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IMMOBILIZATION OF ANABAENA AZOLLAE IN SOLID MATRIX ON AMMONIA EXCRETION

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ABSTRACT

The algal symbiont of Azolla - Anabaena azollae (AS-DS) immobilized in polyurethane foam and hollow fibre recorded higher heterocyst frequency, nitrogenase activity, protein contents and ammonia excretion. Rice seedlings (ADT-36) were raised in acid washed and sterilized sand and inoculated with immobilized A. azollae increased the seedling growth.

Azolla is a free floating water fern which fixes atmospheric nitrogen in association with the N fixing cyanobacterium. **Azolla - Anabaena** symbiosis fixes considerable amounts of nitrogen in flooded rice field ecological condition and contributes 40-60 kg N/ha per rice crop (Kannaiyan, 1992a).

Azolla microphylla a fast growing and higher nitrogen fixing type with tolerance to higher temperature and salinity is used as biofertilizer for rice (Kannaiyan 1992a). The isolation and cultivation of **A. azollae** under free living condition and its nitrogen fixing activity has been reported (Kannaiyan 1991). **A. azollae** (AS-DS) is known to excrete ammonia under laboratory condition (Kannaiyan et. al. 1992). The present paper deals with the immobilization of the algal symbiont **A. azollae** in solid matrix and its inoculation effect on rice seedling growth.

MATERIALS AND METHODS

The solid matrices such as hollow fibre, cotton and silk cotton were cut into small bits of 1.0 cm except polyurethane foam (PU) which was cut into cubes of 1.0 cm. One gram of these materials were washed well and added

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into (250 ml) conical flasks containing 100 ml BG-11 media and sterilized. The flasks were inoculated with 2.0 ml of the actively growing **Anabaena azollae** (AS-DS) and incubated under green house conditions for 5 weeks period. The algal cells were extracted in phosphate buffer (pH 7.0) from the matrices, by grinding them in a pestle and mortar. The heterocyst frequency of **A. azollae** was calculated by taking ten independent counts of vegetative cells and heterocyst for each sample. Heterocyst frequency (H.F.) was calculated by using the following formula.

$$H.F. = \frac{\text{Number of heterocysts}}{\text{Total number of vegetative cells}} \times 100$$

The filterate was taken and used for the analysis of ammonia and the ammonia excreted was estimated by using Nessler's method (Spiller and Gunasekaran, 1991). The nitrogenase activity was determined from 1.0 g of solid matrix immobilized with **A. azollae** by following the method of Hardy et.al. (1969). The amino nitrogen and protein were determined by following the methods of spies (1955) and Lowry et. al. (1951). The rice seedlings (ADT-36) were raised in plastic tubs (area 630 cm^3) filled with acid washed and sterilized sand. The tubs were inoculated with solid matrix immobilized with **A. azollae** on 5th day after sowing. The inoculated tubs were maintained with 2.5 cm water column and sterile water was added intermittently to maintain the water level during the experimental period. The observations on root growth, shoot growth and plant dry weight were taken on 30th day after sowing.

RESULTS AND DISCUSSION

The **Anabaena azollae** (AS-DS) colonized and immobilized well in all the solid matrix. However, the immobilization of **A. azollae** was uniform and much better in polyurethane foam and hollow fibre. The heterocyst frequency and nitrogenase activity of the cells of

Table 1. **Heterocyst frequency, Nitrogenase activity and Ammonia excretion by Anabaena azollae (AS-DS) immobilized in solid matrices.**

Matrics	Heterocyst frequency (%)	Nitrogenase activity (nmoles of ethylene produced/h/g fresh weight)	Ammonia excretion (nmole/g)
Hollow fibre	9.0	20.7	85.71
Cotton	7.0	13.3	86.71
Silk Cotton	10.0	14.2	71.42
Polyurethane foam	7.8	17.7	77.14
Free-living	6.5	4.0	80.00
C.D.	1.77	2.05	7.54

A. azollae were higher under immobilized state than under free-living conditions (Table-1). Among the solid matrices, hollow fibre and polyurethane foam have supported higher heterocyst frequency and nitrogenase activity. The higher nitrogenase activity under immobilized state might possibly be due to the favourable conditions created in the matrix for A. azollae (AS-DS). These results are in agreement with the findings of Brouers et. al. (1988). Ammonia excretion was comparatively higher in hollow fibre and cotton immobilized with A. azollae (AS-DS) (Table 1). Continuous production of ammonia by PU foam immobilized with A. azollae was encountered in a bioreactor system (Kannaiyan et al. 1992). A. azollae (AS-DS) immobilized in different matrices have recorded higher aminonitrogen and protein contents when compared to free-living ones (Table 2). Aminonitrogen production was more with hollow fibre and polyurethane foam. A. azollae immobilized with hollow fibre and silk cotton, have supported higher protein contents. Increase in the contents of protein and aminonitrogen by immobilization of A. azollae could reflect on nitrogen contribution in natural rice soil ecosystem.

Table 2. Amino nitrogen and protein contents of Anabaena azollae (AS-DS) immobilized in solid matrices.

Matrix	Amino nitrogen (Mg/ml)	Protein (Mg/ml)
Hollow fibre	11.6	43.1
Cotton	8.4	27.0
Silk Cotton	2.3	37.6
Polyurethane foam	4.8	28.4
Free-living	1.2	17.3
C.D	1.62	2.83

Table 3. Root length, Shoot length and dry matter production by rice seedlings inoculated with Anabaena azollae (AS-DS) Immobilized in solid matrix.

Matrix	Root length (cm)	Shoot length(cm)	Dry matter (g/tub)
Hollow fibre	12.50	19.60	24.00
Cotton	12.30	18.40	21.50
Silk Cotton	12.40	20.50	23.50
Polyurethane foam	13.25	20.00	24.00
Free-living	12.00	18.00	20.00
Control	10.30	17.00	18.50
C.D	4.44	3.37	1.36

The rice seedlings inoculated with immobilized **A. azollae** (AS-DS) have shown active growth when compared to free-living culture. The growth of uninoculated rice seedlings was poor. The root, shoot and dry matter production by the rice seedlings were significantly higher when inoculated with immobilized **A. azollae** (Table 3). The induction of growth of rice seedings may be due to the continuous supply of ammonia by the immobilized **A.azollae** (AS-DS). The higher growth of rice seedling might possibly be due to the ammonia excretion by the immobilized **A. azollae.** The continuous absorption of ammonia by rice seedlings might have resulted in higher growth. Latorre **et al.**, (1986) have shown the better growth of transplanted rice plants due to the inoculation of a mutant strain of **Anabaena variabilis** and attributed that the higher growth of rice plant was due to ammonia excretion. The ammonia excreting property of **A.azollae** is a positive character and ammonia excretion may be further activated by immobilization. This has practical significance under rice field condition and this may be exploited for continuous ammonia production in rice field for higher productivity.

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PRODUCTIVITY OF TOXIC METABOLITES BY ISOLATES OF RHIZOCTONIA SOLANI

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ABSTRACT

Five different isolates of R.Solani collected from Coimbatore, Madurai, Aliyar, Ambasamudrum and Gobi in Tamilnadu were assessed for the production of toxic metabolites. The culture filtrate of each isolate was considered as a toxic sample and was assayed for toxicity by bioassay of seed germination and radicle length of toxin treated rice seeds. All the isolates were found to reduce significantly the germination percentage of rice seeds and also the radicle length in germination seeds as compared to control indicating the presence of some toxic metabolites produced by the pathogen.

Production of toxin by **R.Solani** has been reported by many workers. (Kohomoto **et.al.** 1973, Ramalingam 1981, Iacobellis and Devey; 1986). In the present study, the isolates collected from various places showed variation among themselves. They differed in their germination of sclerotia, growth in different media, colour of mycelia, branching pattern and growth habit of mycelium, the colour, size, pattern of distribution and number of sclerotia/per unit area. It was also found desirable to see whether there is any difference in the toxic metabolites produced by them and hence this study.

MATERIALS AND METHODS

In order to find out toxin production, if any, by the isolates of **R.Solani**, the various isolates from Madurai, Coimbatore, Aliyar, Ambasamudrum and Gobi in Tamilnadu were grown in Czapek's-dox liquid medium. One hundred ml. of czapek's-dox medium was taken in a

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