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PROPAGATION AND APPLICATION OF *AZOLLA PINNATA* AS AN ORGANIC SOURCE OF NITROGEN FOR WHEAT

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ABSTRACT

Azolla pinnata was grown in three different media (Yoshida, peat moss and soil media) under greenhouse conditions. One gram of *A. pinnata* was transferred into plastic pots and sampled occasionally every 5 days intervals up to 25 days. The growth, N_2–fixation and NPK contents of *A. pinnata* were compared during 25 days of incubation to select the most suitable *Azolla* growing medium. Results indicated that Yoshida medium induced the most positive significant effect on growth parameters (fresh, dry weights, doubling time and NPK content of *A. pinnata*) compared to peat moss or soil media. Moreover, nitrogenase activities reached its maximum values (13.00, 7.52 and 14.72 μ mole C_2H_4 g^{-1} dry $wt^{-1}hr^{-1}$) in Yoshida, peat moss and soil media after 20, 20 and 25 days of incubation, respectively.

In a pot experiment, effects of *A. pinnata* used either as fresh or dry form alone and/or in different combinations with urea on the productivity and yield components of wheat and some soil properties were also evaluated. *Azolla* and urea were gradually mixed together to accomplish the full nitrogen dose (75 kg fed^{-1}) required for wheat production. Results revealed that no significant effects were due to the use of both dry or fresh *Azolla* in single or combined treatment with urea on height, number of panicles $plant^{-1}$ and straw weight of wheat. The use of fresh *Azolla* achieved slightly higher values for these parameters than those recorded due to the use of dry *Azolla* and/or control treatment. Both 1000-grain and grain yield $plant^{-1}$ also responded positively to *Azolla* application especially in the fresh form. The use of *Azolla* in both forms led to a decrease in both soil pH and EC, but increased the soil organic matter content compared to control and/or single urea treatment and the effect was more pronounced in all treatments received fresh *Azolla*. Soil water holding capacity (WHC) was increased compared to control treatment due to dry rather than to fresh *Azolla* application. Fresh *Azolla* application increased the densities of total microbes, *azotobacters* and azospirilla as well as the amount of CO_2 evolved rather than dry *Azolla* and/or urea amendment.

INTRODUCTION

Azolla is known to contain the symbiotic nitrogen-fixing cyanbacteria, *Anabaena azolla*, within its leaf cavities. The cyanobionts furnish the N requirement of *Azolla* plant through the algal symbiont (Singh & Singh, 1990 and Wagner, 1997). The decomposition rate of *Azolla* in the soil

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depends on the C/N ratio, temperature and soil properties. **Ram *et al* (1994)** showed that addition of *Azolla* decreased pH, improved physical soil properties such as aggregation of soil particles, soil structure and permeability, leading to a better water holding capacity and less evapotranspiration. **Kannaian (1993)** reported that the application of *Azolla* increases the N, P contents of the soil. Soil application of *Azolla* increased crop yields in the same degree as application of mineral or organic nitrogen at the rate of 40 -100 kg N ha^{-1} **(Talley & Rains, 1980; Kolhe & Mittra, 1990 and El-Shahat, 1997)**.

Wheat is considered the main source of food in the worlds, especially in Egypt. Raising wheat production through increasing the productivity of land area unit and the cultivated area, represent the most important national target to minimize the gap between the Egyptians production and consumption **(Osman *et al* 2000)**.

The aim of this work is to select the most suitable medium for growing *Azolla pinnata* and its effect on *Azolla* biomass production, doubling time (DT), nitrogenase activity (N_2-ase) and NPK content. As well as to study the effect of two forms of *Azolla* (dry and fresh) inoculation on wheat crop productivity, some soil physico-chemical properties and densities and activity of soil microorganisms.

MATERIALS AND METHODS

Azolla

Azolla pinnata used in the present study was kindly supplied by Soils, Water and Environment Res. Inst (SWERI), Giza, Egypt.

Media used

Three types of media were used to grow *A. pinnata*, i.e., Yoshida medium **(Yoshida *et al* 1976)**. This medium contained the following chemical compositions in ppm: $NaH_2PO_4.2H_2O$ 40, K_2SO_4 40, $CaCl_2.H_2O$ 40, $MgSO_4.7H_2O$ 40, $MnCl_2.2H_2O$ 0.50, $NaMoO_3.2H_2O$ 0.15, H_3BO_3 0.01, $ZnSO_4.7H_2O$ 0.01, $CuSO_4.5H_2O$ 0.01 and Fe-EDTA 2. pH 5.5. Peat moss medium was composed of 20 g of peat plus 600 ml tap water. Peat moss was a product of international LTD Company. Switzerland. It contains (mg/100g) 220-250 K; 100-120 Ca; 80-100 P; 80-100 Mg and 0.8-1.0 N. Soil medium was prepared from soil collected from Kalubia Governorate. One hundred gram soil was added to each pot and covered with 600 mL tap water. Soil samples were analyzed for their chemical and physical properties **(Table1)** according to **Page *et al* (1982)**.

Wheat grains

Wheat grains (*Triticum aestivum* L.) variety Sids 1 were obtained from Wheat Res. Section, Field Crops Research Inst. ARC, Giza.

Experimental Techniques

Evaluation of *A. pinnata* growth in different media

Azolla was surface sterilized with mercuric chloride (0.1%) for 30 Sc. according to **Vandna and Ashwani (1998)**, washed with sterilized water several times and then air dried for 30 minutes. Plastic pots each with 14 cm in diameter and 7cm depth contained 600mL of Yoshida or peat moss or soil medium and sampled after 0, 5, 10, 15, 20 and 25 days and was inoculated with 1g of fresh *Azolla* as standard inoculum. Five replicates for each treatment were applied. The inoculated pots were kept under greenhouse conditions.

Table 1. Physical and chemical analyses of the experimental soil.

Mechanical analysis		Chemical analysis												
Coarse	13.90	Soluble cations (meq/100 g soil)				Soluble anions			W.H.C. (%)	$CaCO_3$ (%)	O.M (%)	E.C (dSm^{-1})	Total N (ppm)	Soil pH
Sand% Fine	9.10													
sand% Silt%	25.30													
Clay%	51.70													
Soil texture	Clay	Ca^{++}	Mg^{++}	Na^{++}	K^{+}	HCO_3^{-}	Cl^{-}	$SO_4^{=}$						
		0.14	0.03	0.07	0.77	0.12	0.08	0.81	55.00	1.95	0.20	0.20	0.11	7.80

Effects of *A. pinnata* applied form as an organic nitrogen source for wheat

A pot experiment was conducted at Soils, Water and Environment Research Institute, Agric. Res. Center, Giza, Egypt, under greenhouse condition to evaluate the effect of either fresh or dry *Azolla pinnata* application on growth and productivity of wheat plant. Pots (35 cm in diameter) were filled with 10 kg soil, amended with superphosphate (15.5% P_2O_5) at a rate of 100 kg fed^{-1} urea (46.5 % N) was applied immediately after seedlings thinning at different doses, i.e., the recommended dose (75 kg $Nfed^{-1}$), 15, 30 and 45 kg $Nfed^{-1}$. Soil was planted with 5 wheat seeds. Upon seedlings development, only 3 healthy wheat seedlings were left/pot. *Azolla* was incorporated into the soil as fresh or dry material mixed with soil before sowing to give the full recommended dose of nitrogen as organic, inorganic or a complementary substitute for nitrogen levels of 15 and 30kg $Nfed^{-1}$ giving the full recommended dose (75 kg $Nfed^{-1}$). Application rate of *Azolla* was calculated on the bases that it contains 4% nitrogen in dry weight reference. The characteristics of soil are presented in **Table (1)**.

Parameters measured

Fresh and dry weight of *Azolla*

Azolla fronds were harvested, washed with deionized water and placed under shade between two thick layers of blotting tissue papers for approximately 1-2 hours before determining fresh weight of *Azolla* in g pot^{-1} according to **EL-Shahat (1997)**. Fresh *Azolla* fronds were then oven dried to a constant weight at 70°C and expressed as g pot^{-1} **(EL-Shahat, 1997)**.

Doubling time

Doubling time (DT) of *Azolla* was calculated according to the equation of **Aziz and Watanabe, (1983)**.

Nitrogenase activity

N_2-ase of *Azolla* was assayed by the acetylene reduction technique as shown by **Hardy *et al* (1973)**.

NPK content

NPK contents (%) of *Azolla* fronds were determined in dried plant materials according to the methods of **Black *et al* (1965)**, **Olsen and Sommers (1982)** and **Brown and Lilliand (1946)**, respectively.

Sampling and determinations of wheat

Soil in pots was sampled at different intervals, i.e., at the time of experimentation and after 15, 30, 45, 60 and 120 days to determine the total microbial densities using plate count technique on Buntt and Rovira medium **(Buntt and Rovira, 1955)**. *Azotobacter* and *Azospirillum* populations using MPN technique on Ashby's modified medium **(Abd El-Malek and Ishac, 1968)** and semi solid malate medium **(Döberiner, 1978)**, respectively. CO_2 evolution was also estimated according to **Monib *et al* (1981)**. At harvest (150 days from sowing), wheat plants were harvested grains and straw yield pot^{-1}, plant height (cm) and 1000-grains weight, number of panicles $plant^{-1}$ were recorded. Total N contents (%) measured by microkjeldahl methods **(Jackson, 1973)** were also estimated.

Statistical analysis

The experimental results were subjected to statistical analysis according to **Gomez and Gomez (1984)**.

RRESULTS

Growth response of *A. pinnata* in different media

Data in **Table (2)** revealed that there was a positive significant relationship between *Azolla* growth and incubation period in all tested media. Hence, the fresh and dry weight of *A. pinnata* reached the maximum level in the three tested media after 25 days of incubation being 26.0, 25.9 and 24.98 g pot^{-1} for fresh weight and 1.8, 1.3 and 1.25 g pot^{-1} for dry weight in yoshida, peat moss and soil media, respectively. However, doubling times (DT) in those media were almost similar after 25 days of incubation, being 5.30, 5.30 and 5.38 days in the same above-mentioned respective order. In spite of recorded variation in early sampling period (5 days), the DT in Yoshida medium was 2.60 days after 5 days of incubation compared

Table 2. Effect of different growth media on fresh, dry weight and doubling time of *A. pinnata*

Parameters / Period (days)	Media								
	Yoshida			Peat moss			Soil		
	F.W.	D.W.	D.T.	F.W.	D.W.	D.T.	F.W.	D.W.	D.T.
0	1.00	0.05	0.00	1.00	0.05	0.00	1.00	0.05	0.00
5	3.70	0.15	2.60	2.90	0.14	3.20	2.42	0.12	3.90
10	6.80	0.70	3.60	4.92	0.25	4.30	3.62	0.21	4.38
15	14.00	1.30	3.90	10.98	0.55	4.30	8.12	0.41	4.96
20	22.00	1.70	4.50	19.62	0.98	4.70	17.79	0.90	4.80
25	26.00	1.80	5.30	25.90	1.30	5.30	24.98	1.25	5.38
L.S.D at 0.05	1.334	0.098	0.139	1.265	0.064	0.238	0.982	0.048	0.365

F.W. =Fresh weigh (g/pot).
D.W. =Dray weigh (g/pot).
D.T. =Doubling time of fresh weight (days).

with the other two tested media, where the corresponding DT were 3.20 and 3.90 days after the same incubation period. Form the above results Yoshida medium recorded the highest growth for *A. pinnata* compared to the other media tested.

Nitrogenase Activity

Acetylene reduction records presented in **Fig. (1)**, generally showed a gradual increase with increasing incubation periods. However, the highest values of acetylene reduction were observed after 20, 20 and 25 days of incubation for Yoshida, peat moss and soil media, being 13.00, 7.52 and 14.72 μ mole C_2H_4/g dry wt./hr, respectively.

NPK contents

Results in **Table (3)** showed a gradual increase in NPK contents of *A. pinnata* with increasing incubation period in all tested growth media. The maximum N content was recorded after 25 days of incubation, being 3.88, 3.10 and 3.51% for Yoshida, peat moss and soil media, respectively. The same trend was also observed for both P and K contents. *A. pinnata* grown in Yoshida medium also gave the highest percentages of P and K% after 25 days of incubation, followed by both peat moss and soil media being 0.83, 0.35, and 0.17 against 1.95, 1.00 and 0.35% for P and K content, respectively.

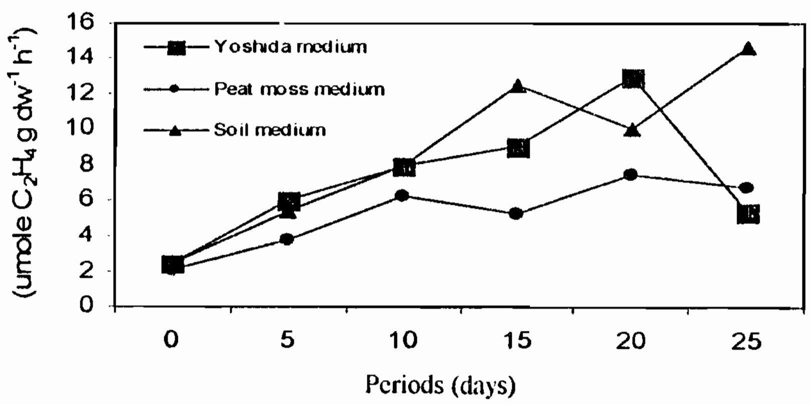

Fig. 1. Nitrogenase activity (N_2-ase) of *Azolla pinnata* grown on different media.

Table 3. NPK content (percentage) of *A. pinnata* as affected by different growth media

Parameter / Period (days)	Media								
	Yoshida			Peat moss			Soil		
	N	P	K	N	P	K	N	P	K
0	2.22	0.04	0.11	2.24	0.04	0.11	2.21	0.04	0.11
5	2.60	0.25	0.35	2.41	0.15	0.20	2.57	0.09	0.13
10	2.77	0.29	0.49	2.46	0.19	0.39	2.58	0.11	0.18
15	2.93	0.32	0.62	2.51	0.23	0.57	2.60	0.12	0.22
20	3.31	0.49	1.05	3.01	0.31	0.93	3.12	0.15	0.37
25	3.88	0.83	1.95	3.10	0.35	1.00	3.51	0.17	0.35
L.S.D. at 0.05	0.891	0.36	0.03	0.880	0.020	0.144	0.135	0.036	NS

Effect of *A. pinnata* applied form as an-organic nitrogen source for wheat

Densities and activities of soil microorganisms

Data in Tables (4 and 5) showed that densities of microbial populations and activities were increased up to 60 days after which both parameters tended to decrease. The highest microbial densities were recorded due to the use of 75 kg Nfed^{-1} as fresh *Azolla* compared to other tested treatments. After 60 days of incubation, total microorganisms reached 210 $\times 10^6$ cfu g. soil^{-1} while *Azotobacters* and *Azospirilla* reached 53.66 $\times 10^3$ and 150$\times 10^4$ cells/g dry soil respectively.

After 120 days of experimentation, densities records were decreased but with a superiority of the treatment received 75 kg Nfed^{-1}as fresh *Azolla*. The use of different rates of *Azolla* and urea increased the densities of the microbial populations at all tested intervals than those recorded by the use of dry *Azolla*. Rates of CO_2 evolution, gave similar trend to those achieved by microbial densities. Therefore, the highest CO_2 evolution rates were also recorded after 60 days of planting compared to the other tested intervals. The priority was also observed for application of 75 kg N fed^{-1} as fresh *Azolla* being 1550 mg CO_2 100g soil^{-1}. Corresponding highest CO_2 evolved rates were 360, 695, 925 and 793 mg CO_2 100 soil^{-1} after 15, 30, 45 and 120 days of planting. However, the use of different rates fresh *Azolla* mixed with different levels of urea was superior to those of dry *Azolla* at all tested intervals.

Growth and yield responses

Data in Table (6) revealed that both tested treatments did not significantly affect plant height, number of panicles plant^{-1} and straw weight of wheat. Higher values of plant height, i.e., 96 95 cm plant^{-1} were recorded due to the application of 75 kg Nfed^{-1} as urea and/or 75 kg Nfed^{-1}as fresh *Azolla*. Regarding No. of panicles plant^{-1}, the highest value was 14 panicles plant^{-1}due to the application of 75 kg N fed^{-1}as fresh *Azolla* but it was insignificantly different from the other tested treatments. Similar observation was noticed with straw weight plant^{-1}. All treatments received fresh *Azolla* either alone or conjugated with different levels of urea were significantly higher in grain yield and 1000-grain weight than those received corresponding treatments using dry *Azolla*. However, the highest grain weight plant^{-1} (45.2 g.) and weight of 1000-grain (35.89 g.) were obtained from the application of 75 kg N fed^{-1}as fresh *Azolla*. The total nitrogen per cent of both grains and straw followed the same pattern showed in other yield components as the highest N percentage were also recorded with the use of fresh *Azolla* particularly at the level of 75 kg Nfed^{-1}, being 1.83 and 0.89% for both grains and straw, respectively.

Effects on soil properties

Data in Table (7) show the effect of *Azolla* and urea either as single or mixed together in different rates on some soil properties after wheat harvesting. Results revealed that all tested treatments

Table 4. Densities of soil total microbes (cfu)[1] and rates of CO_2 (mg CO_2 100 g $soil^{-1}$) evolution as affected by *A. pinnata* applied form combined with different rates of urea nitrogen

Treatment		Incubation period (days)									
		15		30		45		60		120	
		T.C[2]	CO_2 evolved[2]	T.C	CO_2 evolved	T.C	CO_2 evolved	T.C	CO_2 evolved	T.C	CO_2 evolved
Control (initial soil)		12.00	90	23.00	130	20.50	400	41.16	510	10.00	70
Urea	*A. pinnata*										
(kg N/fed^{-1})											
75	-	16.3	182	41.7	200	32.0	600	50.7	790	14.0	120
-	75 dry	27.0	292	62.3	360	64.6	650	102.7	910	38.6	282
-	75 fresh	56.7	360	89.6	695	160.3	925	210.0	1550	90.6	793
15	60 dry	30.7	300	51.3	530	62.7	670	98.3	1100	60.0	505
15	60 fresh	38.0	309	58.7	570	69.7	695	119.0	1180	68.0	560
30	45 dry	39.7	301	61.0	590	104.6	726	134.0	1210	80.6	676
30	45 fresh	45.6	311	66.3	620	116.6	760	156.0	1250	86.0	680
45	30 dry	46.2	331	69.3	630	125.0	790	166.0	1290	90.5	690
45	30 fresh	51.0	340	76.3	661	140.0	830	175.0	1380	98.6	705

[1]cfu = colony forming unit ($\times 10^6$ g^{-1} soil)
[2] T.C = Total microbial count

Table 5. Densities of *Azotobacters* and *Azospirilla* as affected by *A. pinnata* applied form combined with different rates of urea.

Treatment		Incubation period (days)									
		15		30		45		60		120	
		Azoto. (x 10^3 cells g^{-1} soil)	*Azosp.* (x10^4 cells g^{-1} soil)	*Azoto.* (x10^3 cells g^{-1} soil)	*Azosp.* (x10^4 cells g^{-1} soil)	*Azoto.* (x10^3 cells g^{-1} soil)	*Azosp.* (x10^4 cells g^{-1} soil)	*Azoto.* (x10^3 cells g^{-1} soil)	*Azosp.* (x10^4 cells g^{-1} soil)	*Azoto.* (x10^3 cells g^{-1} soil)	*Azosp* (x10^4 cells g^{-1} soil)
Control (initial soil)		2.00	9.30	2.30	16.20	3.10	18.30	4.10	30.50	1.90	7.20
Urea	*A. pinnata*										
(Kg $Nfed^{-1}$)											
75	-	2.7	11.2	3.0	30.0	5.3	60.0	6.6	56.5	4.0	8.1
-	75 dry	5.3	20.0	9.3	46.0	16.0	129.0	18.3	71.3	12.0	23.0
-	75 fresh	7.3	28.7	13.0	66.3	50.3	190.4	53.7	150.0	40.6	120.0
15	60 dry	4.3	19.6	9.0	44.0	18.3	90.7	27.0	92.3	16.0	47.0
15	60 fresh	6.3	24.7	10.7	42.7	20.3	101.3	29.1	96.7	18.0	53.0
30	45 dry	4.7	20.0	7.7	41.7	22.1	116.2	30.9	102.6	20.0	64.0
30	45 fresh	6.7	23.3	8.3	47.0	25.3	125.6	36.6	112.6	23.0	83.5
45	30 dry	7.0	24.6	9.6	48.0	29.0	131.6	40.8	124.0	26.0	89.0
45	30 fresh	7.5	25.3	10.8	50.2	32.0	140.0	50.1	136.0	30.0	93.0

Table 6. Growth, yield and yield components of wheat plant as affected by *A. pinnata* applied form combined with different rates of urea nitrogen

Treatments Urea	*A. pinnata* (Kg N fed^{-1})	Plant height (cm/plant)	No. of pinnacles (plant^{-1})	Straw weight (g plant^{-1})	Grain weight (g plant^{-1})	1000-grain Weight (g)	Total nitrogen (%) Grain	Total nitrogen (%) Straw
75	-	96.0	13.0	50.8	34.0	33.3	1.40	0.40
-	75 dry	87.0	13.0	48.5	35.4	32.5	1.33	0.35
-	75 fresh	95.0	14.0	51.6	45.2	35.8	1.83	0.49
15	60 dry	92.0	12.0	47.1	35.9	31.5	1.46	0.35
15	60 fresh	90.0	13.0	50.2	43.1	32.0	1.56	0.47
30	45 dry	91.4	13.0	48.5	33.8	31.1	1.41	0.37
30	45 fresh	93.0	13.0	50.2	44.3	34.2	1.52	0.44
45	30 dry	92.0	13.0	49.0	36.2	33.0	1.32	0.40
45	30 fresh	93.0	13.0	50.1	43.1	34.2	1.42	0.43
L.S.D. at 0.05		NS	NS	NS	3.07	1.01	0.03	0.02

Table 7. Some soil chemical and physical properties as affected by *A. pinnata* applied form combined with different rates of urea nitrogen

Treatments Urea	*A. pinnata* (Kg Nfed^{-1})	pH	E.C	O.M	TN	WHC
Control (initial soil)		7.80	0.20	0.20	1.17	55
75	-	7.88	0.23	0.23	2.05	54
-	75 dry	7.74	0.22	0.78	2.33	66
-	75 fresh	7.20	0.15	1.90	2.59	61
15	60 dry	7.75	0.22	0.64	2.35	60
15	60 fresh	7.71	0.21	0.69	2.47	58
30	45 dry	7.69	0.19	0.85	2.15	63
30	45 fresh	7.68	0.18	1.00	2.28	60
45	30 dry	7.60	0.18	1.90	2.19	64
45	30 fresh	7.60	0.17	1.80	2.29	60

pH : The hydrogen ion concentration (soil water susp. (1:2.5)

E.C. : Electrical conductivity (dSm^{-1}) O.M. : Organic matter (%)

WHC : Water holding capacity (%) TN.: Total nitrogen (%)

reduced both soil pH and EC compared to the initial soil (control) expect for the addition of 75kg $Nfed^{-1}$ as urea. The least pH (7.20) and EC (0.16) were obtained due to the same amount of N as *Azolla*. On the other hand, increasing the rate of *Azolla* either as fresh or dry material conjugated with decreased urea level led to more decreases in both pH and EC with a more superiority to fresh *Azolla*. Due to soil organic matter content, results showed that the use of *Azolla* either dry or fresh increased soil organic matter compared to control or the application of 75 kg $Nfed^{-1}$ as urea. However, soil organic matter content of 1.90% was obtained due to the use of 75 kg $Nfed^{-1}$ as fresh *Azolla*. Mixing different rates of fresh *Azolla* with different levels of urea increased soil organic matter content than the same treatment comprising dry *Azolla*. Also, the highest total nitrogen content (2.59%) was obtained from the addition of 75 kg $Nfed^{-1}$ as fresh *Azolla*. However, inclusion of *Azolla* either as dry or fresh material at any rates with urea increased soil total nitrogen content over both the control (1.17%) and 75 kg $Nfed^{-1}$ as urea (2.05%). Again fresh *Azolla* appeared to be superior to dry *Azolla* at any rate mixed with urea. Generally, inclusion of *Azolla* either as dry or fresh material increased WHC over control by 55% and by 54% over 75 kg $Nfed^{-1}$as urea. On the contrary, dry *Azolla* application increased WHC by 66% compared to control.

DISCUSSION

The obtained results indicated that Yoshida medium significantly gave the highest growth parameters than both Peat moss and soil media. This may be due to the fact that Yoshida medium contains the essential nutrients needed for *Azolla* propagation. In fact, nearly similar results were obtained by **El-Araby *et al* (1999)** who showed that *A. pinnata* recorded its maximum growth and doubling time with increasing the incubation period up to 25 days. When *A. pinnata* was grown in Yoshida medium, its nitrogen content significantly increased with increasing incubation periods and gave the highest record of nitrogen percentage after 25 days of incubation compared with other media. The present results are also in agreement with **Nour EL-Din (1997)** who found that *Azolla* total nitrogen reached a 5.10% after 25 days of incubation. Recently **Mussa, (2005)**, showed that in Yoshida medium, *A. pinnata* contained the highest total nitrogen ($8.07g.N/m^2$) compared with both soil and Van Hove media after 30th days of incubation. On the other hand, **Sangeeta *et al* (2002)** found that, soil cultures were as good as the nutrient medium for *Azolla* propagation.

In this study *Azolla* propagated under optimal conditions was used as an organic nitrogen source for wheat production compared to urea alone or combined with different rates of either fresh or dry *Azolla* to accomplish the full nitrogen dose (75 kg N fed^{-1}) required for wheat crop production. In this concern, **Kolhe and Mittra (1990)** stated that fresh *Azolla* when applied in rotating rice- wheat cropping system was beneficial for wheat, since this system raised wheat grain yield by 56-69 % over control. **EL-Zeky *et al* (2005)** explained that fresh *Azolla* when incorporated into the soil is quickly mineralized and 75% of its nitrogen becomes available to the cultivated plants within one week. With application of urea alone, onset of nitrogen may be probably lost by leaching, volatilization or denitrification.

Fresh *Azolla* was superior in increasing the counts of *azotobacter*, azospirilla, total microorganisms, as well as the rates of CO_2 evolution. In this concern, **Mandal *et al* (1999)** reported significant increases in biomass and counts of soil microorganisms including *azotobacter* and azospirilla due to *Azolla* incorporation in rice fields. They attributed this behavior to the fact that successive *Azolla* cropping with rice plants increased soil fertility, which enhance growth and biomass of soil microorganisms. The increase in soil microorganisms increased the rate of microbial respiration and subsequently the amounts of evolved CO_2.

Azolla application leads to increase the panicles number/plant, 1000-grain weight and grain as well as straw yields (**EL-Zeky *et al* 2005**). The increase in nitrogen content of straw and grains were also attributed to nitrogen fixed by *Azolla* (30-60 kg N ha^{-1} in 30 days). Nevertheless, **Strik and Staden (2003)** attributed the beneficial effect of fresh *Azolla* to the presence of cytokinins and auxins that enhance plant growth. **Mussa *et al* (2002)** revealed that incorporation of fresh rather than dry *Azolla* suddenly increase the C/N ratio of the soil favoring microbial proliferation and subsequent immobilization of available nitrogen. The mineralization is then released significant amounts of nitrogen within 6-8 weeks because of the decay of added *Azolla*. Consequently *Azolla* released its nitrogen by gradual mineralization, which decreases the loss of nitrogen by leaching, volatilization or denitrification. Although, dry *Azolla* may act similarly as fresh *Azolla* this requires dry

Azolla to become water swelled and this may need more time (10-12 week). Incorporation of dry or fresh *Azolla*, generally, decreased both soil pH and EC. In this concern, Simposon *et al* (1994) noted that incorporation of dry and/or fresh *Azolla* into soil decreased the soil pH while urea raised the pH value. In this respect, fertilization with urea may stimulate algal growth and hence their photosynthetic activity. Therefore the dissolved CO_2 in the soil is reduced during the day time leading to a decrease in soil pH. The increase of soil organic matter in this study due to application of fresh *Azolla* was confirmed by the results of Herzalla *et al* (2002) who showed an increase of 27.6% in soil organic carbon due to *Azolla* applied in rice field. *Azolla*, upon its decomposition has also enhanced microbial proliferation in the soil which increased soil organic matter content (Abd-El Rasoul *et al* 2004). Moreover, *Azolla* mineralization led to the release of nitrogen into the soil and consequently increases in soil nitrogen content (EL-Zeky *et al* 2005). However, dry *Azolla* increased WHC more than fresh *Azolla*. This finding may be due to the ability of dry *Azolla* to absorb more water than fresh *Azolla* (Herzalla *et al* 2002).

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حوليات العلوم الزراعية
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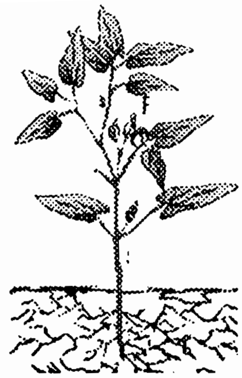

إكثار و استخدام الأزولا بيناتا كمصدر للنيتروجين العضوى لمحصول القمح

[٢٤]

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نميت الأزولا بيناتا علي ثلاث بيئات مختلفة هـي اليوشيدا، البيت موس والتربة وذلك تحـت ظـروف الصوبة فى أصص بلاستيكية بواقـع واحـد جرامـاً أزولا كلقاح فى كل بيئة ثم أخذ عينات بعـد فتـرات مختلفة من التحضين (صفر ، ٥، ١٠، ١٥، ٢٠، ٢٥ يوم) لتقدير وزن المادة الطازجة و الجافـة و معـدل تضاعف الأزولا ومحتواها من الأزوت والفوسـفور والبوتاسيوم ونشاط أنزيم النيتروجينيـز. وأظهـرت النتائج أن بيئة اليوشيدا أعطت أفضل النتائج من حيث الوزن الطازج والجاف ومعدل التضـاعف مقارنـة ببيئات البيت موس والتربة. و تـم الحصـول علـى أقصى معدلات تثبيت أزوت الهواء الجوى (اختـزال الاسيتيلين) عند استخدام بيئة اليوشيدا بعدَ ٢٠ يوم منْ التحضين كما ثبت أنّ هنـاك علاقـة إيجابيـة بـين محصول النمو و زمن التحضين.

ثم أُجريت تجربة أصص تحت ظروف الصوبة لتقيم تأثير الأزولا بيناتا طازجة أو جافة وبحالة منفردة أو مخلوطة بمعدلات مختلفة فى اليوريا للحصول على المعدل الموصى به من النيتروجين المعدني (٧٥ كجم نيتروجين للفدان) لمحصول القمح ومكوناته وكذا علي بعض خواص التربة والنشاط البيولوجي لها متمثلاً في أعداد الازوتوباكتر والأزوسبيريللم والعدد الكلي لميكربات التربة وكمية ثاني أكسيد الكربون المتصاعدة. وقد أظهرت النتائج أنه لا يوجد تأثير معنوي لاستخدام الأزولا مع المعدلات المختلفة لليوريا علي طول النبات ،عدد السنابل/ نبات ووزن القش ولكن تفوقت الأزولا الطازجة على الأزولا الجافة أو معاملة المقارنة بدرجة غير معنوية فى حين كان تأثيرها معنوياً علي وزن الـ ١٠٠٠ حبة ومحصول القمح بالمقارنة مع استخدام الأزولا الجافة ومعاملة الكنترول. ومن ناحية أخرى فقد أدّى استخدامُ الأزولا سواء طازجة أو جافة إلي انخفاض كل من درجتى الحموضة (pH) والملوحة (EC) للتربة وزيادة محتوى المادة العضوية والنيتروجين الكُلى للتربة مع وجود أفضلية لاستخدام الأزولا الطازجة بالمقارنة مع معاملتى الكنترول وإضافة ٧٥ كجم نيتروجين للفدان. وعلى العكس من ذلكَ فقد أدّى استخدام الأزولا الجافة إلى زيادة السعة المائية للتربة بالمقارنة مع استخدام الأزولا الطازجة. فى حين أدّى استخدام الأزولا الطازجة والجافة إلى زيادة لأعداد الكلية للميكروبات بالتربة وكذا أعداد كل مِن الأزوتوباكتر والأزوسبيرللم وكمية ثاني أكسيد الكربون المتصاعدة من التربة مع تفوق الأزولا الطازجة فى هذَا الصدد أيضاً.

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