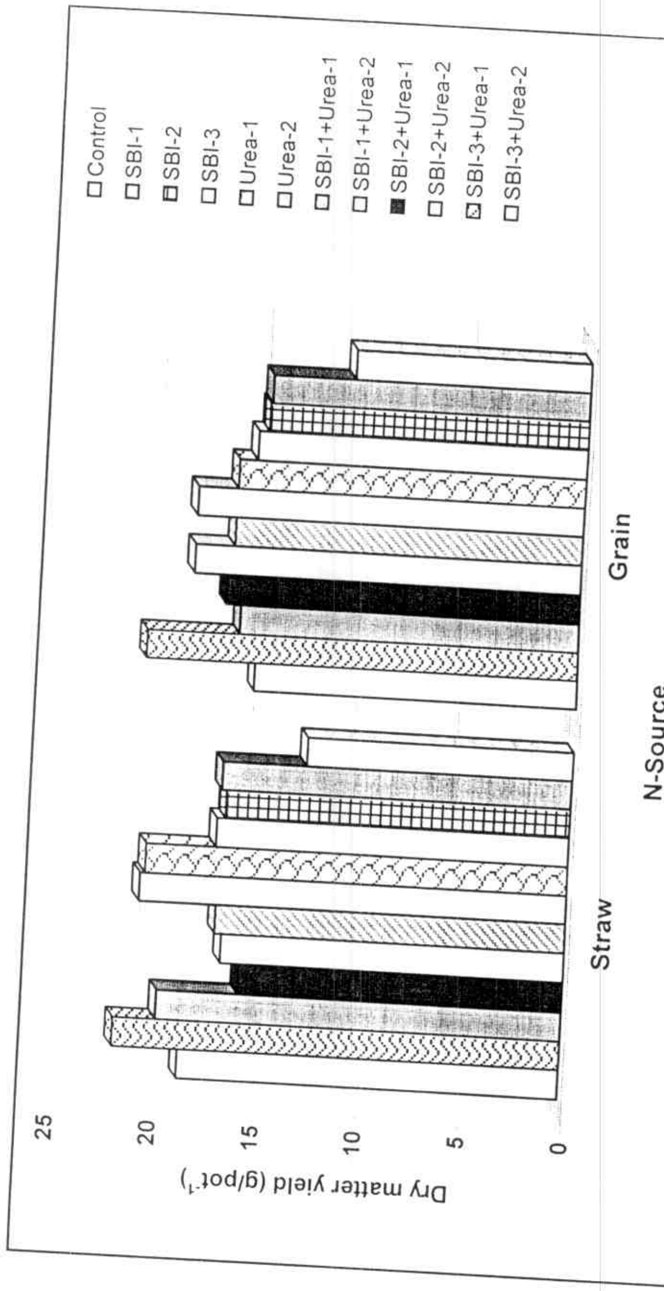

Fig. (15): Residual effect of SBI and urea-N on straw and grain yieds of wheat crop.

The same trend was obtained with the combination between SBI and urea. The highest grain yield was 20.90 g pot^{-1} was given by (SBI-3 +urea). This high value was significantly different from most of the treatments used.

B: Plant height:

All treatments increased the plant height except those of SBI-2 and (SBI-2 + urea-2) (Table 7 and Fig. 16). The significant increases were achieved with using the rate of SBI-1 and both rates of urea (urea-1 and urea-2), besides the following combinations: (SBI-2 + urea-1), (SBI-3 + urea-1) and (SBI-3 + urea-2). The highest plant height of 76.77 cm was due to (SBI-3 + urea-1). This plant height was comparable to that of 76.67 cm due to urea-2 treatment (full mineral nitrogen rate).

C: 1000-grain weight:

The residual effect of urea-1, urea-2 (SBI-2 + urea-1) and (SBI-3 + urea-1) treatments recorded the significant increases in 1000-grain weight over the control treatment (Table 7 and Fig. 16). The produced weight values for such treatments were 46.00, 46.23, 45.57 and 50.77 g against 40.60 g for the control treatment. Treatments of SBI-2 + urea-1 and SBI-3 + urea-1 were the only combinations possess a significant effect on 1000-grain weight parameter. The highest 1000-grain weight of 50.77 g that was given by (SBI-3 + urea-1) was significantly higher than all of those attained due to all treatments except that was achieved with urea-2 (46.23 g) and urea-1 (46.00 g).

D: Protein percentage:

All the recorded protein percentages (Table 7 and Fig. 17) of treatments receiving SBI and / or urea were significantly

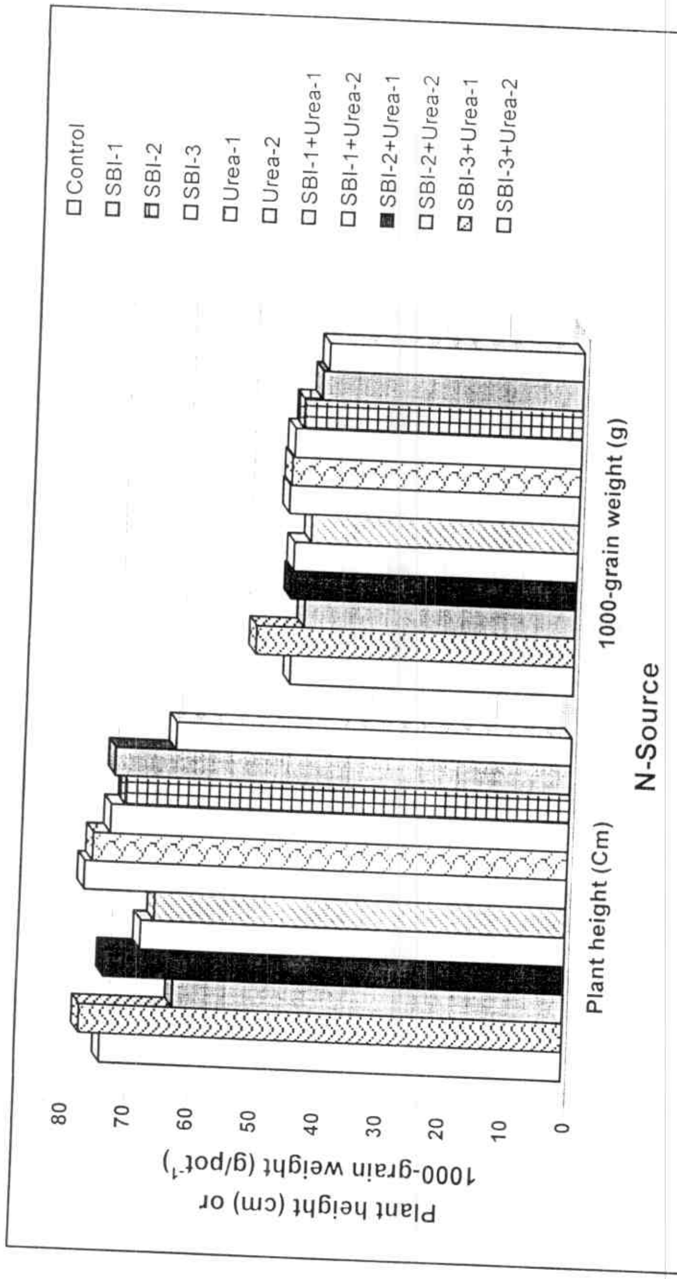

Fig. (16): Residual effect of SBI and urea-N on plant height and 1000- grain weight of wheat crop.

higher than that of the control treatment. The protein percentage value of 9.53 due to (SBI-3 +urea-1) treatment was the highest one among the tested treatments. However, it was not significantly different from the other treatments. Using SBI at the tested levels significantly increased protein percentage in wheat grains with no significant difference between each other. Similar significant effect was attained with applying urea either at the high rate or at the low one. Combination of SBI and urea significantly increased protein content in grains, except those of (SBI-2 + urea-1) or (SBI-2 + urea-2) which showed no significant increase in protein percentage.

E: <u>Total nitrogen uptake</u>:

Data of Table (7) and Fig. (18) reveal the residual effect of SBI inoculation and urea fertilization on the total nitrogen uptake content (straw and grain) of wheat crop.

It could be concluded that the treatments which received either SBI inoculation or urea fertilization during rice cultivation gave a significant increase in total N uptake. The highest value of total N-uptake (350 mg N pot^{-1}) was achieved with (SBI-3 + urea-1) treatment, this value was not significantly different from values achieved with the other treatments except SBI-1, SBI-2, (SBI-1 + urea-2) and (SBI-1 + urea-1) treatments.

These results may indicate that the effect of SBI inoculation and urea either alone or in combination was persisted after rice harvesting. It is also worthy to state that the total N-uptake values recorded with (SBI-3 + urea-2) "310 mg N pot^{-1}" and (SBI-3 + urea-1) "350 mg N pot^{-1}" were not significantly

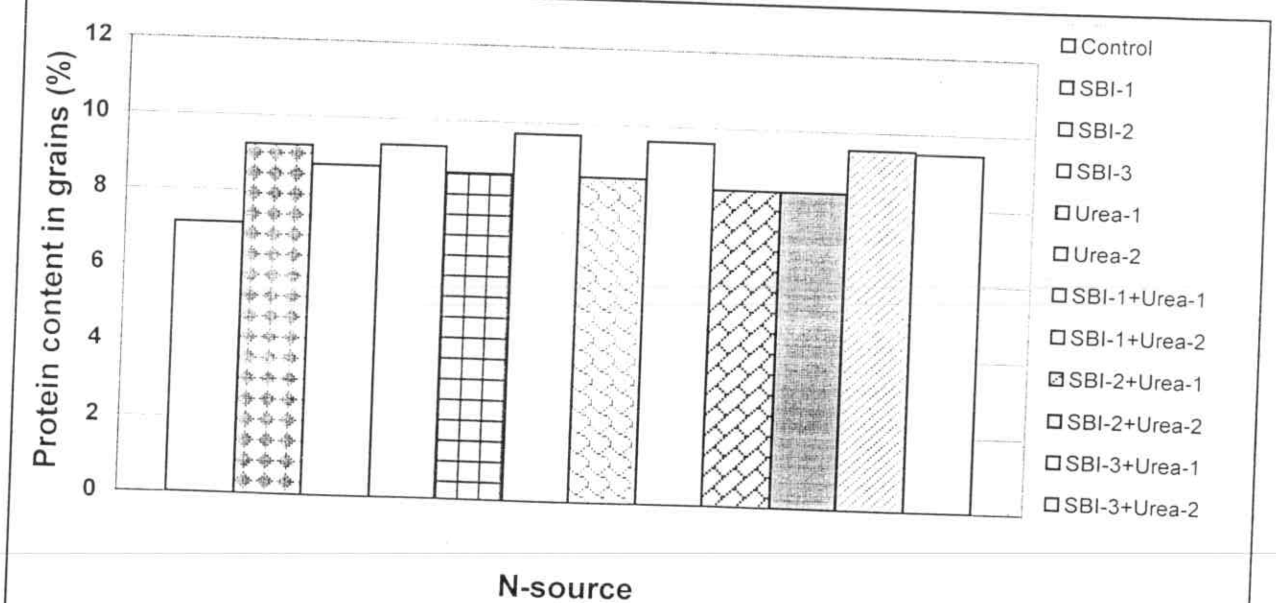

Fig. (17): Residual effect of SBI and urea-N on protein (%) in grains of wheat plant.

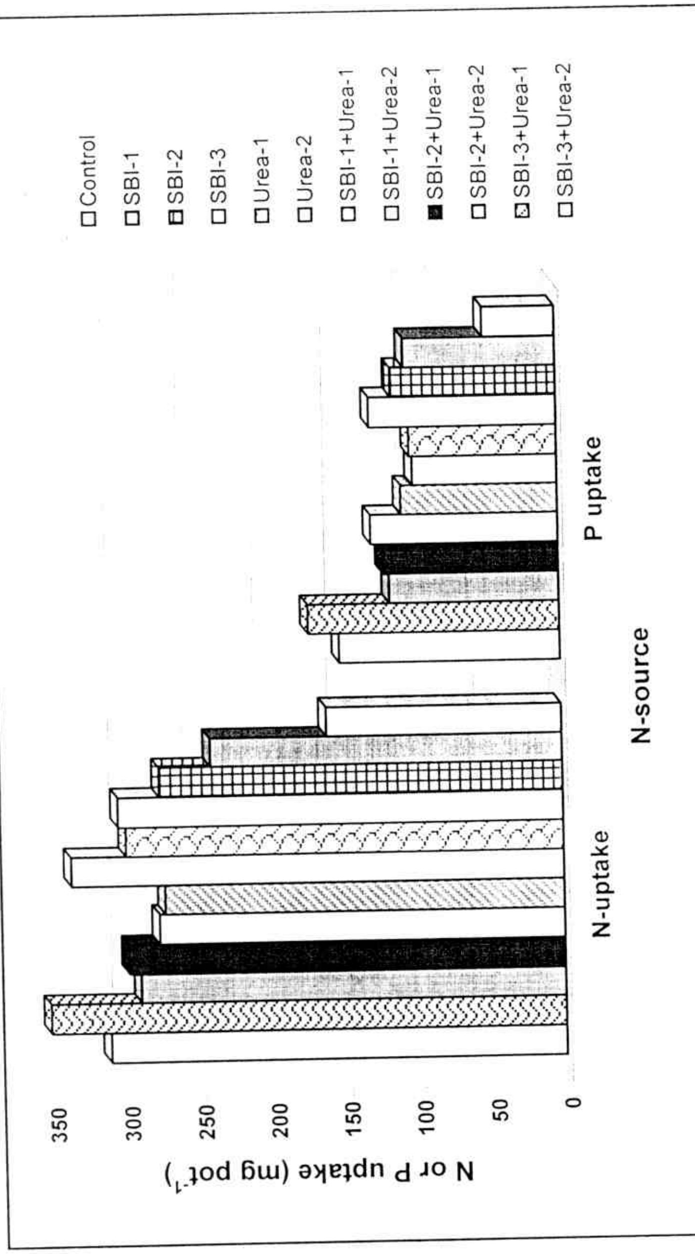

Fig. (18): Residual effect of SBI and urea-N on N and P uptake of wheat plant different from those recorded by urea-2 (335 mg N pot^{-1}) and urea-1 (298 mg N pot^{-1}).

F: <u>Total phosphorus uptake</u>:

The residual effect of SBI inoculation and urea each applied alone or in combination on the total phosphorus uptake (in straw + grain) of wheat crop resulted in significant increase in total-P uptake (Fig. 18). Significant increases in P uptake were obtained with the combination between SBI and urea when they were applied at their different rates. The highest values of P uptake were recorded with the following treatments: (SBI-3 + urea-1) "171 mg P pot^{-1}", (SBI-3 + urea-2) "150 mg P pot^{-1}" and (SBI-1 + urea-2) "127 mg P pot^{-1}". The highest value of total P-uptake (171mg P pot^{-1}) was obtained with (SBI-3 + urea-1).

G: <u>Overall effect on plant</u>:

Few studies are performed to investigate the residual effect of cyanobacteria for crops grown subsequent to the crop to which they were applied, such as wheat crop after rice. The results obtained in the current study are in accordance with those of **Suri et al., (1995)** who reported that cyanobacteria inoculated to rice increased significantly grain and straw yields for the following wheat crop.

4.2.2. <u>Residual effect of SBI and urea on soil chemical properties:</u>

Data in Table (8) indicate the residual effect of cyanobacteria inoculation (SBI) and urea fertilization applied alone at different levels or both in combination to rice on some chemical properties of soil such as total nitrogen, total phosphorus, available phosphorus and organic matter percentage.

Table (8): Residual effect of cyanobacteria (SBI) inoculation and urea fertilization on soil N, P and O.M. after wheat harvesting.

Treatment	Total N (mg kg^{-1})	Total P (mg kg^{-1})	Available P (mg kg^{-1})	O. M (%)
Control	300	340	58.73	2.40
200 g fed^{-1} cyanobacteria (SBI-1)	600	520	62.03	3.15
1.5 kg fed^{-1} cyanobacteria (SBI-2)	490	600	65.99	3.00
3 kg fed-1 cyanobacteria (SBI-3)	690	530	68.34	3.13
30 kg-N fed^{-1} (Urea-1)	400	510	60.48	2.17
60 kg-N fed^{-1} (Urea-2)	550	560	63.66	2.45
SBI-1 + Urea-1	680	610	63.55	3.29
SBI-1 + Urea-2	730	675	69.99	2.97
SBI-2 + Urea-1	570	625	70.73	3.25
SBI-2 + Urea-2	610	650	71.52	3.20
SBI-3 + Urea-1	800	800	78.83	3.35
SBI-3+ Urea-2	750	770	76.47	3.32
L. S. D. < 0.05	194	330	12.10	0.39

A: **Total nitrogen**:

The residual effect of both SBI inoculation and urea fertilization applied previously to rice significantly increased contents of total nitrogen (Table 8 and Fig. 19) of the soil remained after the following crop wheat. It was observed that when urea was applied along with cyanobacteria, the amount of total-N was higher than when urea was applied alone. Also inoculation with SBI alone had recorded higher total-N contents than those of urea applied alone. The highest total-N value of 800 mg N pot^{-1} was due to (SBI-3 + urea-1) treatment. This value was only significantly higher than those of SBI-1, SBI-2, urea-2, urea-2 and (SBI-2 +urea-1) treatments. Their corresponding total-N values were 600, 490, 550, 400 and 570 mg N pot^{-1}, respectively. However, it could be stated that the cyanobacteria inoculation either alone or combined with the low rate of urea nitrogen in rice soils could improve the nitrogen status in the soil and their residual effect could be extended after the following crop harvesting **(Mandal et al., 1999)**.

B: **Total phosphorus**:

Total-P content in soil after wheat harvest significantly increased when (SBI-1 +urea-2), (SBI-3 +urea-1) and (SBI-3 +urea-2) treatments were used (Table 8 and Fig. 19). The highest total-P value (800 mg P pot^{-1}) was obtained with the treatment of (SBI-3 + urea-1). This value was also higher, although it was not significantly higher than those of other treatments.

These results indicate that cyanobacteria inoculation at its highest rate supported with the lowest rate of urea during rice

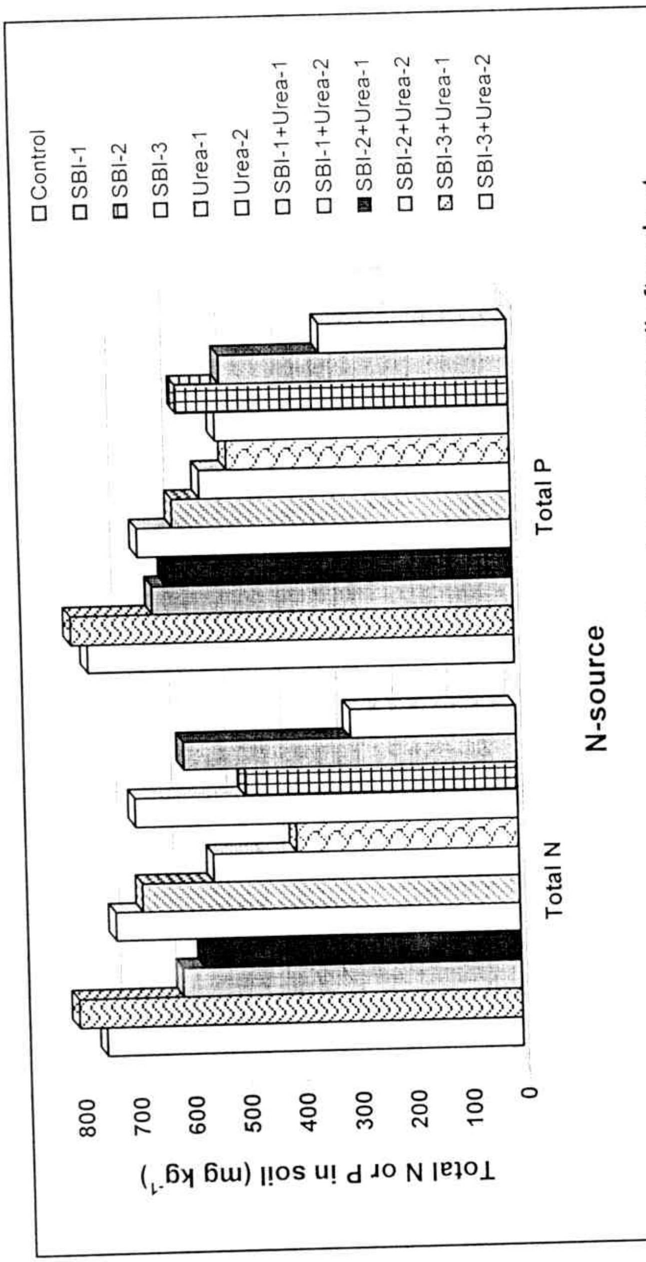

Fig. (19): Residual effect of SBI and urea-N on total N and P in soil after wheat harvesting.

cultivation can ensure residual total phosphorus content of the soil after harvesting of rice, to the following crop (wheat).

C: Available phosphorus:

Concerning the available P (Table 8 and Fig. 20), it is obvious that only the (SBI-3 + urea-1), (SBI-3 +urea-2) and (SBI-2 +urea-2) treatments recorded the highest amounts of 7.88, 7.65 and 7.15 mg P pot^{-1}, respectively. These values were significantly higher than that of the control treatment (5.87 mg P pot^{-1}). Also available-P values of these aforementioned treatments were not significantly different from the SBI-3, (SBI-1 + urea-2) or (SBI-2 +urea-1) treatments. The residual effect of the SBI inoculation along with urea fertilization during rice cultivation regarding the available P content in the soil after wheat harvesting was confirmed that cyanobacteria inoculation combined urea increased available P in soil to meet the demand of the subsequent wheat crop and the crop following it to P **(Rao and Burns 1990)**.

D: Organic matter percentage:

The residual effects of the entire cyanobacteria and urea treatments on soil organic matter content after wheat harvesting are presented in Table (8) and illustrated in Fig. (21). All the organic matter percentages recorded in response to the residual effect of the treatments receiving SBI and / or urea were significantly higher than that of the control treatment except those of urea-1and urea-2 treatments. The highest organic matter percentage of 3.35 % was due to (SBI-3 +urea-1). This value was significantly different from those recorded with control, urea-1 and urea-2 treatments.

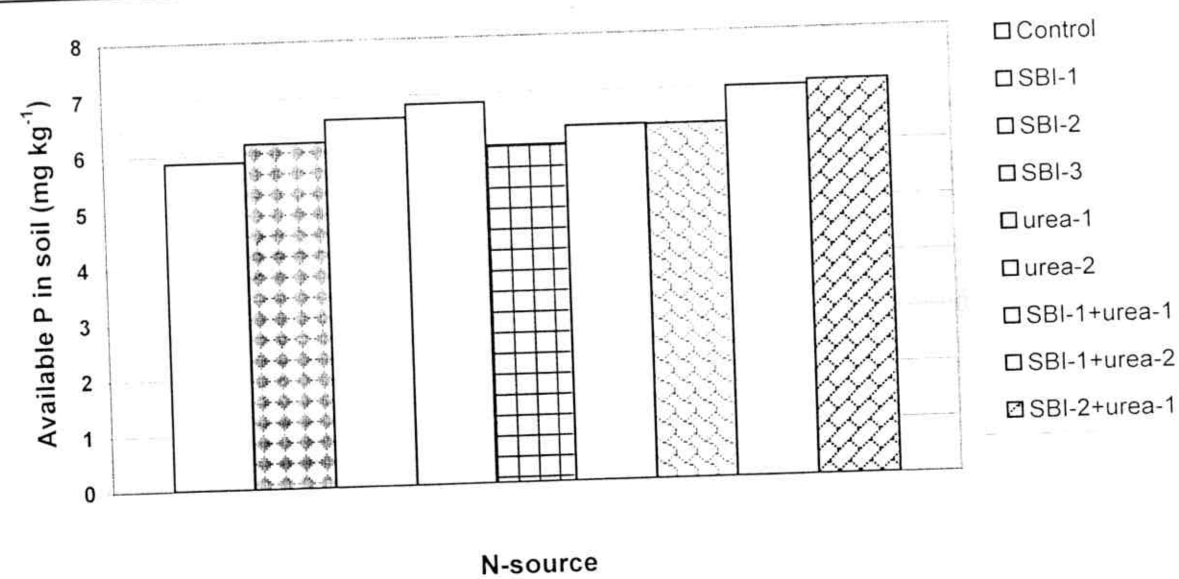

Fig. (20): Residual effect of SBI and urea-N on available P in soil after wheat harvesting.

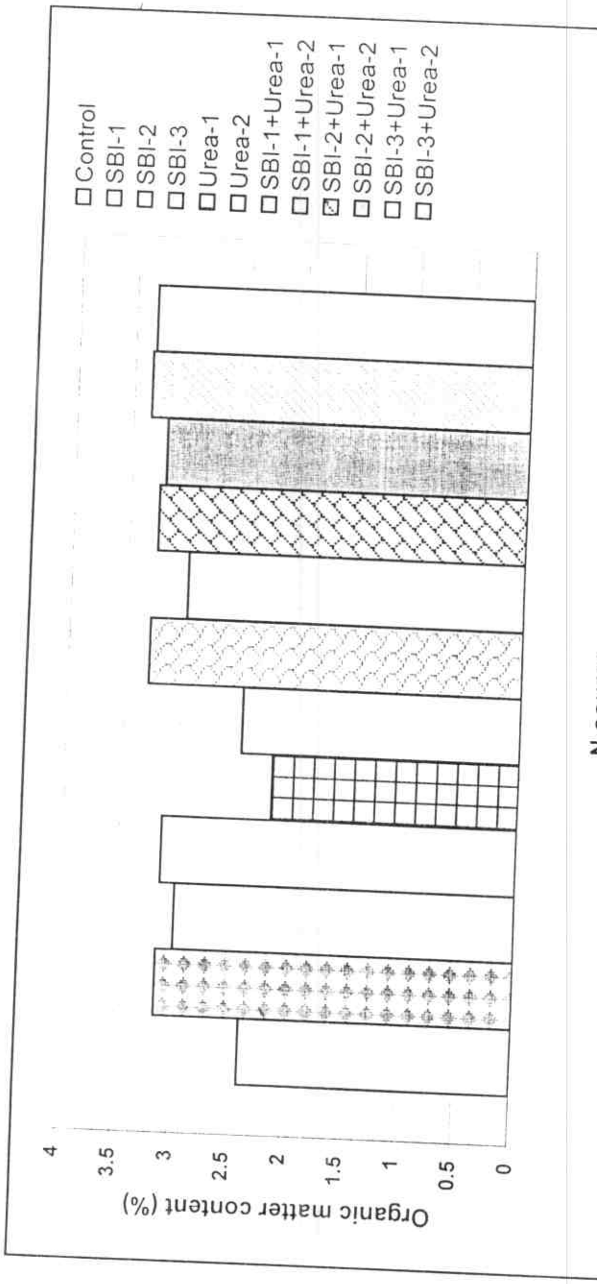

Fig. (21): Residual effect of SBI and urea-N on organic matter in soil after wheat harvesting.

E: Overall effect on plant:

It has been suggested that besides fixing atmospheric nitrogen, cyanobacteria produce vitamins and plant growth stimulating hormones, excrete polysaccharides thereby improving soil aggregation, stimulate some beneficial soil microorganisms, improve soil water holding capacity, increase soil organic matter, and make phosphates soluble as mentioned by **Roger and Kulasooriya (1980 and Rao and Burns (1990).** They also explained that the soil enzyme activities such as urease and phosphatase increased by 2.8 and 5.5 folds in soils inoculated with cyanobacteria after 21 weeks. These increases in enzymatic activities ensure adequate amounts of nitrogen and phosphorus in soils due cyanobacteria inoculation. **Mandal et al. (1999)** indicated that under favorable conditions a good cyanobacterial bloom in rice fields gave yields in average ranging from 6 to 8 t ha^{-1} fresh biomass. The persistence of such biomass in soil as organic matter, however, depends on its decomposability. Such high organic matter in rice fields upon its decomposition during rice life, would liberate nutrients to became available to the following crops like maize, potato, wheat oilseeds.

4.2.3. Residual effect of *Azolla* inoculation and / or urea application on yield components and some chemical properties of soil:

Data in Tables (9 and 10) explain the residual effect of *Azolla pinnata* inoculation and urea fertilization each alone at different levels or in combination on wheat yield components, the total nitrogen and phosphorus uptake by wheat crop as well as on some soil chemical properties.

Table (9): Residual effect of *Azolla* inoculation and urea fertilization on wheat yield components.

Treatment	Yield (g pot^{-1})		Plant height (cm)	1000-grain Weight (g)	Protein (%) in grains	Total-N uptake (mg pot^{-1})	Total-P uptake (mg pot^{-1})
	Straw	Grain					
Control	11.87	10.40	62.77	40.60	7.12	160	49
30 kg N *Azolla* fed-1 (Azol -1)	20.00	18.17	84.77	48.73	9.63	324	118
60 kg N *Azolla* fed-1 (Azol -2)	20.12	19.30	86.67	48.63	9.10	390	123
30 kg-N fed^{-1} (Urea-1)	19.20	18.20	83.77	48.57	9.27	378	110
60 kg-N fed^{-1} (Urea-2)	19.70	21.63	82.43	48.10	9.17	402	115
Azol -1 + Urea-1	21.77	22.83	90.00	50.93	10.97	460	150
Azol -1+ Urea-2	20.13	21.10	76.23	47.97	9.60	425	135
Azol -2 + Urea-1	18.83	20.13	79.67	47.73	9.70	344	136
Azol -2 + Urea-2	19.30	18.17	84.67	47.07	10.23	342	124
L. S. D. < 0.05	6.65	4.6	6.72	3.64	2.13	92	50

4.2.3.1. Residual effect of *Azolla* and urea on wheat plant:

A: Straw and grain yields:

Straw and grain yields of wheat were significantly increased by the application of *Azolla* and urea either alone or in combination to the previous rice crop (Table 9 and Fig. 22). Application of *Azolla* alone at its two rates showed high residual effect on straw yield compared with urea alone or combined application of (Azol-2 + urea-1) or (Azol-2 + urea-2) However, combined application of (Azol-1 and urea-1) or (Azol-1 and urea-2) showed the highest straw yield as compared with the other treatments. The residual effect produced by the application of biofertilizer and urea increased straw yield by 58.6 to 83.4 %.

Concerning grain yield, the effect of applying *Azolla* alone was not superior to that of urea alone when the first rate of *Azolla* was compared with the first rate of urea and the second rate of *Azolla* was compared with the second rate of urea. Insignificant superiority *was* shown by urea when applied alone compared with application of *Azolla* alone. Combination of *Azolla* and urea was more effective compared with solely application of *Azolla* or urea in particular with (Azol-1 + urea-1) and (Azol-1 + urea-2) treatments. The increases obtained in grain yield ranged from 79.70 % to 119.50 % as compared with control. The highest straw and grain yields were attained with (Azol-1 + urea-1) treatment. The corresponding weight values of straw and grain were 21.77 and 22.83 g pot^{-1}, respectively. These two high values for straw and grain yields were not significantly different from those achieved by both *Azolla* and urea applied either alone or together. These results led to a conclusion that the

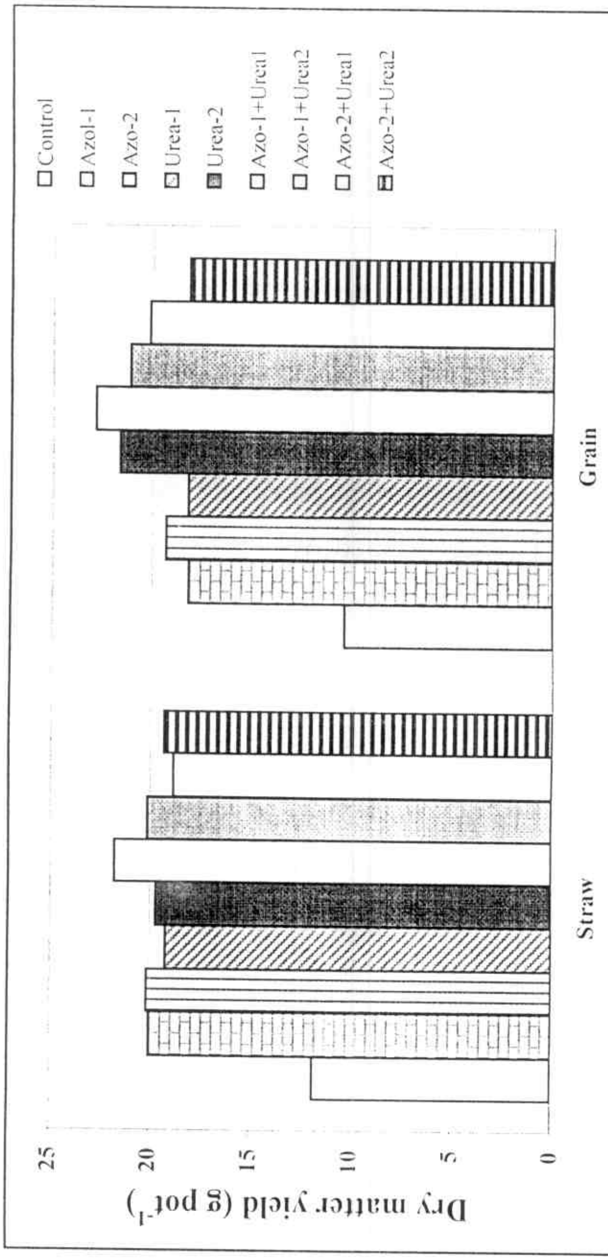

Fig. (22): Residual effect of Azolla and urea-N on straw and grain yields of wheat crop.

residual effect of *Azolla* previously applied to rice could be extended to support the following wheat crop.

B: Plant height:

Plant height of wheat significantly increased over that of the control treatment with fertilizers *Azolla* and urea (Table 9 and Fig. 23). *Azolla* application alone significantly increased plant height and produced taller plants compared with urea alone. The positive effect of *Azolla* decreased slightly when combined with urea except the treatment of (Azol-1 + urea-1) which produced the tallest plants in the experiment (90 cm). This highest plant height value was significantly different from those due to urea-2 (82.43 cm), (Azol-2+ urea-1) "79.67 cm'' and (Azol-1 +urea-2) "76.23 cm'' treatments. These results also indicate the potential of *Azolla* to extend its effect to the following crop next to rice.

C: 1000-grain weight:

The 1000-grain weight (Table 9 and Fig. 23) was significantly increased with the application of *Azolla* or urea. However, the highest 1000-grain weight (50.93 g) was obtained in response to the residual effect of the (Azol-1+ urea-1) treatment. Treatments which had received *Azolla* alone or urea alone produced the heaviest1000-grain weight. However, those treated with combined treatments (*Azolla* + urea) produced less values of 1000-grain weight except those received (Azol-1 + urea-I) treatment.

D: Protein percentage:

Protein content (Table 9 and Fig. 24) in wheat grains was significantly increased due to the application of *Azolla* and urea

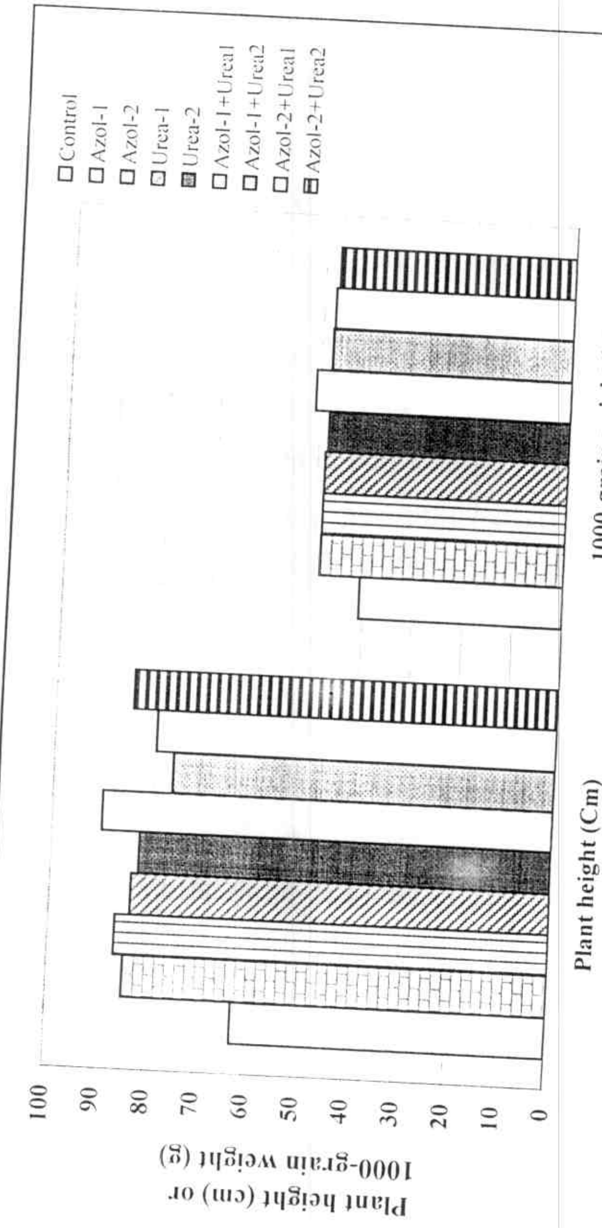

Fig. (23): Residual effect of Azolla and urea-N on plant height (Cm) and 1000- grain weight (g) of wheat crop.

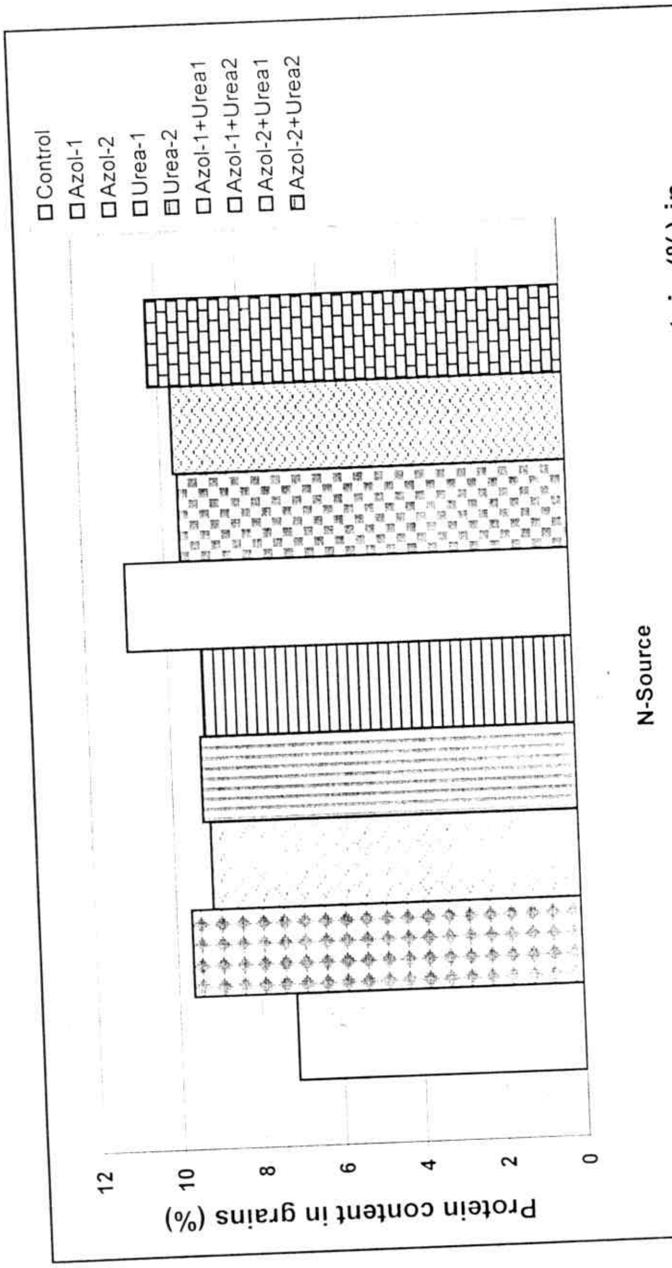

Fig. (24): Residual effect of Azolla and urea-N on protein (%) in wheat grains.

to rice except with the treatments of Azolla-2 and urea-2 where the increases in protein content in grains were not significant.

E: Total nitrogen uptake:

Data of Table (9) and Fig. (25) show the residual effect of *Azolla* inoculation and urea fertilization on the total nitrogen uptake content of wheat crop.

It could be observed that the treatments which received either *Azolla* inoculation or urea fertilization in rice cultivation gave significantly higher values of the total N uptake than that of the control treatment. Application of urea alone at its two rates significantly increased total N uptake and the increase which resulted from each level of N was higher than that of the corresponding level of *Azolla*. The highest value of total N-uptake (460 mg pot^{-1}) was scored due to the residual effect Azol-1+urea-1 treatment. This total N-uptake value was not significantly different from those recorded due to the response of the other treatments which received *Azolla* and / urea.

F: Total phosphorus uptake:

Application of *Azolla* and urea each separately or combined together to the previous rice increased significantly the total phosphorus uptake (Table 9 and Fig. 25). The control treatment showed (49 mg P pot^{-1}). The highest P-uptake value (150 mg P pot^{-1}) was attained due the residual effect of (Azol-1+ urea-1) treatment. This high P-uptake value was not significantly different from those obtained due to the residual influences of the other treatments which had received *Azolla* and / urea. This trend means that *Azolla* applied to rice can save phosphorus to wheat crop cultivated after rice as same as urea.

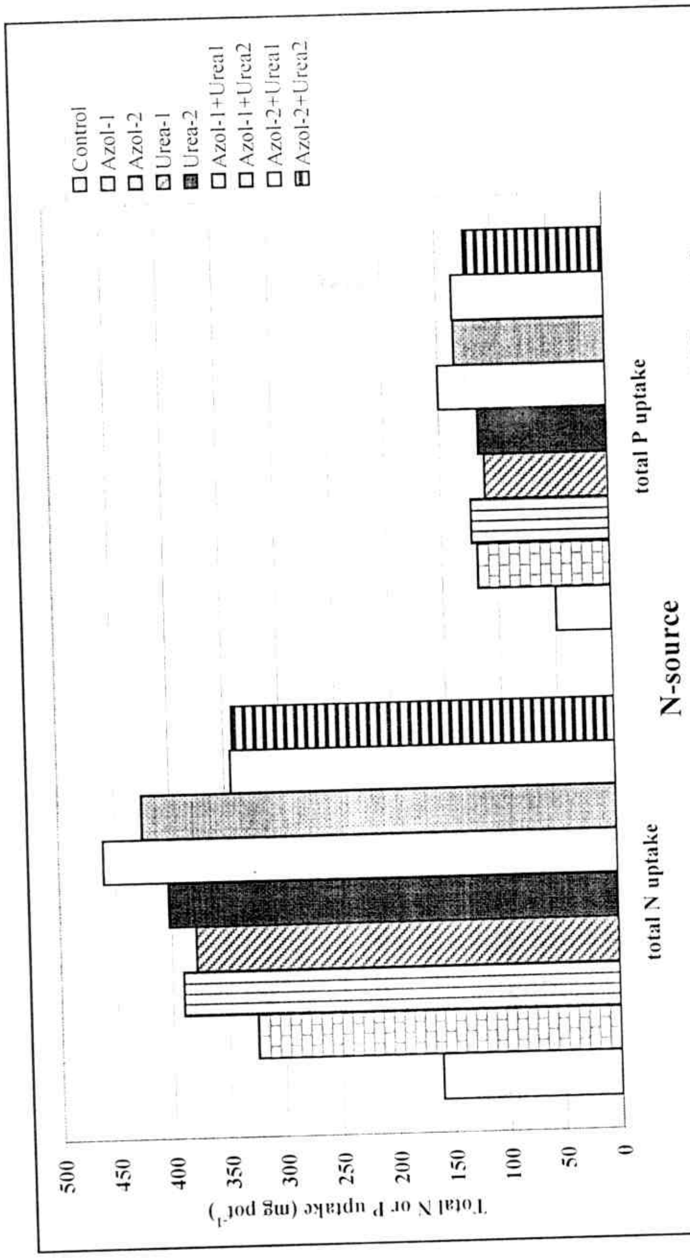

Fig. (25): Residual effect of Azolla and urea-N on total N-uptake and P uptake by wheat crop.

G: Overall residual effect on plant:

Such results are in argument with **Mahapatra et al. (1987)** who reported a presence of a significant residual effect of *Azolla* in combination with mineral N applied to rice on succeeding wheat crop. In the current study, inoculation with (Azo-1 + urea-2) resulted in increasing grain yield, dry matter, N-uptake and P uptake over the control for the subsequent wheat crop. *Azolla* can be beneficial to many target crops other than rice. For instance it is beneficial to wheat when applied in a rotating rice-wheat cropping system as reported by **Kolhe and Mittra, (1990)** who mentioned that *Azolla* applied as monocrop between the wheat and rice crops, or applied as intercrop with rice, has a significant beneficial effect on subsequent wheat crop. **Marwaha et al. (1992)** reported that fresh *Azolla* applied to rice increased grain yield for the succeeding wheat crop, though straw yield and the number of tillers per plant were largely unaffected. **Wagner (1997)** found that the application of *Azolla* with *Sesbania* had beneficial residual effects on subsequent wheat crops, raising grain yield by 56-69 %. **Zia et al. (1998)** stated that the residual effect of applied N, P and *Azolla* green manure applied to rice significantly increased straw and grain yield of the subsequent wheat crop.

It could be concluded that *Azolla* can be considered as a beneficial biofertilizer for rice as well as for the subsequent upland crops like wheat. This is due to that *Azolla* produce growth factors that enhance the beneficial soil microorganisms to grow actively, decompose organic materials in soil, support the current and subsequent growing plants with vital nutrients and fix atmospheric nitrogen; in addition to *Azolla* itself which contains high N-content caused by an active cyanobacteria (*Anabaena azollae*) as a symbiont **(El-Shahat et al. 2002).**

4.2.3.2. Residual effect of *Azolla* and urea on soil chemical properties after wheat harvesting:

The soil chemical properties of the wheat postharvest soil in response to previously rice treated with urea and / or inoculated with *Azolla* either alone or in combination with different levels are shown in Table (10).

A: Total nitrogen:

All the treatments which had received *Azolla* and /or urea showed significant increase on the total content of soil nitrogen (Table 10 and Fig. 26). The results showed that application of *Azolla* alone at its two rates increased total nitrogen and more increases were recorded with urea alone. The combination of *Azolla* and urea increased total N content to be higher than the individual application of each N source. The highest total nitrogen amount of 1018 mg kg^{-1} was due to the residual effect for (Azol-1 +urea-1) treatment. This high value was particularly significantly greater than those resulted from the residual effects of Azol-1, Azol-2 and urea-1 treatments.

B: Total phosphorus:

Total phosphorus content (Table 10 and Fig. 26) remained in soil after wheat harvesting were significantly higher in treatments given *Azolla* and / urea than that of the control treatment except for urea-1 treatment which showed a non-significant increase. The highest total-P amount (850 mg P kg^{-1}) was due to the residual effect of (Azol-1 +urea-1). This high

Table (10): Residual effect of *Azolla* inoculation and urea fertilization on soil N, P and O.M. after wheat harvesting.

Treatment	Total N (mg kg^{-1})	Total P (mg kg^{-1})	Available P (mg kg^{-1})	O. M (%)
Control	300	340	58.73	2.4
30 kg N *Azolla* fed-1 (Azol -1)	650	620	59.81	3.42
60 kg N *Azolla* fed-1 (Azol -2)	800	690	65.90	3.52
30 kg-N fed^{-1} (Urea-1)	750	400	63.89	3.44
60 kg-N fed^{-1} (Urea-2)	980	700	66.91	3.02
Azol -1+ Urea-1	1018	850	80.20	3.79
Azol -1 + Urea-1	980	800	74.15	3.56
Azol -2+ Urea-1	900	620	71.67	3.39
Azol -2 + Urea-2	970	740	70.49	3.43
L. S. D. < 0.05	177	230	11.47	0.47

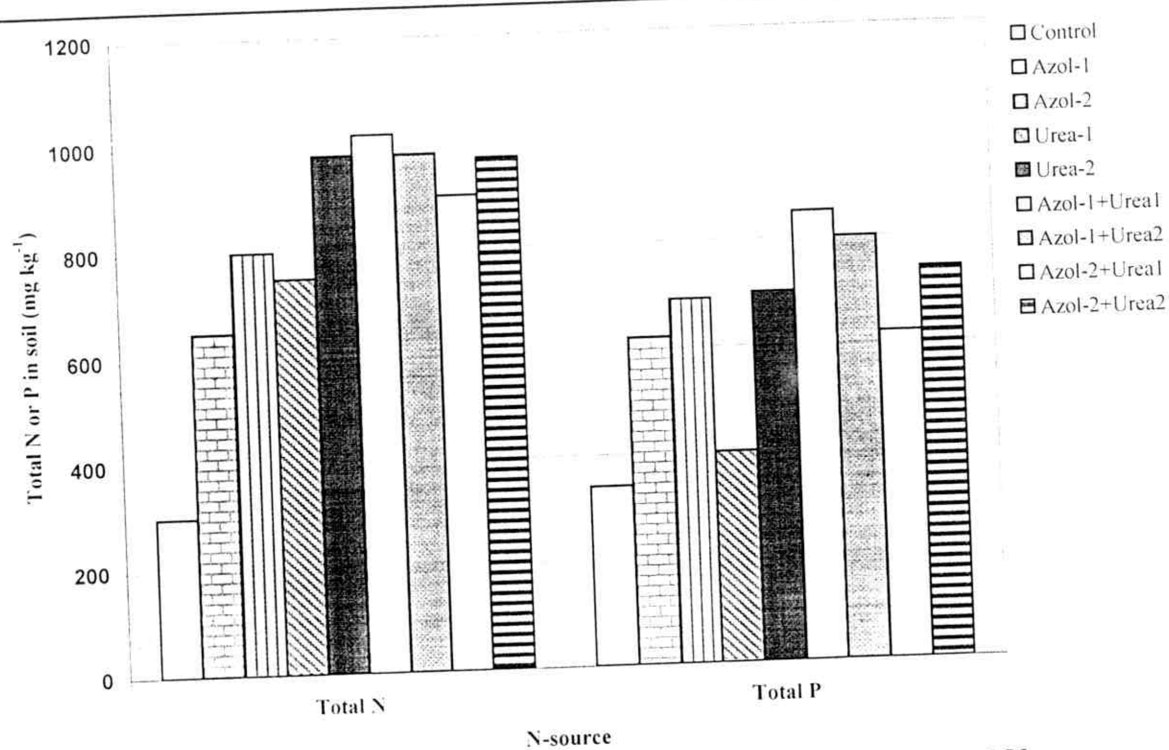

Fig. (26): Residual effect of Azolla and urea-N fertilization in total N aand P on soil after wheat harvesting total-P value was not significantly different from those of Azol-2, urea-1 or (Azol-2 + urea-2) treatments.

C: Available phosphorus:

The available phosphorus amounts (Table 10 and Fig. 27) remaining after wheat harvesting in soil due to the residual effect of urea and *Azolla* applied during rice cultivation were significantly higher than that of the control treatment. The highest available-P amount of 8.02 mg P kg^{-1} was attained due to the residual effect of (Azol-1 +urea-1) treatment. This high available-P value was not significantly greater than those recorded for (Azol-2 + urea-2) "7.05 mg Pkg^{-1}", (Azol-1+urea-1) "7.17 mg Pkg^{-1}" and (Azol-1 +urea-2) "7.42 mg P kg^{-1}".

D: Organic matter content:

The soil organic matter content (Table 10) and Fig. 28) after wheat harvesting was significantly increased due to the residual effect of applying either *Azolla* or urea alone or in combination at different rates during rice cultivation. No significant differences were noticed in the organic matter percentages due to the residual effect of all tested treatments. However the highest organic matter percentage (82.20) was attained due to the residual effect of (Azol-2 +urea-2) treatment.

E: Overall residual effect on soil:

These results are in harmony with the observations recorded by **Nazeer and Prassad (1984)** who found that due to *Azolla* application in rice soil, an increase in nitrogen and organic matter content of the soil was noticed.

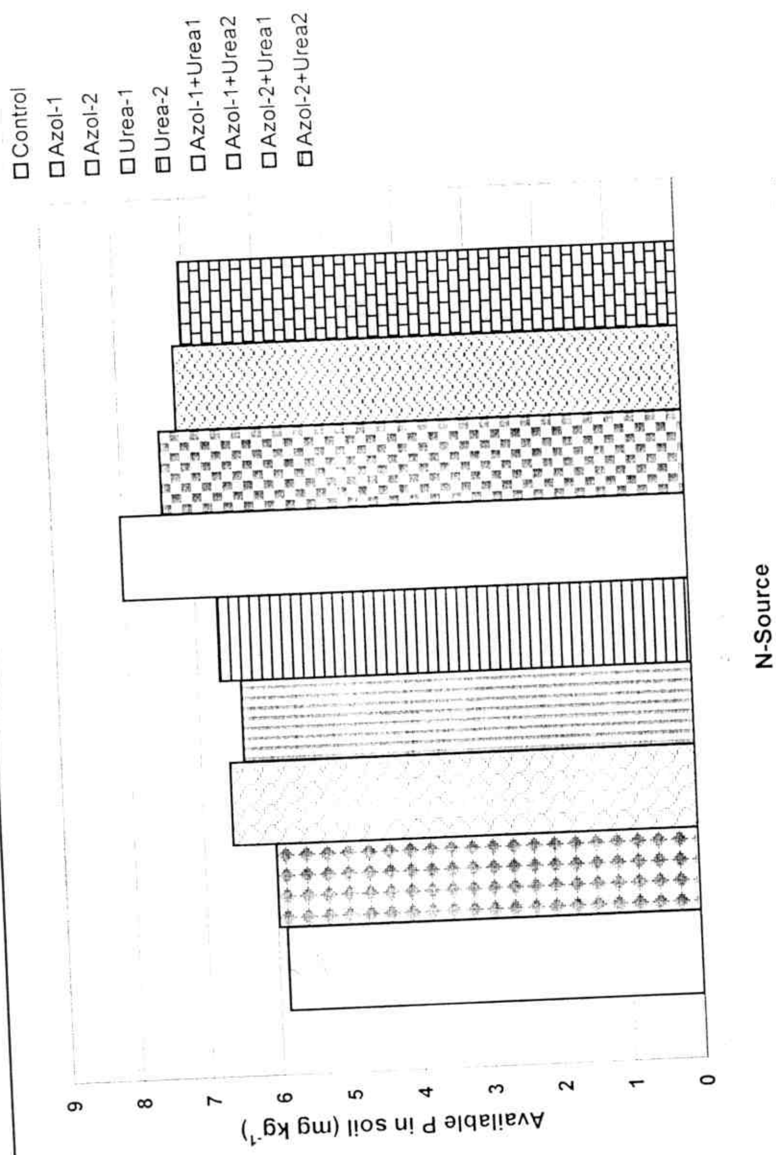

Fig. (27): Residual effect of Azolla and Urea-N on available P in soil after wheat harvesting

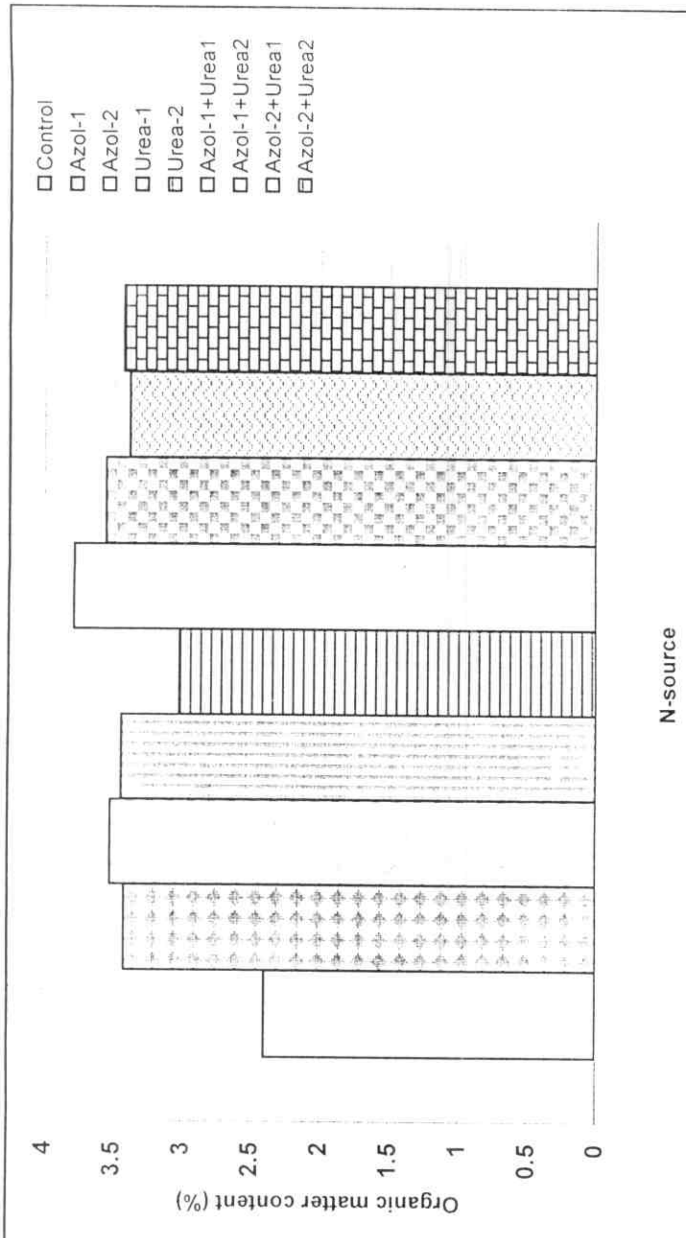

Fig. (28): Residual effect of Azolla and Urea-N on organic matter content in soil after wheat harvesting

Staden, (1990) reported that using *Azolla* builds up organic matter content and can cycle mineral elements into forms more readily available to rice plants. **Tanagaraju and Kannaiyan (1993)** indicated that *Azolla* application had improved soil fertility by increasing total nitrogen; organic carbon and available phosphorus in the soil. *Azolla* sown in paddy fields had significant effect on nitrogen balance **(Chen et al., 1994)**. *Azolla* utilized in rice field as nitrogen source, increased the soil organic carbon over the control by 27.6 % and 42.5 % for urea and *Azolla* combination treatment **(Herzallah et al., 2002).**

These findings could explain that the use of *Azolla* as a biofertilizer nitrogen source might preserve and support the soil fertility to several following crops.

4.3. Effect of iron and zinc-enriched *Azolla* as a slow-release biofertilizer on maize plants grown in a sand culture system:

A pot experiment was carried out using a sand culture technique to study the effect of both enriched Fe and / or Zn dried *Azolla* (Fe and Zn organic sources) in comparison with those of Fe EDTA (Fe, 5 %), Fe EDDHA (Fe, 6 %) and Fe SO_4 (Fe, 56 %), Zn EDDHA (Zn, 17.7%) and $ZnSO_4.7H_2O$ (Zn, 22.6%) as Fe and Zn inorganic chemical fertilizer sources on the growth of maize plants, concentration and uptake of Fe and Zn in plants.

4.3.1. Effect of iron:

Data presented in Table (11) and illustrated in (Figs. 29 and 30) indicate the significant and positive effect of different iron

Table (11): Effect of iron forms on dry matter yield and iron concentration in shoots and roots of corn plants grown in sand culture.

Treatment	Dry matter (g pot^{-1})		Iron concentration (mg kg^{-1})	
	Shoots	Roots	Shoots	Roots
Control	6.43	3.53	330.30	694.70
Fe EDTA (5 % Fe) 5mg kg^{-1}	17.10	6.67	721.70	2206.70
Fe EDDHA (6 % Fe) 10 mg kg^{-1}	17.60	8.30	995.70	2216.30
$Fe SO_4$ (56 % Fe) 20 mg kg^{-1}	15.87	7.67	544.00	1059.00
Azol Fe_1 (8.6 % Fe) 5mg kg^{-1}	19.20	8.57	1066.70	998.00
Azol Fe_2 (8.6 % Fe) 10 mg kg^{-1}	17.87	14.03	700.00	1156.70
Azol Fe_3 (8.6 % Fe) 20 mg kg^{-1}	16.30	8.40	933.70	1350.00
L.S.D. 5%	3.88	4.36	229.37	324.22

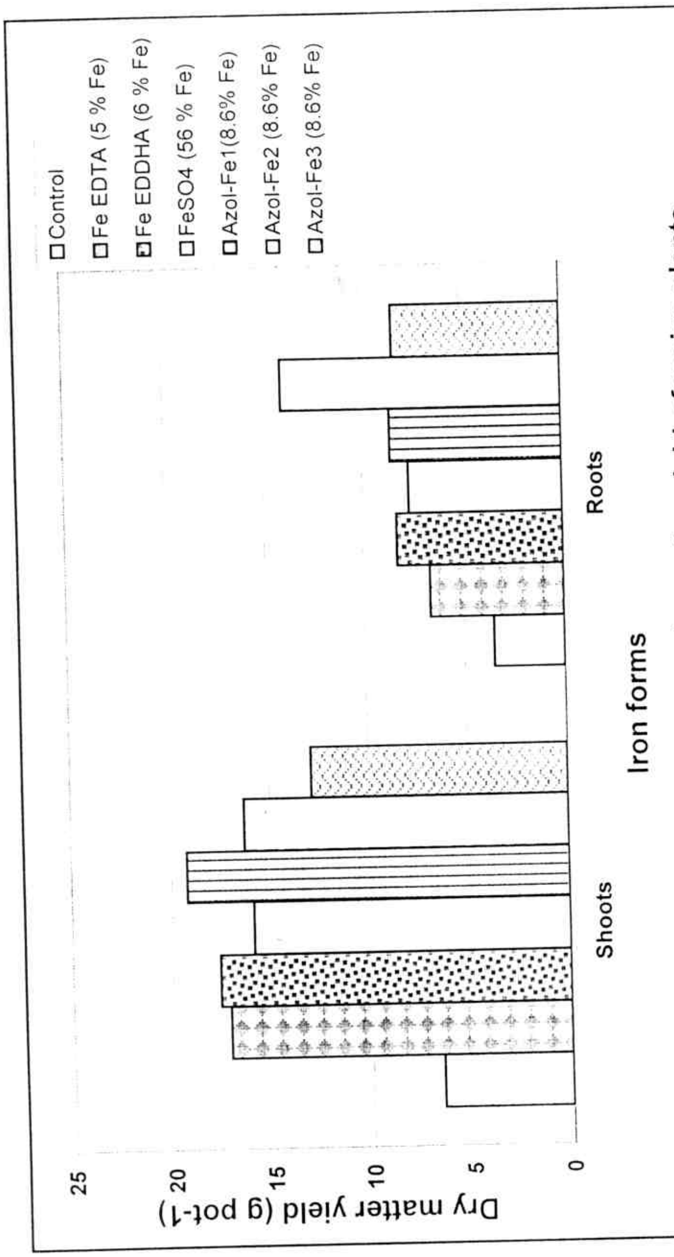

Fig. (29): Effect of iron forms on dry matter yield of maize plants grown in sand culture

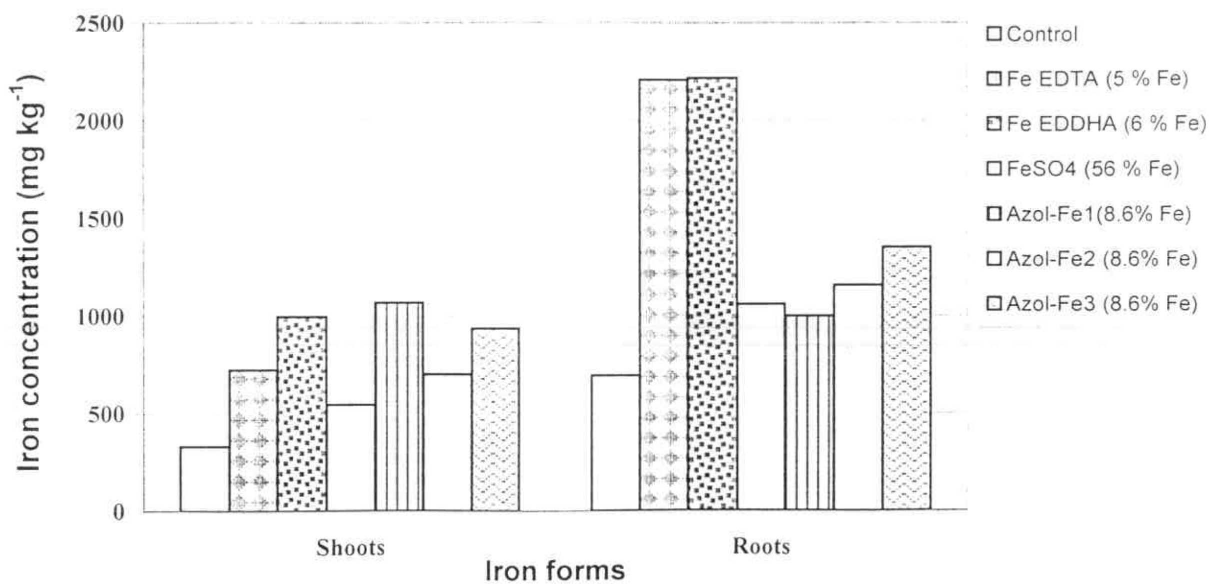

Fig. (30): Effect of iron forms on iron concentration in shoots and roots of maize plants grown in sand culture.

forms on the dry weight of maize plants as represented by the weight of dry shoots and roots in grams pot^{-1}, as well as iron concentration in both shoots and roots.

4.3.1.1. **Dry matter yield of shoots and roots**:

All the tested iron forms significantly increased shoot dry weight of maize. The highest shoot dry yields of 19.20 g pot^{-1} was attained due to the Azol-Fe_1, treatment followed by 17.87, 17.60, 17.10, 16.30 and 15.87 g pot^{-1} for the iron forms of Fe EDDHA, Fe EDTA, Azol Fe_3 and Fe SO_4,respectively. However, this high yield of shoots (19.20 g pot^{-1}) was comparable with and not significantly different from those attained due to the other tested treatments receiving Fe. The lowest shoot yield (15.87 g pot^{-1}) was due to the inorganic Fe source treatment (Fe SO_4).

Concerning yield of roots as affected by different inorganic and organic iron forms, it was noticed that all iron forms significantly increased roots yield except for Fe EDTA (6.67 g pot^{-1}) and Fe SO_4 (7.67 g pot^{-1}) treatments.

The highest yield of root yield (14.03 g pot^{-1}) was due to Azol-Fe_2 treatment. This high yield significantly exceeded those of other treatments which were accompanied with inorganic and organic Fe sources. The lowest dry weight of roots (7.17 g pot^{-1}) was achieved with Fe EDTA.

4.3.1.2. **Iron concentration**:

Concerning iron concentration in shoots and roots (Table 11) and (Fig 30), results reveal that all treatments receiving Fe significantly increased iron concentration in shoots except for that of $FeSO_4$ treatment (544.00 mg kg^{-1}). However, the highest Fe concentration of shoots (1066.70 mg kg^{-1}) was obtained with

Azol- Fe_1 treatment. This high concentration was not significantly different from those recorded with EDDHA and Azol- Fe_3 treatments.

Table (11) indicates that iron concentration in roots increased significantly due to applying Fe except for the treatment of Azol- Fe_1 (998.00 mg kg^{-1}) where the increase was not significant. The highest iron concentration in roots (2216.30 mg kg^{-1}) was associated with Fe EDDHA treatment. This high iron concentration value was significantly higher than those attained with $FeSO_4$, Azol- Fe_1, Azol- Fe_2 and Azol- Fe_3 treatments. It is also observed that iron concentration in roots attained by $FeSO_4$, Azol- Fe_1, Azol- Fe_2 and Azol- Fe_3 treatments were not significantly different from each other.

4.3.1.3. <u>Iron uptake</u>:

Table (13) and (Fig.33) represent iron uptake by maize shoots and roots. Results reveal that all the tested iron forms increased significantly Fe-uptake by shoots and roots. The highest Fe-uptake value by shoot (20.44 mg pot^{-1}) is recorded with Azol- Fe_1. This high value was significantly higher than those attained with Fe EDTA (13.50 mg pot^{-1}), $FeSO_4$ (8.64 mg pot^{-1}) and Azol- Fe_3 (15.16 mg pot^{-1}) treatments. The lowest Fe-uptake value corresponded to $FeSO_4$ (8.64 mg pot^{-1}) was the lowest one amongst all the tested iron forms.

On the other hand, Fe-uptake by roots recorded the highest value of 18.37 mg pot^{-1} with Fe EDDHA treatment. This high value was significantly higher than those recorded with $FeSO_4$, Azol- Fe_1 and Azol- Fe_3 treatments. Their corresponding Fe-uptake values were 8.26, 8.55 and 11.42 mg pot^{-1}, respectively. However, the value of 16.36 mg pot^{-1} due to the Azol- Fe_2 treatment was not significantly different from that of the highest one of 18.37 mg pot^{-1} resulting from the (Fe EDDHA) treatments.

4.3.2. Effect of zinc:

4.3.2. 1. Dry matter weight of shoots and roots:

Effect of different inorganic and organic zinc forms on both shoots and roots dry weight is presented in Table (12) and illustrated in (Fig.31). It is observed that all zinc forms significantly increased both shoots and roots dry weight. The highest shoot dry weight value (17.30 g pot^{-1}) was obtained with Azol-Zn_2 treatment. This high value was not significantly different from values obtained with other zinc forms. The same treatment (Azol-Zn_2) gave the highest dry weight of root (8.40 g pot^{-1}), which was not significantly different from those recorded with the other zinc forms.

4.3.2.2. Zinc cocentration:

All the inorganic and organic zinc forms significantly increased zinc concentration of shoots (Table 12 and Fig. 32). The highest concentration of zinc in shoots (853.33 mg kg^{-1}) was due to Azol- Zn_3 treatment. The highest zinc concentration in shoots resulted from Azol-Zn_3 was significantly different from those of Zn EDTA (236.68 mg kg^{-1}) and $ZnSO_4$ (243.33 mg kg^{-1}) treatments.

As shown in Table (12) and Fig. (32) all the zinc forms significantly increased zinc in roots. (23.00 mg kg^{-1}). The highest zinc concentration value in roots (336.70 mg kg^{-1}) was achieved with $ZnSO_4$ treatment. This high zinc concentration value was not significantly different from the other treatments receiving Zn. Also, zinc concentration values, which were accompanied with the other treatments, were not significantly different from each other.

Table (12): Effect of zinc forms on dry matter yield and zinc concentration in shoots and roots of corn plants grown in sand culture.

Treatment	Dry matter (g pot^{-1})		Zinc concentration (mg kg^{-1})	
	Shoots	Roots	Shoots	Roots
Control	5.43	2.83	73.00	23.30
Zn EDTA (17.7 % Zn) 5mg kg^{-1}	16.30	7.10	236.68	266.67
Zn $SO_4.7H_2O$ (22.6 % Zn) 20 mg kg^{-1}	16.47	7.20	243.33	336.70
Azol Zn_1 (5.27 % Zn) 5 mg kg^{-1}	16.00	8.13	760.00	233.33
Azol Zn_2 (5.27 % Zn) 10 mg kg^{-1}	17.30	8.40	760.00	250.00
Azol Zn_3 (5.27 % Zn) 20 mg kg^{-1}	16.13	7.70	853.33	273.33
L.S.D. 5%	3.34	2.61	163.82	217.58

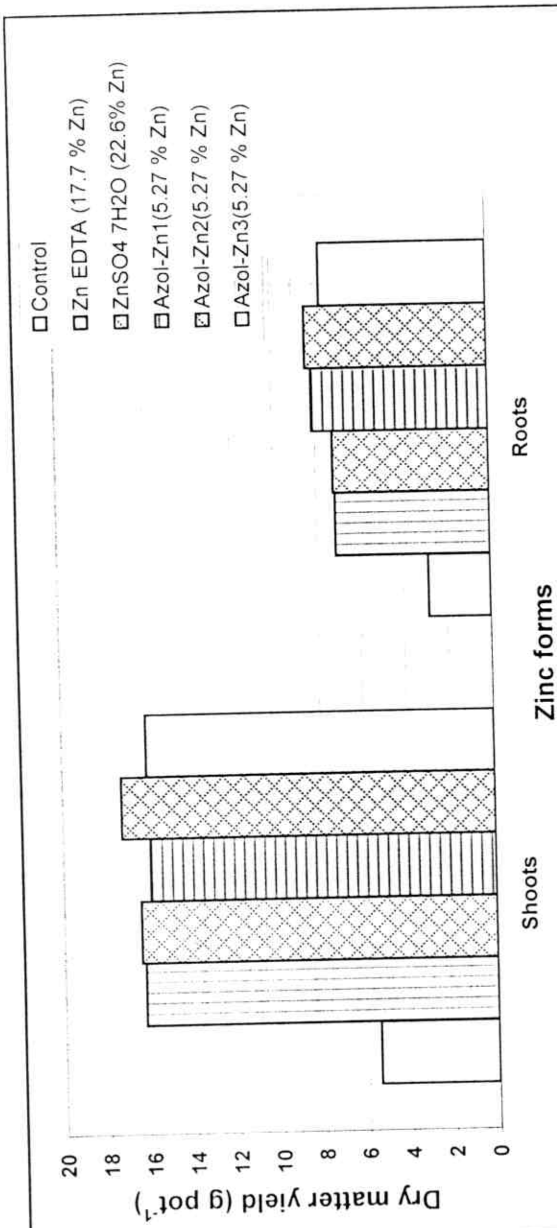

Fig. (31): Effect of zinc forms on dry matter yield of shoots and roots of maize plants grown in sand culture.

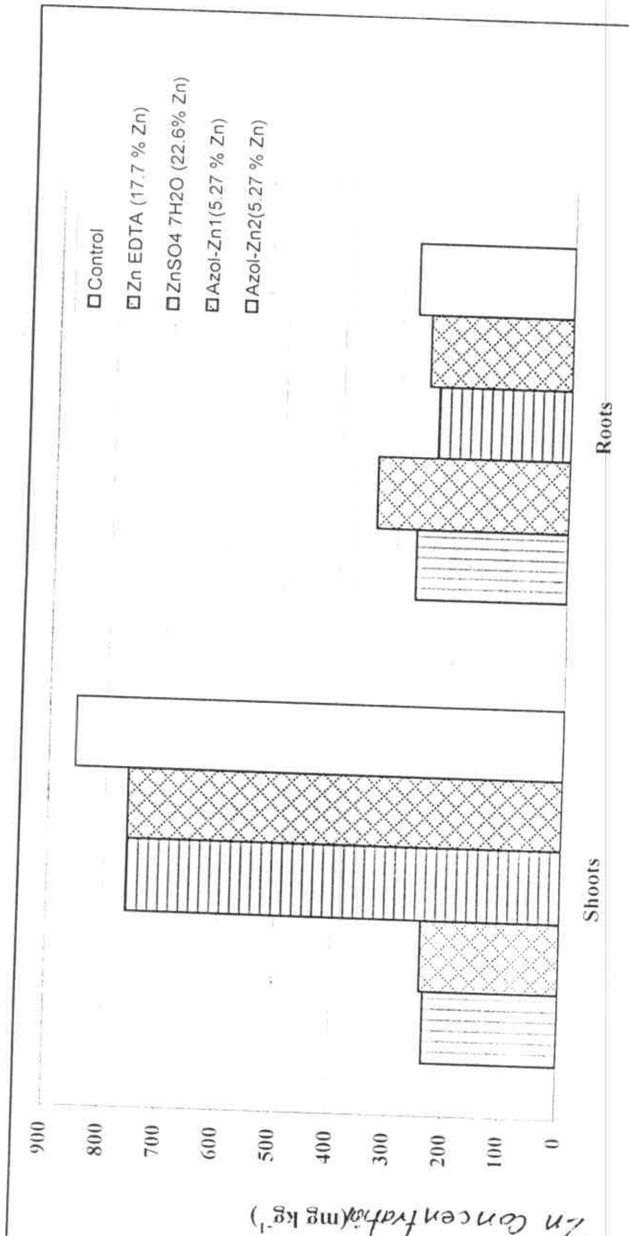

Fig. (32): Effect of zinc forms on zinc concentration in roots and shoots of corn plants grown in sand culture.

4.3.2.3. Zinc uptake:

Results presented in Table (13) and depicted in Fig. (34) revealed that all tested treatments receiving Zn significantly increased Zn-uptake by both shoots and roots over the control treatment. The highest shoots zinc uptake value of 13.63 mg pot^{-1} was achieved with Azol-Zn_3 treatment. This value was higher than and significantly different from those of 3.87 and 5.66 mg pot^{-1} due to Zn EDTA and Zn $SO_4.7H_2O$, respectively. However, this high value was slightly higher than but not significantly different from those achieved with Azol Zn-$_1$ (12.07 mg pot^{-1}) and Azol Zn-$_2$ (13.07 mg pot^{-1}) treatments.

4.3.3. Overall effect of Fe- or Zn- enriched *Azolla* on plant:

The results obtained indicated that *Azolla* when enriched with iron and / or zinc could be considered as being equivalent and effective source of iron or zinc to those of chelated sources. Few reports had discussed this phenomenon. **Heller (1989)** reported that dried *Azolla* enriched with 2 % Fe on dry weight basis was applied to peanut plants grown on a highly calcareous soil resulted in leaf chlorophyll content equal to or higher than those in plants treated with Fe-EDDHA. **Plessner (1998)** noticed that iron-starved plants exhibited fast regreening of the chlorotic interveinal tissues after the addition of Fe-Azolla complex to nutrient solution. They also postulated that iron starvation decreased the activity of catalase; iron-starved plants exhibited recovery of catalase activity upon applying Fe compared to the low level activity measured with untreated Fe-starved plants. The current study show that iron-enriched *Azolla* was equivalent to Fe-EDTA and Fe-EDDHA as a source of iron.

Table (13): Iron and zinc by corn plants (shoots and roots)

Treatment	Fe uptake (mg pot^{-1})		Treatment	Zn uptake (mg pot^{-1})	
	Shoots	Roots		Shoots	Roots
Control	2.15	2.31	Control	0.40	0.66
Fe EDTA (5 % Fe)	13.50	14.04	Zn EDTA (17.7 % Zn)	3.87	1.88
Fe EDDHA (6 % Fe)	17.37	18.37	Zn $SO_4.7H_2O$ (65 % Zn)	5.66	2.41
Fe SO_4 (56 % Fe)	8.64	8.26	Azol Zn_1 (5.27 % Zn)	12.07	1.90
Azol Fe_1 (8.6 % Fe)	20.44	8.55	Azol Zn_2 (5.27 % Zn)	13.07	2.15
Azol Fe_2 (8.6 % Fe)	12.44	16.36	Azol Zn_3 (5.27 % Zn)	13.63	1.90
Azol Fe_3 (8.6 % Fe)	15.16	11.42			
L.S.D. 5%	3.42	5.48		2.77	0.64

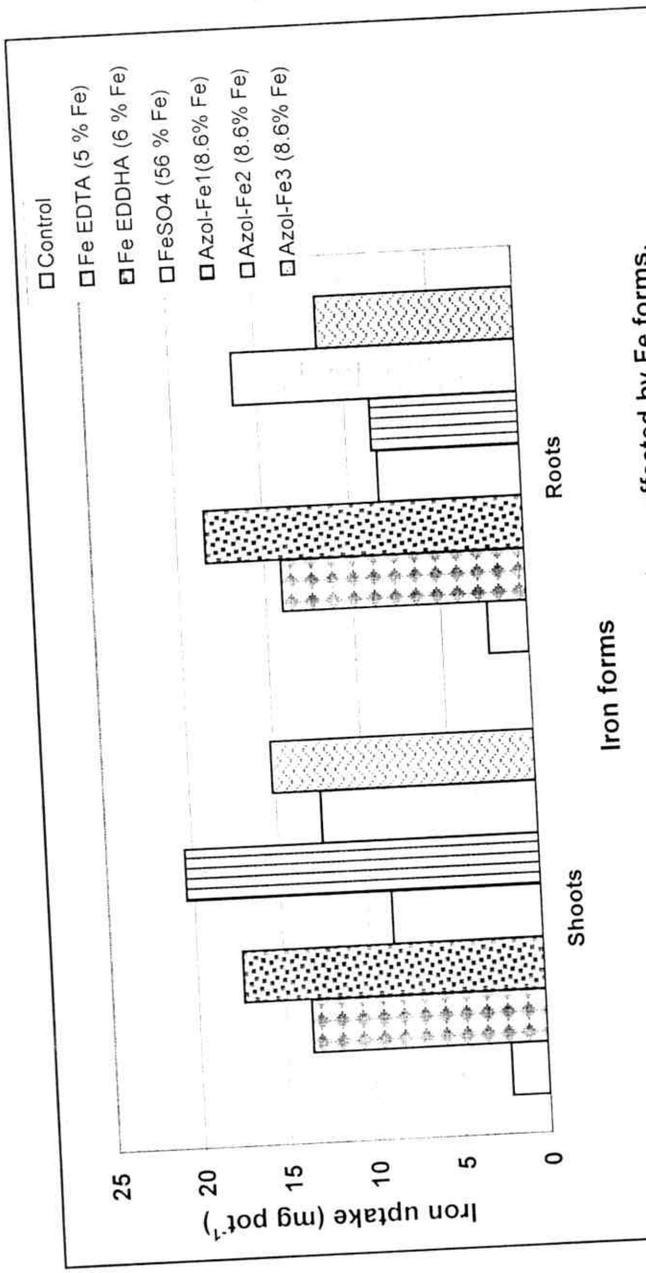

Fig. (33): Iron uptake by corn shoots and roots as affected by Fe forms.

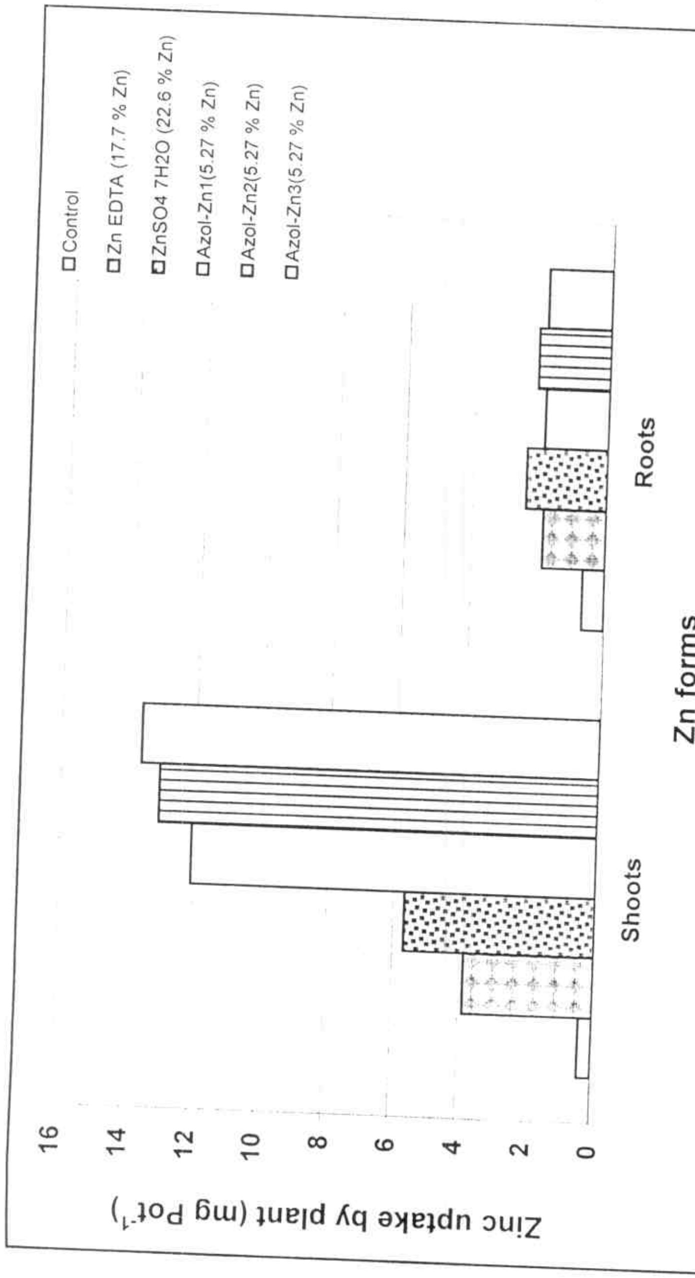

Fig. (34): Zinc uptake by corn shoots and roots as affected by Zn forms.