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STUDIES ON THE ECOLOGY OF *AZOLLA PINNATA* R. BR. OF ORISSA[1]

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ABSTRACT

Ecological studies were initiated in September, 1981 to record the distribution and seasonal variation in the growth N_2-fixation, pigment status, P and K content of *Azolla* in a pond and a ditch of Jajpur locality of Orissa state and to collect information about its associates at different places of Orissa having varied pH and nutrient levels of water. The rise in biomass with respect to fresh and dry matter tissues was corroborated in the increase in N_2-fixation, P, K and Chlorophyll content and frond size of individuals and in the decrease in the anthocyanin content of the plant. *Azolla* grew well specially in ditches with slightly acidic pH and higher P concentration irrespective of seasons. *Azolla* disappeared during March to July. Inorganic nutrient status of water gradually increased by March and decreased by July.

INTRODUCTION

The water fern *Azolla* with its algal symbiont *Anabaena azollae* is accumulating increasing international attention for the promise it holds as an efficient biofertilizer for rice. Its capacity to manifest both photosynthesis and Nitrogen fixation in leaves makes it agronomically important. Modern agriculturists find in *Azolla-Anabaena* consortium a healthy alternative to the increasing cost and the widening gap between demand and supply of inorganic nitrogenous fertilizers in the developing countries. Researches carried out in India and other parts of the world have proved that the fern has a significant role in nitrogen economy of rice soils and can be exploited for increasing rice yield (Singh, 1977, 1979; Rains and Talley, 1979; Watanabe, 1977). The ecological survey attempts to bring true the feasibility of large scale *Azolla* culture in shaded ponds and ditches in order to provide a massive inoculum required for rice cultivation. Occurrence of *Azolla* in temporary ponds of Varanasi and several waterbodies of Orissa was reported by Gopal (1967) and Satapathy *et al.* (1982) respectively. Detail ecological studies of *Azolla* are, however, lacking especially as to its seasonal productivity in relation to the P, K, Ca and pH of surface water.

We initiated in the month of September, 1981 an ecological investigation and sampling programme in ponds and ditches of different places of Orissa to collect extensive physical, chemical

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and biological data (i) to document the seasonal variation in the *Azolla* productivity in both ponds and ditches and (2) to provide information about its distribution in the state of Orissa.

This paper presents the observations of our first year of study.

MATERIAL AND METHODS

Azolla is a small aquatic pteridophyte with alternate overlapping leaves on rhizome and simple roots hanging down into the water. Each leaf is bilobed, the upper lobe is chlorophyllous containing *Anabaena azollae* in its cavity while the lower thin lobe mostly remaining submerged in water is devoid of chlorophyll. Several temporary ponds, and roadside ditches of twenty eight different places of Cuttack and Puri district of the State were surveyed. The time of *Azolla* appearance, pH, and Po_4 content of the surface water and the occurrence of other free-floating macrovegetation associated with *Azolla* were recorded.

A pond and a ditch of Jajpur locality of Orissa were chosen for detail studies and these were under investigation for a period of one year (from September, 1981 to August, 1982). These are temporary water bodies situated in North-East part of Orissa 70 Km. away from the Bay of Bengal. *Azolla* growth, its chemical composition and the physico-chemical properties of surface water were analysed during the period, while the fern was present. Sampling was done in the end of every month as follows :

Azolla growth:

The biomass was determined by random sampling with the help of a metallic quadrate of $1 \times 1 m^2$. The average of 15 observations is presented. Dry matter yield was computed after drying the *Azolla* in the oven at 70°C for 24 hours. The plant size was estimated by plotting the outline of the frond on a mm graph paper. Measuring the length and breadth of the pond on the banks, the area of coverage was calculated and then the percentage of coverage was worked out in relation to the area of the pond.

Plant analyses:

Chlorophyll pigments were extracted with 80% cold acetone and its absorbance was measured at 652 nm using Bausch and Lomb spectronic-20 Spectrocolorimeter (Yoshida *et al.* 1971). Anthocyanin pigments were extracted with methanolic 0.01% hydrochloric acid and then pigment absorbance was measured at 500 nm (Ribereau-Gayon, 1959).

Total nitrogen was analysed by the modified Kjeldahl's method (Jackson, 1967). The dried plant materials were digested with triacids and the aliquots were used for the determination of phosphorus colorimetrically by sulphomolybdate blue colouration method and potassium directly with the help of flame spectrophotometer (Jackson, 1967).

Physico-chemical measurements of water:

Water samples were collected with a PVC Van Dorn Water sampler and were placed on ice for transport to the Laboratory. The samples were analysed within 10 hours of collection using procedures (APHA, 1965) as follows. The K and Ca content were determined directly by feeding the water samples to flame spectrophotometer. The orthophosphate analysis was made by Stannous chloride blue-colouration method. Ammoniacal-nitrogen was measured by direct nesslerization method (Onken and

Sunderman, 1977). Daily water temperature was recorded with a mercury thermometer. Hydrogen ion concentration was determined with the help of a portable pH meter. The data obtained from water bodies were statistically analysed for critical difference (C. D.) upto 1% level of significance.

RESULTS

The fern was commonly observed in temporary ponds and ditches along with other free-floating plants like *Lemna*, *Spirodela* and *Pistia*. In some water bodies, species of *Eichhornia*, *Salvinia*, *Utricularia*, *Ipomoea*, *Marsilea*, *Commelina*, *Limnophylla*, *Trapa*, *Nymphaea* and *Limnanthemum* were also found associated with *Azolla* in different combinations in different places. However, *Azolla* collections of twenty eight different sites of Cuttack and Puri district of Orissa were identified as the singular species *Azolla pinnata* R. Br. containing a heterocystous blue-green alga *Anchaena* species in the dorsal leaf cavity. *Azolla* appeared with the free floating vegetation during the end of July while a thick growth of *Azolla* was found in many temporary ponds of these two districts of Orissa by the end of October. However, the percentage coverage of the pond, frond-size and N, P content of *Azolla* varied with places of different water pH and phosphate concentration. It is evident from the result (Table I) that significantly higher

TABLE I

Azolla GROWTH AND PHYSICO-CHEMICAL PROPERTIES OF POND WATER AT DIFFERENT SITES OF CUTTACK DISTRICT IN THE MONTH OF OCTOBER, 1981

Sites	% of coverage	Average frond size in cm^2	N % of *Azolla on* dry wt. basis	P% of *Azolla* on dry wt. basis	pH of water	Phosphate (PO_4) content of water in ppm
Pratapnagari	80	1.2	4.5	0.35	6.8	4.1
Tulasipur	100	0.95	3.9	0.28	6.5	3.6
Kujanga	60	0.55	2.3	0.18	7.8	2.1
Jadupur	50	0.48	2.2	0.15	7.6	2.3
Bahugram	100	0.9	3.5	0.3	7.0	2.9
Salipur	90	0.85	3.8	0.3	6.9	3.2
Kendupatna	90	0.7	3.4	0.28	6.9	2.6
Chhatia	100	1.3	4.4	0.34	6.7	4.2
Rambag	100	1.4	4.8	0.4	6.4	4.5
Singhpur	100	0.8	3.9	0.32	6.9	3.6
C. D. at 5%	6.45	0.157	0.338	0.0021	0.278	0.251
1%	8.84	0.216	0.463	0.0028	0.381	0.344

coverage (100%), frond-size (1.4 cm²), N content (4.8%) and P content (0.4%) of *Azolla* were observed at Rambag with higher phosphate concentration of 4.5 ppm, and pH of 6.4 in the surface water. While significantly lower coverage of 60% and 50%, frond size of 0.55 cm² and 0.48 cm², N content of 2.3% and 2.2%, P content of 0.18% and 0.15% of *Azolla* were measured at Kujanga and Jadupur ponds with lower phosphate concentration of 2.1 and 2.3 ppm and higher pH of 7.8 and 7.6 respectively. It was also observed in other places that the growth and N, P content of *Azolla* were affected adversely with higher pH and lower phosphate concentration of surface water of the pond (Table I).

Azolla growth through Seasonal Cycles:

The periodical (monthly) observations of *Azolla* growth in a pond and a a road-side ditch revealed a significant effect of climatic parameters such as light and temperature on its growth, N, P, chlorophyll and anthocyanin contents. *Azolla* recorded significantly higher growth in terms of fresh matter during the month of November, 1981 attaining an average of 14,0 t. ha^{-1} and 18.0 t. ha^{-1} in the pond and ditch of Jajpur locality of Orissa respectively (Table II, Fig. 3). During this month

TABLE II

SEASONAL FLUCTUATION OF *Azolla* GROWTH AND N_2-FIXATION IN THE WATER BODIES OF JAJPUR LOCALITY OF ORISSA

Sampling time	Fresh matter t/ha		Dry matter Kg/ha		N_2 fixed Kg/ha		Chlorophyll mg/gm of fresh matter		Anthocyanin content in O. D.	
	Pond	Ditch	Pond	Ditch	Pond	Ditch	Pond	Ditch	Pond	Ditch
September	2.5	4.1	100	164	3.0	6.56	0.68	0.7	0.37	0.28
October	8.2	10.6	328	424	11.48	17.81	0.71	0.72	0.35	0.24
November	14.0	18.0	700	900	25.2	40.5	0.74	0.76	0.32	0.22
December	9.6	15.0	480	750	14.88	29.25	0.69	0.74	0.37	0.22
January	8.8	10.0	484	550	14.04	19.25	0.66	0.71	0.43	0.27
February	3.4	4.8	170	240	4.42	7.8	0.51	0.69	0.46	0.3
March	1.1	—	66	—	1.32	—	0.48	—	0.50	—
April	—	—	—	—	—	—	—	—	—	—
May	—	—	—	—	—	—	—	—	—	—
June	—	—	—	—	—	—	—	—	—	—
July	—	—	—	—	—	—	—	—	—	—
August	0.8	2.2	32	88	0.96	3.61	0.64	0.55	0.44	0.38
C. D. at 5%	0.158	0.122	16.14	25.29	0.18	0.20	0.09	0.07	0.11	0.03
1%	0.22	0.171	22.40	35.46	0.25	0.28	0.13	0.10	NS	0.04

average water temperature was noted as 19.6°C in the pond and 20.0°C in the ditch. The month of August experienced the minimum biomass production of 0.8 t. ha^{-1} and 2.2 t. ha^{-1} in both pond and ditch respectively. *Azolla* was virtually absent during April, May and June in both the waterbodies and also during March in the ditch due to desiccation and reappeared in the month of August. No appreciable *Azolla* growth was recorded during July. The results indicated a gradual increase in fresh matter from September to November and thereafter a sharp decline in growth upto March in the pond and upto February in the roadside ditch (Table II).

It is interesting to note that the dry matter, N_2-fixation and % coverage followed a similar trend as the fresh matter yield. The maximum dry matter, N_2-fixation, % coverage and frond size, of 0.7 t. ha^{-1} and 0.9 t. ha^{-1}; 25.2 Kg. ha^{-1} and 40.5 Kg. ha^{-1},; 100% each; 1.25 cm^2 and 1.35 cm^2 were recorded during the month of November in the pond and ditch respectively, when these measurments were found significantly lower during the months of July and August (Table II, III; Fig. 1).

TABLE III

EFFECT OF SEASONS ON THE FROND SIZE, NITROGEN, PHOSPHORUS AND POTASSIUM CONTENT OF *Azolla* IN THE WATER BODIES OF JAJPUR LOCALITY OF ORISSA

Sampling time	% of coverage		Frond size (cm^2)		Nitrogen % (on dry wt basis)		Phosphorus % (on dry wt. basis)		Potassium % (on dry wt. basis)	
	Pond	Ditch	Pond	Ditch	Pond	Ditch	Pond	Ditch	Pond	Ditch
September	80	100	0.8	1.0	3.0	4.0	0.26	0.35	2.15	2.2
October	100	100	1.25	1.3	3.5	4.2	0.3	0.38	2.3	2.4
November	100	100	1.25	1.35	3.6	4.5	0.35	0.41	2.28	2.4
December	95	90	0.9	1.1	3.1	3.9	0.28	0.31	1.95	2.0
January	80	85	0.85	0.95	2.9	3.5	0.26	0.28	1.7	1.85
February	60	50	0.9	0.95	2.6	3.25	0.2	0.26	1.68	1.76
March	30	—	0.75	—	2.0	—	0.15	—	1.6	—
April	—	—	—	—	—	—	—	—	—	—
May	—	—	—	—	—	—	—	—	—	—
June	—	—	—	—	—	—	—	—	—	—
July	15	25	0.35	0.3	3.1	3.9	0.28	0.34	2.0	2.1
August	30	40	0.68	0.75	3.0	4.1	0.28	0.36	2.1	2.3
C. D. at 5%	11.53	8.77	0.35	0.15	0.59	0.56	0.08	0.19	0.23	0.29
1%	15.89	12.17	0.49	0.21	0.81	0.78	0.11	0.27	0.32	0.40

The N, P and K percentage and chlorophyll content of the *Azolla* plant body also undertook, more or less, similar course as the biomass, however, it exhibited higher N, P, K and chlorophyll content during the month of July and August. Unlike other growth attributes, anthocyanin content followed a reverse trend. The minimum anthocyanin content of 0.32 and 0.22 relative O. D. were observed during the month of November in the pond and ditch respectively, whereas, its maximum value was recorded during the month of February and March (Table III, Fig. 2).

Summing up a comparison between the two waterbodies, the ditch showed higher growth, N_2-fixation, N, P and K percentage, % of coverage and chlorophyll content with lower anthocyanin content relative to the *Azolla* grown in pond. (Table II, III; Fig. 1, 2).

Monthly changes in the nutrient status and pH of water :

The water nutrient status varied from one month to another as evident from the phosphate, ammonium, potassium calcium content and pH of water at the end of every month. It was observed that the level of PO_4, Ca, K and NH_4^+ in surface water increased from the time of peak *Azolla* growth period (November) to its disappearance (i. e., March in pond and February in ditch). Again all these nutrient levels decreased from time of *Azolla* appearance to its peak growth period (Table IV, Fig. 3). However, pH of water did not obey any order but ranged from 6.5—7.3 and 6.5—6.8 in the pond and ditch respectively.

Between the two waterbodies, the ditch exhibited comparatively more nutrient levels and less pH than the

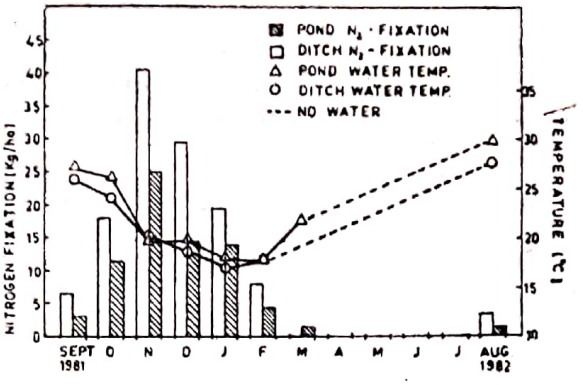

Fig. 1. Seasonal variation in *Azolla* nitrogen fixation in relation to temperature of the water-bodies of Jajpur locality.

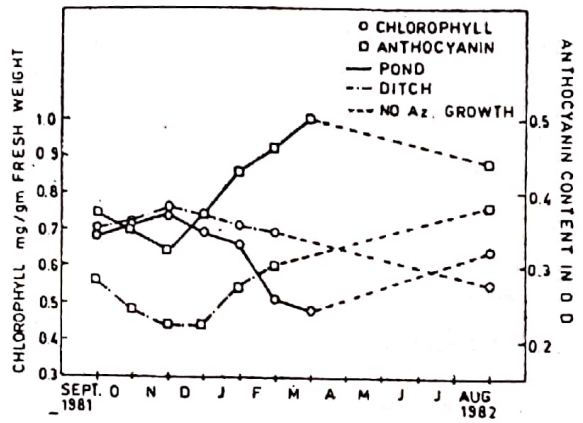

Fig. 2. Seasonal variation of *Azolla* pigment in the water-bodies of jajpur locality.

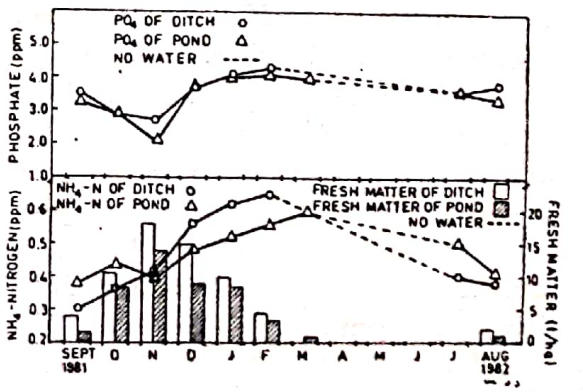

Fig. 3. Seasonal variation in *Azolla* fresh matter yield in relation to phosphate and ammoniacal nitrogen of the water-bodies of Jaipur locality.

TABLE IV

EFFECT OF SEASONS ON THE PHYSICO-CHEMICAL PROPERTIES OF A POND AND DITCH WATER OF JAIPUR LOCALITY OF ORISSA

Sampling time	Temperature in °C		pH		Phosphate (PO_4) in ppm		Ammonia (NH_4) in ppm		Potassium (K) in ppm.		Calcium (Ca) in ppm.	
	Pond	Ditch	Pond	Ditch	Pond	Ditch	Pond	Ditch	Pond	Ditch	Pond	Ditch
September	31.1	29.0	7.3	6.6	3.3	3.5	0.38	0.3	8	15	16	20
October	25.7	24.0	6.8	6.8	2.86	2.9	0.44	0.36	6.5	12	14	18
November	19.6	20.0	6.9	6.7	2.08	2.7	0.4	0.41	7	11	14	19
December	19.3	18.5	7.0	6.8	3.8	3.65	0.48	0.56	10	18	18	28
January	17.5	17.0	6.8	6.5	3.95	4.1	0.52	0.62	12	22	26	28
February	17.5	17.5	6.5	6.7	4.1	4.25	0.56	0.65	18	25	27	27
March	21.9	—	6.6	—	3.98	—	0.6	—	16	—	26	—
April	—	—	—	—	—	—	—	—	—	—	—	—
May	—	—	—	—	—	—	—	—	—	—	—	—
June	—	—	—	—	—	—	—	—	—	—	—	—
July	31.6	30.0	7.2	6.5	3.6	3.6	0.5	0.4	15	18	24	20
August	29.9	27.5	7.0	6.8	3.44	3.8	0.41	0.38	11	16	18	16
C. D at 5%					0.26	0.358	0.045	0.059	4.28	0.335	3.13	2.9
1%					0.36	0.497	0.069	0.081	5.9	0.465	4.32	4.02

pond. The PO_4 content of surface water ranged from 2.08 (November) to 4.1 (February) and 2.7 (November) to 4.25 (February) ppm in pond and ditch respectively. On the other hand, the NH_4+ level of surface water varied from 0.38 (September) to 0.6 (March) ppm and 0.3 (September) to 0.65 (February) ppm, K level from 7 (November) to 18 (February) ppm and 11 (November) to 25 (February) ppm, Ca level from 14 (Novembr) to 27 (February) ppm and 16 (August) to 28 (January) ppm in the pond and ditch respectively (TableI V).

DISCUSSION

Azolla requires P, Ca, Mg as macronutrients and trace elements like Mo, Fe and Co for its optimal growth in nitrogen free solution (Moore, 1969; Singh, 1979). Besides nutrients, the climatic factors also play an important role in the development of *Azolla* in natural condition.

The present study finds *Azolla* mostly in shallow temporary ponds and ditches, the growth being better in the latter. The absence of *Azolla* in per-

manent ponds might be due to the low availability of nutrients at surface water. Also the higher *Azolla* productivity in ditches than the temporary ponds might be due to its higher nutrient status. Hence it is the concentration and not the total amount of nutrients present in the water that decides the growth, N, P, K and Chlorophyll content of *Azolla*. Subudhi and Watanabe (1979) observed from their nutrient uptake studies that the concentration of phosphorus is the key nutrient that controls P uptake.

As evident from the present survey, *Lemna*, *Spirodela* and *Pista* species are three common aquatic free-floating weeds associated with *Azolla* in almost all the water bodies investigated. Neal (1965) also reported the associations of *Lemna* sp. with *Azolla* in the temporary ponds, ditches and rice fields. The appearance of *Azolla* at the end of July could be due to the germination of sporocarp that had remained dormant during March to June in the pond or ditch. The maximum growth of *Azolla* as observed in this study during the end of October and onset of November is in agreement with the findings of Gopal (1967). The observations on the *Azolla* growth in different places of varied pH and PO_4 concentration suggested that it grows well in the slightly acidic water bodies with higher PO_4 concentration. Singh (1977) reported that slightly acidic or neutral pH range was found most suitable for *Azolla* growth. Liu Chung Chu (1979) and Singh *et al.* (1982) considered that next to pH, the most decisive factor was the available P content of the soil, which directly influences the growth of *Azolla*. It was reported (Watanabe *et al.* 1980) that the P concentration of 0.3 to 1 ppm P_2O_5 in the flood water appeared to be necessary for meeting *Azolla's* requirement.

The productivity of *Azolla* was dependent on seasonal variation round the year. The maximum biomass, N_2-fixation, chlorophyll, P and K content of *Azolla* were observed during November when water temperature (19-20°C) was favourable. It was reported that 20-30°C was the optimum temperature for the better growth of *Azolla* (Singh, 1979, Satapathy and Singh, 1980). Wide variation in chlorophyll and anthocyanin content of *Azolla* was also noticed throughout the year and a correlation was observed between the quantity of chlorophyll and anthocyanin content with the biomass. Leith (1965) observed a direct correlation between the chlorophyll content and dry matter productivity. The increase in biomass and chlorophyll content during peak period (November) was associatedwith the decrease of anthocyanin pigment and the reverse was true for other months. More of anthocyanin pigments exhibiting a red brown colouration in *Azolla* plant body indicate a period of stress during the adverse conditions such as pH and temperature extremes (Lumpkin and Plucknett, 1980; Singh, 1979). The disappearance of *Azolla* during March to July from the temporary water bodies was due to dryness.

It is evident from the observations that there was a gradual increase in water nutrients level from the month of November to March. This increase in nutrient status of the water might be due to the gradual decomposition and mineralization of *Azolla* from the lower side of the thick mat in the water. The decrease in the water nutrients from September to November could be due to the vigorous multiplicaion as well as nutrient uptake of *Azolla* during these periods.

To sum, up, it is the ready availa-

bility of *Azolla* in the temporary ponds and ditches, its adaptability to wide pH range and temperature extremes, preference for low water depth and quicker mineralization of its body mineral nutrients in water are the plus points for its co-cultivation with rice in the medium and low land rice field for higher crop productivity.

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