INNOVARE JOURNAL OF AGRICULTURAL SCIENCE

Vol 2, Issue 3 , 2014 ISSN: 2321-6832

Original Article

# USE OF *AZOLLA* BIOFERTILIZER IN POT CULTURE STUDIES WITH PADDY CROP *ORYZA SATIVA*

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*Received: 24 July 2014, Revised and Accepted: 14 August 2014*

**ABSTRACT**

The pure cultures of *Azolla* species were mass multiplied and used as potential biofertilizer to study the growth performance of paddy (BPT paddy variety) in pot culture. Pots were prepared with different compositions of Red soil, Vermicompost, NPK and *Azolla* cultures. Healthy seeds of BPT paddy variety were collected from Raj Agro Form, Dindigul and showed in pots with thirteen treatments and watered regularly. The various growth parameters such as percentage of Seed germination, Shoot length, Root length, No of leaflets, No of root branches, Wet and Dry weight of whole crops of different treatment were observed periodically for 21 d. The total chlorophyll and carotenoid contents were estimated in all treatments including control. The rapid growth rate of paddy crop was recorded between 15 to 21 d of pot cultures due to the influence of *Azolla* biofertilizers. This study will attract the farmers to use *Azolla* biofertilizers for paddy cultivation.

**Keywords:** *Azolla*, BPT Paddy, *Oryza sativa*, Biofertilizer. Nitrogen fixation

## INTRODUCTION

The quick multiplication rate and rapid decomposing capacity of *Azolla* has become an important factor to used as green manure cum biofertilizer in paddy field [1]. This free-floating freshwater fern, fixes atmospheric nitrogen through the symbiotic association with *Anabaena azollae* that lives inside the dorsal lobes of *Azolla* leaves, potentially supplying a substantial amount of $N_2$ to the paddy crop [2]. The genus of *Azolla* species is very sensitive to lack of water in aquatic ecosystems such as stagnant waters, ponds, ditches, canals or paddy fields. These areas may be seasonally covered by a mat of *Azolla* associated with other free-floating crops species such as *Lemna minor* [3]. Application of *Azolla* not only increases the growth and yield of paddy crops but also improves the soil fertility [4]. The mass cultivation of *Azolla* species is achieved in fresh water under strictly controlled conditions. The fresh water system is wildly available in populated countries like India; large scale production of *Azolla* is feasible in tropical conditions in developing countries, where land costs and labor are comparatively cheaper. The mass cultivation of *Azolla* in dairy effluent with various combinations of cow dung, whey water and tap water as low cost medium was reported [5]. Hence, utilization of waste water from dairy, industry also recommendable for mass cultivation of *Azolla*. This aquatic fern can be exploited as a potential source of bioferitilizer to increase the production of paddy variety [6]. Hence; Preliminary studies were carried out to evaluate the growth performance of paddy crop using *Azolla* biofertilizer.

## MATERIALS AND METHOD

### Mass multiplication of *Azolla* species

The large cement tub was filled with 2 Kg of fine red soil. Slurry was made by mixing one Kg of cow dung, 15 gm of super phosphate in 5 liters of water. Only live and healthy culture of half kg *Azolla* was inoculated in the prepared tub bed. The optimum temperature 27$^0$ C and pH 5.0 to 5.7 is maintained for mass multiplication [7].

### Population estimation of *Azolla* species

Pure cultures of *Azolla* species were procured from TNAU, Coimbatore. The Population of *Azolla* was estimated by direct count method. Such as 50 gms wet wt of the culture was taken and spread over the marked 1 sq. cm area of the clean grid petriplate. Then it was observed under 50 lux white light source. The number of individual *Azolla* were counted per grid field and the populations were estimated by using Conn's direct observation method [8].

### Pot culture studies with paddy crop using *Azolla* biofertilizer

Pots were prepared with thirteen different treatment using red soil, vermicompost NPK and *Azolla* cultures. Healthy seeds of BPT paddy variety were collected from Raj Agro Farm dindigul and sowed in pots and watered regularly. The various growth parameters such as percentage of seed germination, Shoot length, Root length, No of leaflets, No of branches, Wet and Dry weight of whole crops of different treatment were observed periodically for 21 d.

### Analysis of Seed Germination Percentage of paddy crop

The germination percentage of paddy seed in various treatments were calculated using the following Formula [9].

Germination (%) = Number of seeds germination / Total number of seeds X 100

### Analysis of Shoot Length and Root Length of the paddy crop

The shoot length of *Azolla* species treated paddy crops were measured in all 30 d treated crops. The average shoot length is expressed in centimeter for each treatment and in control. Simultaneously the length of root was measured from a fixed point below the surface of the soil to the end of the root [10].

### Analysis of Fresh and Dry Weight of the paddy Crop

The paddy crop from pots of each treatment were carefully uprooted on 15d & 21d, washed with water and weighted the fresh weight using weighing balance. Followed by the whole Crops were dried in hot air oven at 50$^o$C by gradually increasing the temperature up to 120$^o$ C for every two hrs for 24hrs. The dry weight of whole crop was weighed accurately and the results were expressed in grams [11].

### Estimation of Chlorophyll and Carotenoid Contents in Paddy Crop

The chlorophyll & carotenoid contents of paddy crop grown in various treatments were estimated by standard method (Arnon, 1949). One gram of leaf from paddy crop was homogenized in 20 ml acetone (80%) and centrifuged at 10,000 rpm for 5 min. The contents of total chlorophyll (T-Chl), chlorophyll a (Chl-a), chlorophyll b (Chl-b) and carotenoid in the supernatant were determined by spectrophotometrically at OD of 645, 662 and 470

nm. The chlorophyll and carotenoid contents were calculated using the following formulae [12]:

Chlorophyll a (mg/g fw) = 11.75*A662-2.35*A645

Chlorophyll b (mg/g fw) = 18.61*A645-3.96*A662

Total chlorophyll (mg/g fw) = Chlorophyll a + b

Carotenoids (µmole g fw) =1000 X A470 - 2.29*chlorophyll (a) – 81.4* chlorophyll (b) / 227.

**Statistical Analysis of Selected Parameters**

Statistical analysis of the data was carried out by using Prism Software (PV6). Mean standard deviation (SD) and percent variation was calculated. 'One Way Analysis of Variance' (ANOVA) was tested in order to check the statistical significance in parameters like Root and Shoot length, No of leaflets, No of root branches, Fresh and Dry wt of treated paddy crops. The data was analyzed for three different levels of significance based on the 'p' values

**RESULT AND DISCUSSION**

*Azolla* is a free floating aquatic fern, which can be widely found in freshwater environments in temperate and tropical regions all over the world. It ranks among the fastest growing crops on earth and due to its association with the nitrogen fixing Cyanobacteria *Anabaena azollae*, it is independent of external organic nitrogen. In that way *Azolla* species is not only known to fix huge amounts of carbon, but as well to produce vast amounts of organic nitrogen. The nutrient, which mainly limits the growth of *Azolla,* is phosphorous. *Azolla* comes under the family of *Salviniaceae* [13].In the present study the procured strains of *Azolla* were mass multiplied under controlled condition (FIG 1) without *Lemna* species contamination. The dead *Azolla* cultures were removed periodically in order to enhance the oxygen content for a time. The well grown *Azolla* cultures have been treated with the selected paddy cultivars for 21 days.

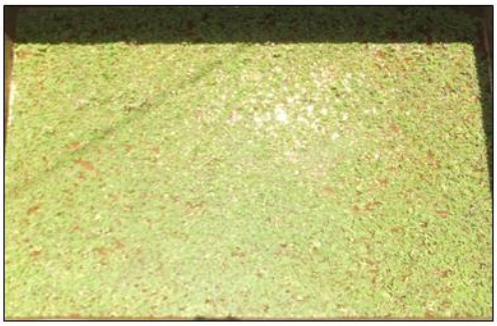

**Fig. 1: Mass multiplied *Azolla* Species**

Seed germination is the growth of an embryonic crop contained within a seed; it results in the formation of the seedling, in the present study the pots were prepared in different combinations of red soil, vermicompost, NPK and *Azolla*. The sowed paddy seeds germination percentage was observed with in 7 to 10 d under controlled conditions. Approximately 95% seeds are germinated well compared with control pot (TABLE 1). The growth of shoot length on 15d & 21d was observed as 8.9±1.05 & 16.83±0.351 cm in treatment 12, this drastic growth may be the influence of *Azolla.* The graphical comparison of shoot length on 15th and 21st d given in (FIG 2)

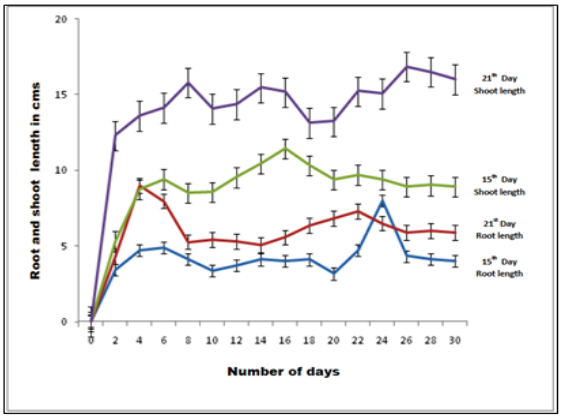


**Fig. 2: Root and shoot length of paddy cultivars grown in various treatments on 15d and 21d .**

Well development of leaflets in (Treatment 12) and root branches T12 were observed in the *Azolla* treated paddy crops during the same period of course. The more number of leaflets may enhance the rate of photosynthesis which helps to produce more number of carbohydrate productions in the form of reserved food materials in seeds due to presence of increased level of chlorophyll contents [14] The enhanced level of chlorophyll content in the treated crops was higher compared to control (FIG 2 and TABLE 4), which was observed in the treatment pots 1, 7 and 12 has high level of chlorophyll content. The mean value of total chlorophyll content was expressed in mg/g in TABLE: 4. the enormous development of roots in the crops plays a major role in absorption of minerals and available nutrients in the soil under controlled condition [15] (TABLE 1). Comparatively increased level of wet and dry weight was observed in 15th and 21st d of paddy cultivars of *Azolla* treated crops. (TABLE 3) This indirectly indicates the enhancement of Biomass level [16].The statistical analysis of selected parameter (TABLE 5) showed statistically significant result due to the impact of *Azolla* in the present study.

**Table 1: Percentage of seed Germination of paddy O*riza sativa* in various treatments on 7d**

| Treatment | Treatment Condition | No of seeds Germination | % of germination |
|---|---|---|---|
| T0 | Sand + Red sail (1:1) - Control | 17±1 | 85.00 |
| T1 | Sand + Red soil (1:1) + Recommended dose NPK (100%) | 14.66±1.52 | 73.33 |
| T2 | Sand + Red soil(1:1) + Vermicompost (100%) | 17±1 | 85.00 |
| T3 | Sand + Red soil (1:1) + *Azolla microphylla* (100%) | 18.33±0.57 | 91.67 |
| T4 | Sand + Red soil(1:1) + *Azolla filiculoids* $C_4$ hybrids (100%) | 17.66±0.57 | 88.33 |
| T5 | Sand + Red soil (1:1) + *Azolla microphylla (50%) + Azolla filiculoids* $C_4$ hybrids (*50%)* | 18.66±0.57 | 93.33 |
| T6 | Sand + Red soil (1:1) + | 17±1 | 85.00 |

| | | | |
|---|---|---|---|
| | *Azolla microphylla* (50%) + NPK (50%) | | |
| T7 | Sand + Red soil (1:1) + *Azolla filiculoides* $C_4$ hybrids (50%) + NPK (50%) | 17±1 | 85.00 |
| T8 | Sand + Red soil(1:1) + *Azolla filiculoids* $C_4$ hybrids (25%) + *Azolla microphylla* (25%) + NPK (50%) | 15.66±1.52 | 78.33 |
| T9 | Sand + Red soil(1:1) + *Azolla microphylla* (50%) + vermicompost (50%) | 18±1.73 | 88.33 |
| T10 | Sand+ Red soil(1:1) + *Azolla filiculoides* ($C_4$ hybrids (50%) + vermin compost (50%) | 19.33±0.57 | 96.67 |
| T11 | Sand+ Red soil (1:1) + *Azolla microphylla (25%) + Azolla filiculoids* $C_4$ hybrids (25%) + Vermicompost (50%) | 18.66±0.57 | 95.33 |
| T12 | Sand + Red soil (1:1) + *Azolla microphylla* (25%) *Azolla filiculoids* $C_4$ hybrids (25%) +vermicompost (25% ) + NPK (25% ) | 19±0 | 95.00 |

**Values are mean of triplicate ± standard error**

**Table 2: Number of leaf lets and Root branches in paddy crop on 15 d and 21 d**

| Treatment | Treatment Condition | Number of leaf lets | | Number of root branches (in cm) | |
|---|---|---|---|---|---|
| | | 15 d | 21 d | 15 d | 21 d |
| T0 | Sand + Red sail (1:1) - Control | 1 | 2 | 4.66±0.57 | 5.0 ±1.0 |
| T1 | Sand + Red soil (1:1) + Recommended dose NPK (100%) | 2 | 2 | 4.66 ±1.52 | 8.0 ±1.73 |
| T2 | Sand + Red soil(1:1) + Vermicompost (100%) | 2 | 2 | 4.0 ±1.73 | 6.33 ±1.52 |
| T3 | Sand + Red soil (1:1) + *Azolla microphylla* (100%) | 2 | 2 | 5.0 ±1.0 | 6.66 ±0.57 |
| T4 | Sand + Red soil(1:1) + *Azolla filiculoids* $C_4$ hybrids (100%) | 2 | 2 | 3.33 ±0.57 | 6.0 ±2.0 |
| T5 | Sand + Red soil (1:1) + *Azolla microphylla (50%) + Azolla filiculoids* $C_4$ hybrids (*50%)* | 3 | 4 | 5.0 ±1.0 | 6.0 ±1.0 |
| T6 | Sand + Red soil (1:1) + *Azolla microphylla* (50%) + NPK (50%) | 2 | 2 | 3.66 ±2.08 | 10.0 ±1.0 |
| T7 | Sand + Red soil (1:1) + *Azolla filiculoides* $C_4$ hybrids (50%) + NPK (50%) | 3 | 4 | 3.0 ±1.0 | 8.66 ±1.52 |
| T8 | Sand + Red soil(1:1) + *Azolla filiculoids* $C_4$ hybrids (25%) + *Azolla microphylla* (25%) + NPK (50%) | 2 | 3 | 4.33 ±1.15 | 7.66 ±1.52 |
| T9 | Sand + Red soil(1:1) + *Azolla microphylla* (50%) + vermicompost (50%) | 2 | 3 | 5.33 ±0.57 | 6.33 ±0.57 |
| T10 | Sand+ Red soil(1:1) + *Azolla filiculoides* ($C_4$ hybrids (50%) + vermin compost (50%) | 2 | 3 | 4.66 ±1.52 | 5.33 ±1.15 |
| T11 | Sand+ Red soil (1:1) + *Azolla microphylla (25%) + Azolla filiculoids* $C_4$ hybrids (25%) + Vermicompost (50%) | 2 | 3 | 4.66 ±1.52 | 6.66 ±0.57 |
| T12 | Sand + Red soil (1:1) + *Azolla microphylla* (25%) *Azolla filiculoids* $C_4$ hybrids (25%) +vermicompost (25% ) + NPK (25% ) | 2 | 3 | 3.66 ±2.08 | 10.0 ±1.0 |

**Value are mean of triplicate ± standard error**

**Table 3: Fresh weight and Dry weight of whole paddy crop on 15 d and 21 d**

| Treatment | Treatment Condition | Fresh weight in whole paddy crop(in mg) | | Dry weight in whole paddy crop(in mg) | |
|---|---|---|---|---|---|
| | | 15 d | 21 d | 15 d | 21 d |
| T0 | Sand + Red sail (1:1) - Control | 0.02 ± 0.01 | 0.022±0.04 | 0.008 ± 0.002 | 0.011±0.0017 |
| T1 | Sand + Red soil (1:1) + Recommended dose NPK (100%) | 0.02±0.04 | 0.043±0.001 | 0.0093 ±0.0032 | 0.027 ±0.002 |
| T2 | Sand + Red soil(1:1) + Vermicompost (100%) | 0.04±0.01 | 0.050±0.031 | 0.014 ±0.0025 | 0.029 ± 0.021 |
| T3 | Sand + Red soil (1:1) + *Azolla microphylla* (100%) | 0.03±0.008 | 0.044±0.012 | 0.028 ±0.005 | 0.038 ±0.044 |
| T4 | Sand + Red soil(1:1) + *Azolla filiculoids* $C_4$ hybrids (100%) | 0.033±0.006 | 0.038±0.003 | 0.008 ±0.0028 | 0.024 ±0.004 |
| T5 | Sand + Red soil (1:1) + *Azolla microphylla (50%) +* *Azolla filiculoids* $C_4$ hybrids (*50%)* | 0.033±0.005 | 0.041±0.0005 | 0.009 ±0.0046 | 0.082 ±0.010 |
| T6 | Sand + Red soil (1:1) + *Azolla microphylla* (50%) + NPK (50%) | 0.045±0.013 | 0.041±0.014 | 0.008 ±0.0026 | 0.023 ±0.005 |
| T7 | Sand + Red soil (1:1) + *Azolla filiculoides* $C_4$ hybrids (50%) + NPK (50%) | 0.03±0.02 | 0.045±0.0050 | 0.009 ±0.0020 | 0.027 ±0.005 |
| T8 | Sand + Red soil(1:1) + *Azolla filiculoids* $C_4$ hybrids (25%) + *Azolla microphylla* (25%) + NPK (50%) | 0.03±0.09 | 0.035±0.008 | 0.007 ±0.0045 | 0.017 ±0.009 |
| T9 | Sand + Red soil(1:1) + *Azolla microphylla* (50%) + vermicompost (50%) | 0.04±0.04 | 0.037±0.005 | 0.008 ±0.001 | 0.030 ±0.004 |
| T10 | Sand+ Red soil(1:1) + *Azolla filiculoides* ($C_4$ hybrids (50%) + vermin compost (50%) | 0.04±0.02 | 0.045±0.005 | 0.011 ±0.015 | 0.016 ±0.004 |
| T11 | Sand+ Red soil (1:1) + *Azolla microphylla (25%) +* *Azolla filiculoids* $C_4$ hybrids (25%) + Vermicompost (50%) | 0.03±0.01 | 0.040±0.001 | 0.008 ±0.0047 | 0.012 ±0.005 |
| T12 | Sand + Red soil (1:1) + *Azolla microphylla* (25%) *Azolla filiculoids* $C_4$ hybrids (25%) +vermicompost (25% ) + NPK (25% ) | 0.03±0.007 | 0.051±0.008 | 0.006 ±0.0015 | 0.01 ±0.007 |

**Value of mean triplicate ± standard error**

**Table 4: Chlorophyll and Carotenoid contents of paddy crop grown in various treatmentson 21d**

| Treatment | Treatment condition | Chlorophyll a (mg/g fw) | Chlorophyll b (mg/g fw) | Total Chlorophyll (mg/g fw) | Carotinoids (µ mole g /fw) |
|---|---|---|---|---|---|
| T0 | Sand + Red sail (1:1) – Control | 4.50±0.636 | 2.242±0.253 | 6.74 | 1.68±0.044 |
| T1 | Sand + Red soil (1:1) + Recommended dose NPK (100%) | 10.08±1.732 | 1.210±0.590 | 11.29 | 3.89±0.015 |
| T2 | Sand + Red soil(1:1) + Vermicompost (100%) | 5.06±0.999 | 2.820±1.04 | 7.88 | 1.46±0.04 |
| T3 | Sand + Red soil (1:1) + *Azolla microphylla* (100%) | 5.17±0.612 | 2.305±0.932 | 7.475 | 1.19±0.125 |
| T4 | Sand + Red soil(1:1) + *Azolla filiculoids* $C_4$ hybrids (100%) | 4.48±0.670 | 2.89±0.60 | 7.37 | 1.55±0.020 |
| T5 | Sand + Red soil (1:1) + *Azolla microphylla (50%) +* | 5.15±1.558 | 2.905±1.254 | 8.05 | 2.18±0.025 |

| | | | | | |
|---|---|---|---|---|---|
| | *Azolla filiculoids* $C_4$ hybrids (*50%)* Sand + Red soil (1:1) + | | | | |
| T6 | *Azolla microphylla* (50%) + NPK (50%) Sand + Red soil (1:1) + | 7.57±1.515 | 2.762±1.172 | 10.332 | 2.75±0.043 |
| T7 | *Azolla filiculoides* $C_4$ hybrids (50%) + NPK (50%) Sand + Red soil(1:1) + | 6.91±2.035 | 3.265±0.70 | 10.175 | 2.93±0.02 |
| T8 | *Azolla filiculoids* $C_4$ hybrids (25%) + *Azolla microphylla* (25%) + NPK (50%) Sand + Red soil(1:1) + | 8.34±0.57 | 2.19±1.28 | 10.53 | 2.946±0.037 |
| T9 | *Azolla microphylla* (50%) + vermicompost (50%) Sand+ Red soil(1:1) + | 7.55±0.023 | 2.976±0.96 | 10.526 | 2.56±0.01 |
| T10 | *Azolla filiculoides* ($C_4$ hybrids (50%) + vermin compost (50%) Sand+ Red soil (1:1) + | 8.63±0.23 | 1.218±0.245 | 9.848 | 3.106±0.032 |
| T11 | *Azolla microphylla (25%) + Azolla filiculoids* $C_4$ hybrids (25%) + Vermicompost (50%) Sand + Red soil (1:1) + | 7.15±0.23 | 3.601±0.93 | 10.751 | 2.033±0.028 |
| T12 | *Azolla microphylla* (25%) *Azolla filiculoids* $C_4$ hybrids (25%) +vermicompost (25% ) + NPK (25% ) | 8.32±0.576 | 3.042±0.894 | 11.362 | 2.166±0.015 |

**Value of mean triplicate ± standard error**

**Table 5: Statistical analysis on different growth parameters of paddy cultivars growth in various treatments**

| Parameters | 15th d treatment p value | Level of significance With mean | 21st d treatment p value | Level of significance With mean |
|---|---|---|---|---|
| Root Length in cm | 0.0269 | *4.573 ± 0.4818 | < 0.0001 | ****6.372 ± 0.3524 |
| Shoot Length in cm | < 0.0001 | ****9.517 ± 0.2485 | < 0.0001 | ****14.75 ± 0.3582 |
| No of Leaflets | < 0.0001 | ****2.552 ± 0.08535 | <0.0004 | ***2.083 ± 0.05921 |
| No of Root branches in cm | < 0.0001 | ****7.024 ± 0.4968 | < 0.0001 | ****7.303 ± 0.4524 |
| Fresh wt of whole crop in mg | < 0.0001 | ****0.0340 ± 0.002026 | < 0.0001 | ****0.0425 ± 0.001406 |
| Dry wt of whole crop in mg | 0.1653 | Not significant | 0.0051 | **0.02792 ± 0.005442 |

**Significant (p = 0.01 to 0.05), ** Very Significant (p = 0.001 to 0.01) and *** Extremely Significant (p < 0.001) **** strongly significant (p<0.0001)**

## CONCLUSION

In the present preliminarily study, the procured *Azolla* species were mass multiplied in controlled condition and the same was utilized for the growth of paddy crops for 30 d. During the course of studies for 30 d the fundamental parameters were investigated. It was observed that, *Azolla* species started to show their impact from the 15thd and was completely degraded in the prepared tub bed on the 21std. Logarithmic growth rate of paddy crop was observed between 15th to 21st d of treatment. Hence further supplementation is recommended to enhance the growth and yield of paddy crops for longer time till harvesting. The functional role and interaction between nitrogen fixation and utilization by paddy crop networks per square feet area namely measurement of *Azolla* response, grain yield, Crop Nitrogen uptake, excretion of $NH_3$ by *Azolla* species in free state conditions, rate of reproduction through sporulation, preparation of dried inoculum along with long shelf life of different *Azolla* species are yet to be analyzed well. An extensive research work has to be carried out to produce more quintals of paddy yield using organic fertilizers, which will help to increase the economic status of our rural parts of India.

## ACKNOWLEDGMENT

Authors are grateful to TNAU for providing the various cultures of *Azolla* species and Raj agro farms – Dindigul, for the supply of BPT paddy seeds.

## AUTHOR'S CONTRIBUTION

MPU, MK and SMG designed the experimental setup. MK, SMG carried out the implementation of various methods. MPU, AN interpreted the results and AN prepared the manuscript. All authors read and approved the manuscript.

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