IN VIVO EXPERIMENTAL STUDIES ON AZOLLA PINNATA R.Br.

Azolla pinnata R.Br. was cultured in plastic treys under in vivo systems, to find out the effects of modified medium which supported optimal biomass production, under in vitro conditions, would equally/better support biomass production of Azolla, under these experimental conditions.

The rates of ammonification of Azolla in its vegetative and sporulating stages of development were investigated. Ammonification of Azolla nitrogen started after the plants were incorporated in the flooded soil and they started to decompose. In addition, the various minerals of Azolla biomass also got incorporated into the soil. Therefore, mineral composition of A. pinnata was carried out. Application studies were conducted on incorporation of Azolla in soil in which rice (paddy) variety IR 28 had been grown.

SECTION A : Biomass production of Azolla

Experiment 14

Biomass production of Azolla in Watanabe and its modified medium under in vivo conditions

Two grams of Azolla plants from in vitro stock cultures were inoculated in one litre of Watanabe medium and modified

medium in plastic trays. Cultures were incubated in culture room at 25 ± 2°C in 1000 lux light intensity for 16/8 hours light/dark cycle. Weekly renewal of culture media was done and biomass production was calculated after three weeks experimental period.

Eight fold increase in biomass production was recorded in Azolla cultured in Watanabe medium while twelve fold increase in Azolla biomass occurred when grown in modified medium (Table 23). The surface of modified medium was completely covered by Azolla plants after three weeks culture period (Plate 13).

By culturing Azolla under in vivo conditions, large quantities of it was available at a time which could be used for further experimental work.

SECTION B : Mineralisation of Azolla

Experiment 15

Measurement of rates of ammonification of Azolla nitrogen

Azolla incorporated in flooded soil started decomposing during which its accumulated nitrogen in the form of ammonia was released. In the present experiment rates of ammonification of Azolla nitrogen during its vegetative and sporulating stages of development were measured, according to

Table 23 : Effect of Watanabe medium and modified medium on biomass production of *A. pinnata* under *in vivo* conditions

Inoculum : Fresh wt. = 2000 ± 70 mg
Dry wt. = 85 ± 10 mg

Weekly observations	Watanabe medium		Modified medium	
	Fresh wt. (mg)	Dry wt. (mg)	Fresh wt. (mg)	Dry wt. (mg)
1	4920±60	205±40	5870±70	247±30
2	9310±120	398±30	11980±90	509±60
3	18320±190	802±90	24600±700	1050±70

Mean of six replicates with S.D.

Plate 13 Biomass production of *in vivo* grown *A. pinnata* after three weeks

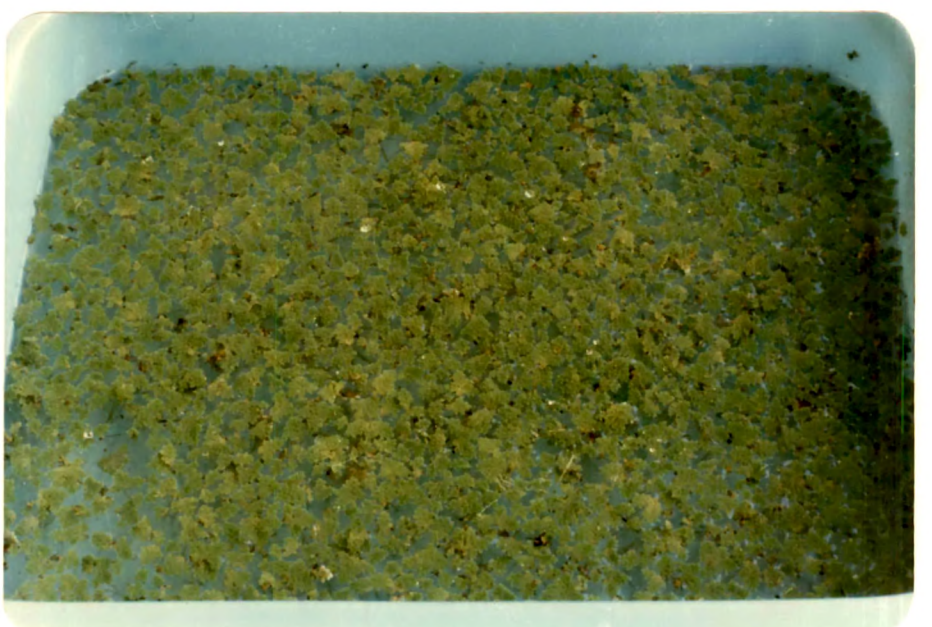

Plate 13

the method described in Chapter II, Materials and Methods (12).

Azolla (green) in vegetative stage or (pink) sporulating stage of its development started decomposing the first week, after being incorporated in flooded soil. The amount of ammonia released from vegetative and sporulating Azolla by the end of first week was almost same. At the end of fifth week of incorporation of Azolla in the soil, higher ammonification occurred from sporulating Azolla when compared with vegetative Azolla (Fig. 14). At the end of four weeks about 50% ammonification from vegetative Azolla was recorded. For about 80% ammonification from both the types of Azolla, eight weeks were needed indicating that Azolla to be a slow nitrogen releasing biofertilizer.

Experiment 16

Mineral composition of A. pinnata

In vivo grown Azolla plants were taken in a nickel crucible and heated in a muffle furnace (500 °C) and the ash was analysed for its constituents according to the procedure described in Chapter II, Materials and Methods (9).

The ash contents of Azolla was found to be 10% on dry weight basis. The ash contained potassium 3.31%, phosphorus 1.27%, magnesium 1.14%, calcium 1.08% and iron 0.34% as seen in Table 24. Presence of few other micro-

Fig. 14 Rates of ammonification of vegetative (green) and sporulating (pink) A. pinnata during a period of eight weeks.

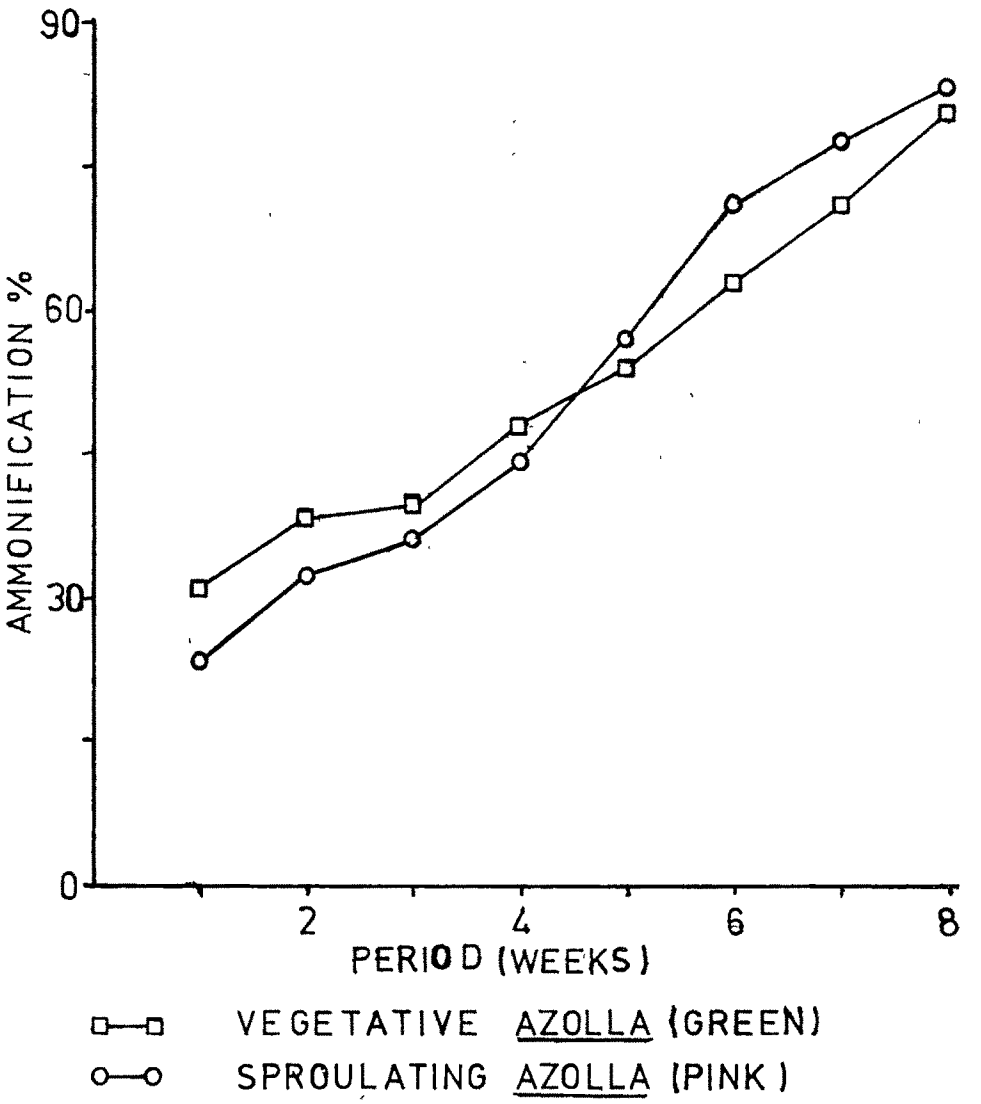
90
60
30
0
AMMONIFICATION %
2
4
6
8
PERIOD (WEEKS)
VEGETATIVE AZOLLA (GREEN)
SPROULATING AZOLLA (PINK)

Fig. 14

Table 24 : Mineral composition of *Azolla pinnata*

Constituents	Percentage on dry weight basis
Phosphorus	1.27
Potassium	3.31
Calcium	1.08
Magnesium	1.14
Sulphur	0.84
Sodium	0.67
Chloride	0.82
Iron	0.34
Silicon	0.13
Manganese	0.0322
Copper	0.0042
Zinc	0.0046

Mean of three replicates

elements viz. manganese, copper and zinc in small quantities was also recorded.

SECTION C : Application studies on *A. pinnata* as a biofertilizer

Experiment 17

Effect of *Azolla* and/or nitrogen fertilizer on the growth and yield of rice variety IR 28

Experiments were conducted in summer and kharif seasons (dates of transplantation 4-2-86 and 3-9-86 and harvest 25-5-86 and 7-12-86 respectively) in the year 1986, to study the effects of *Azolla* and/or inorganic fertilizer nitrogen incorporated in soil, on the growth and yield of rice variety IR 28. Twenty five days old seedlings of rice variety IR 28 were grown in soil incorporated with *Azolla*, inorganic fertilizer (ammonium sulphate), a mixture of *Azolla* with inorganic fertilizer (ammonium sulphate) and control receiving no treatment as described in Chapter II, Materials and Methods.

Results recorded on height of rice plants showed significant increase at 30 days, 60 days and 90 days of transplantation in summer and kharif seasons when grown in soil incorporated with only *Azolla*, only ammonium sulphate and *Azolla* combined with ammonium sulphate (Plate 14).

Plate 14 Growth of rice plants, variety IR 28 after 60 days (summer season) (Co = control, Az = *Azolla* alone, F = Ammonium sulphate alone and F + Az = Ammonium sulphate + *Azolla*)

Plate 14

Highest increase in height, biomass as well as nitrogen contents were recorded according to the sequence of fertilizers used as stated earlier (Tables 25 and 26).

Application of Azolla alone to the rice plants increased tiller numbers by 42% and 74% in rice grown in summer and kharif seasons respectively over the control values. In response to ammonium sulphate alone, tiller numbers increased by 79% and 98% over the control values in rice grown in summer and kharif seasons respectively, still further increase in these values was noted in response to application of Azolla combined with ammonium sulphate.

Results recorded at various time intervals, clearly showed that the increase in height, fresh weights, dry weights and nitrogen contents of rice plants were reflections of the availability of nitrogen fertilizers supplied in various forms.

The effect of incorporation of Azolla alone, or ammonium sulphate and both combined on the yield components and yield of rice plants recorded increase in these growth parameters (Table 27). An increase in panicle length by 18% and 23% were recorded over the control in Azolla application, 22% and 36% in ammonium sulphate application and Azolla combined with ammonium sulphate, the increase

Table 25 : Effect of Azolla and fertilizer nitrogen on plant height, biomass and nitrogen content of rice crop (Summer)

Treatments	Plant height (cm)	Fresh wt. of shoot (g)	Dry wt. of shoot (g)	Fresh wt. of root (g)	Dry wt. of root (g)	N content of shoot (mg/g dry wt.)	N content of root (mg/g dry wt.)
After 30 days							
Control	16.2	0.760	0.323	0.690	0.260	13.1	10.5
Azolla alone	20.0	1.483	0.693	1.560	0.576	18.4	13.0
Ammonium sulphate	21.7	2.096	1.030	2.643	0.976	21.1	18.8
Azolla + Ammonium sulphate	22.9	2.203	1.083	2.766	1.093	23.7	20.1
C.D. at 5%	1.1	0.28	0.12	0.36	0.17	0.7	1.1
After 60 days							
Control	25.8	3.136	1.506	3.550	1.313	24.6	20.9
Azolla alone	29.1	6.203	3.020	6.643	2.590	28.3	21.6
Ammonium sulphate	33.6	10.990	5.290	11.080	5.083	31.0	24.6
Azolla + Ammonium sulphate	35.1	12.510	6.096	13.400	6.113	32.3	26.2
C.D. at 5%	2.3	1.09	0.67	2.12	0.92	0.9	1.2

Table 26 : Effect of Azolla and fertilizer nitrogen on the plant height, biomass and nitrogen contents of rice (Kharif)

87

Treatments	Plant height (cm)	Fresh wt. of shoot (g)	Dry wt. of shoot (g)	Fresh wt. of root (g)	Dry wt. of root (g)	N content of shoot (mg/g dry wt.)	N content of root (mg/g dry wt.)
After 30 days							
Control	17.3	0.820	0.384	0.720	0.324	12.8	10.0
Azolla alone	21.7	1.198	0.569	2.070	0.940	17.9	12.0
Ammonium sulphate	22.1	2.270	1.090	2.715	1.195	20.9	16.2
Azolla + Ammonium sulphate	24.6	2.920	1.374	3.010	1.354	22.7	18.7
C.D. at 5%	1.6	0.38	0.38	0.82	0.08	1.6	1.7
After 60 days							
Control	31.1	3.258	1.466	3.125	1.406	23.9	21.2
Azolla alone	33.8	7.483	3.346	6.215	2.798	27.0	21.7
Ammonium sulphate	38.1	11.420	5.140	10.840	4.859	29.7	23.1
Azolla + Ammonium sulphate	41.2	13.680	6.255	12.720	5.724	31.7	24.2
C.D. at 5%	1.9	1.89	0.96	1.14	0.84	2.0	0.9

Table 27 : Effect of Azolla and fertilizer nitrogen on the yield components and yield of rice crop (Summer and Kharif seasons)

Treatments	Tiller (nos/hill)	Panicle length (cm)	Grain yield/hill (g)	Straw yield/hill (g)	100 grain weight (g)
Summer crop					
Control	10.6	13.8	4.350	11.762	1.099
Azolla alone	15.0	16.3	6.660	18.010	1.391
Ammonium sulphate	19.0	16.8	8.030	21.520	1.540
Azolla + Ammonium sulphate	22.0	17.3	9.790	36.433	1.736
C.D. at 5%	1.3	1.2	0.95	3.21	0.13
Kharif crop					
Control	8.6	13.3	6.050	9.450	1.107
Azolla alone	15.0	16.4	8.280	11.730	1.412
Ammonium sulphate	17.0	18.1	8.705	17.210	1.620
Azolla + Ammonium sulphate	19.0	19.2	13.530	24.115	1.758
C.D. at 5%	1.8	1.6	1.05	2.29	0.12

Mean of six replicates

was by 25% and 44% respectively in summer and kharif seasons (Fig. 15 and 16).

Incorporation of Azolla increased the yield of grain and straw by 53% in rice variety IR 28 grown in summer season, while that grown in kharif season the increase was 37% and 24% in grain and straw yield when compared with the control values. Application of the inorganic fertilizer, ammonium sulphate resulted in an increase of upto 85% and 83% in yield in grain and straw respectively in rice grown in summer season. Application of the ammonium sulphate could bring 44% and 82% increase in yield of grain and straw respectively when compared with control grown in kharif season. The application of Azolla combined with ammonium sulphate reached to its maximum increase in yield of grain and straw by 125% and 210% respectively in rice grown in summer when compared with the control values. The same treatment resulted in 124% and 155% increase in yield of grain and straw in rice plants grown during kharif season over the control values.

A positive correlation was observed in biomass production and nitrogen contents at thirty days (r=0.988), sixty days (r=0.680) and ninety days (r=0.991) in rice plants grown in summer season and treated with Azolla, ammonium sulphate, and Azolla combined with ammonium

Fig. 15 Effects of incorporation of

1 = Azolla alone,

2 = Ammonium sulphate alone and

3 = Azolla + ammonium sulphate

on tiller number, grain yield, ponicle length and straw yield of rice plants IR 28 variety (summer reason).

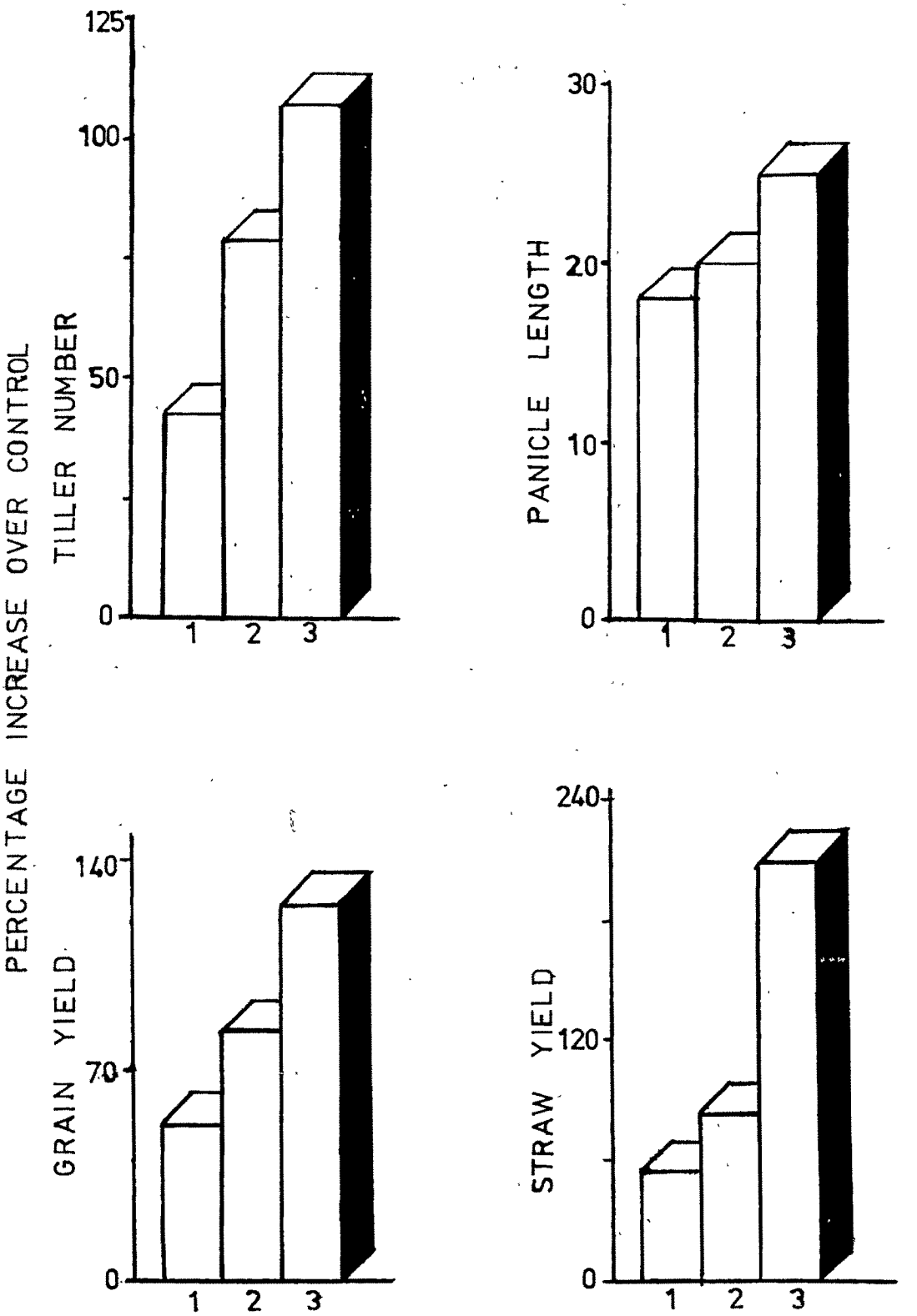
PERCENTAGE INCREASE OVER CONTROL
TILLER NUMBER
125
100
50
0
1
2
3
PANICLE LENGTH
30
20
10
0
1
2
3
GRAIN YIELD
140
70
0
1
2
3
STRAW YIELD
240
120
0
1
2
3

Fig. 15

Fig. 16 Effects of incorporation of

1 = Azolla alone,

2 = Ammonium sulphate alone, and

3 = Azolla + ammonium sulphate

on tiller number, grain yield, panicle length and straw yield of rice plants IR 28 variety (kharif season).

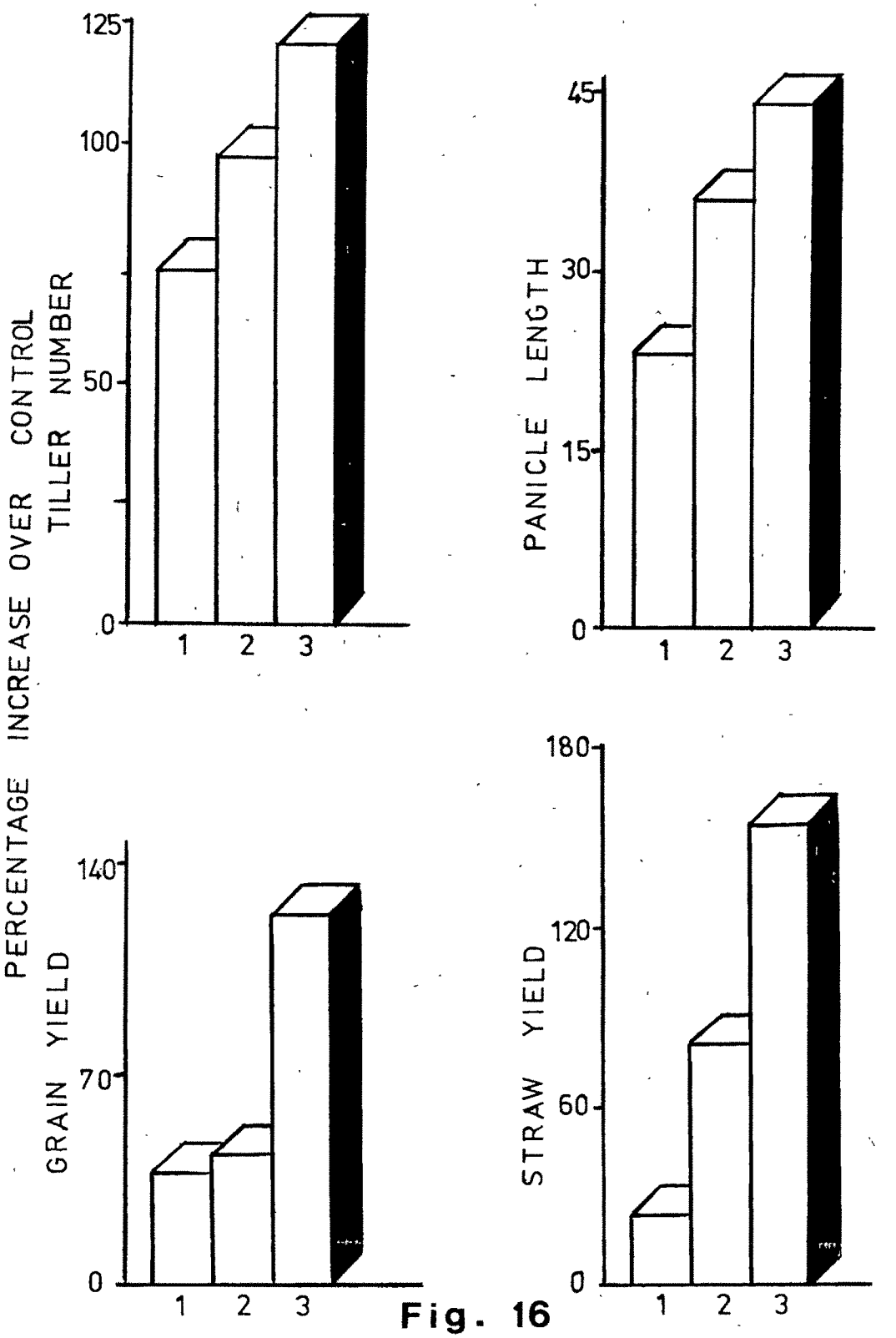
PERCENTAGE INCREASE OVER CONTROL
TILLER NUMBER
125
100
50
0
1 2 3
PANICLE LENGTH
45
30
15
0
1 2 3
GRAIN YIELD
140
70
0
1 2 3
STRAW YIELD
180
120
60
0
1 2 3

Fig. 16

sulphate. Similarly, a positive correlation existed in biomass production and nitrogen contents of rice grown in kharif season and treated with Azolla, ammonium sulphate, and Azolla combined with ammonium sulphate at thirty days (r=0.988), sixty days (r=0.986) and ninety days (r=0.889) after transplantation.

Thus it was evident that the nitrogen released from decomposing Azolla was sufficient to significantly increase rice yield of IR 28 variety.