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Influence of different organic matter on growth parameters and yield of *Azolla microphylla*

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Abstract
The present study intends to experimentally evaluate the response of different levels of organic matter on growth and yield of *Azolla microphylla.* The study was undertaken at Plant Pathology Section, College of Agriculture, Nagpur in cement tanks arranged in Completely Randomized Design (CRD) with 10 treatments in three replications for 60 days. The treatments encompassed vermicompost 1 kg, 1.5 kg and 2 kg/tank., compost 1 kg, 1.5 kg and 2 kg/tank and FYM 1 kg, 1.5 kg, 2 kg/ tank and uninoculated control. Growth and yield parameters *viz*., fresh weight, dry weight, chlorophyll content, root length, frond size, heterocyst per cent, N and P per cent at 15, 30, 45 and 60 DAI were evaluated. It was observed that fresh weight, dry weight, chlorophyll content, fond size, heterocyst per cent, N per cent and P per cent were significantly higher with the supplementation of compost at the rate 2 kg/tank followed by 1.5 kg/tank and 1 kg/tank of compost. On the contrary maximum root length was recorded in 2 kg/tank FYM treatment. Treatment T_{10} (Uninoculated control) gave the lowest result in all growth parameters indicating the need for nutrient supplementation. It is inferred from the results of this experiment that compost supplementation at the rate of 2 kg/tank would serve as a good system for the obtaining maximum growth and yield of *Azolla microphylla.*

Keywords: *Azolla microphylla*, organic matter, growth parameters

1. Introduction

Azolla is a genus of free floating aquatic pteridophyte belonging to the family Salviniaceae. This aquatic fern is native to Central and South America and Western North America and is widely established in Europe,
Morocco, southern Africa, Australia, New Zealand, Hawaii and Asia (Sadeghi *et al*., 2013) [17]. *Azolla* grows on floating water surfaces in the temperate and sub-tropical regions (Katole *et al.*, 2017) [9]. Under ideal conditions, it grows exponentially, doubling its biomass every 2-5 days (De *et al.*, 2016 and Kathirvelan *et al*., 2015) [3, 10]. The initial reports of using *Azolla* as a cheap source of nitrogen was realized by the Chinese farmers and there were several records on the use of *Azolla* as manure in the beginning of 17th century in China and Vietnam (Raja *et al.*, 2012) [16].

Tremendous interest on *Azolla* has been generated among the scientific community due to its symbiotic association with a diazotroph. The pteridophyte in its dorsal leaf cavity contains symbiotic atmospheric nitrogen (N_2) fixing microorganism, *Anabaena azollae*. Due to its symbiosis, the primary production of the plant is hardly ever N-limited under natural conditions (Temmink *et al.*, 2018) [19]. Using nitrogenase enzyme the diazotroph reduce atmospheric nitrogen (N_2) to ammonium (NH_4^+), which is excreted in the leaf cavity and taken up by the fern (Peters 1976) [14]. The cyanobiont supplies fixed nitrogen to the phytobiont and in return the phytobiont supplies photosynthates to the cyanobiont.

Preceding studies have evaluated the effect of different organic matters, nitrogen free medium and soil on biomass growth of *Azolla*. The repercussion of organic manures and inorganic fertilizers on biomass growth and protein content have been analyzed in prior investigations. Organic matter is crucial for the luxuriant growth and multiplication of *Azolla*. However, the effect of different levels of organic matter on growth and yield of *Azolla* is yet to be explored. Therefore, the present study was initiated with an objective to evaluate the effect of different levels of organic matter on growth and yield of *Azolla* in cement tank experiment.

2. Materials and Methods

The *Azolla microphylla* cultures were acquired from the Section of Plant Pathology, College of Agriculture, Nagpur. The culture contained blotted *Azolla* fronds freshly procured from mother culture. The obtained *Azolla* inoculum were grown in demineralized water for three days to prevent carryover of nutrients. Fresh weight and dry weight of the fern was used to record biomass growth observations. Vermicompost and FYM were procured from Animal Science and Diary Science Department, College of Agriculture, Nagpur. Compost was obtained from a local farm. The chemical composition of various organic matter is presented in Table 1.

Table 1: Chemical content of organic matters

Parameters	Vermicompost	Compost	FYM
pH	8.5	7.8	7.8
EC (mS/ppm)	0.311	0.326	0.322
N (per cent)	0.48	0.35	0.44
P (per cent)	0.38	0.27	0.41
K (per cent)	0.31	0.60	0.31

Growth of *Azolla* was assessed with different organic matters with different treatments *viz*., T_1- Vermicompost (1kg/tank), T_2- Vermicompost (1.5kg/tank), T_3- Vermicompost (2 kg/tank), T_4- Compost (1 kg/tank), T_5- Compost (1.5 kg/tank), T_6- Compost (2 kg/tank), T_7- FYM (1 kg/tank), T_8- FYM (1.5 kg/tank), T_9- FYM (2 kg/tank) and uninoculated control. The dimensions of the tanks were 66 x 44 x 34 cm^3. The capacity of tank was 98.5 litters approximately. Tap water was filled to $3/4^{th}$ capacity of tank *i.e.* 74 litters water per tank. Organic matters of required doses were added in each tank except for the control. *Azolla* inoculums grown in demineralized water for three days were inoculated at the rate of 50 g/tank. Observations were recorded at 15, 30, 45 and 60 DAI. The details of the treatments were the experiment was conducted in Completely Randomized Design (CRD) in three replications with 10 treatments during 2022-2023.

Azolla were blotted dry to record the fresh weight then the plants were dried at 60^0C in hot air oven to record the dry weight. Total chlorophyll content (mg g^{-1}) of the dried leaves was estimated by colorimetric method as suggested by Subudhi and Watanabe (1981) [18]. The nitrogen content in leaves was determined by Micro Kjeldhal's method given by Ali and Watanabe (1986) [1]. The phosphorus content in leaves was determined by Vanadomolybdate yellow colour method given by Jackson (1967) [8]. Heterocyst percentage was determined and calculated according to Konde and Kannaiyan by using the following formula.

$$\text{Heterocyst percentage} = \frac{\text{No. of heterocyst cell}}{\text{Total no. of vegetative cell}} \times 100$$

During the experiment the pH of medium was maintained at 6.5.

3. Results and Discussion

The observations divulge significant differences resulted by the application of different levels of organic matter on growth and yield of *Azolla* at all intervals throughout the experiment over uninoculated control. It was revealed from the data presented in Table 2 that the maximum fresh weight of 161.17 g/ tank at 15 DAI, 314.50 g/ tank at 30 DAI, 400.33 g/tank at 45 DAI and 511.17 g/tank at 60 DAI were recorded with Treatment T_6 and it was found significantly superior over all other treatments. These observations are in line with the reports of Andreelie *et al*. (2015) [2], Veerabhau (2015) [20] and Gatole (2019) [4]. Similarly, dry weight of *Azolla* was recorded at 15, 30, 45 and 60 DAI. The results indicated significant differences over uninoculated treatment in respect of dry weight of *Azolla* Significantly higher dry weight was obtained by Treatment T_6 (7.31, 14.57, 18.54 and 23.46 g/tank) at 15, 30, 45 and 60 DAI, respectively. The increase in dry weight of biomass may be due to more availability of nutrients required for the growth of *Azolla*. Comparable results of increase dry weight of *Azolla* were observed with the findings of Ghate (2013) [5], Gatole (2019) [4] and Potdukhe *et al*.(2020) [15]. With regard to chlorophyll content maximum chlorophyll content was recorded under Treatment T_6 recording (1.57, 1.77, 1.68 and1.62 mg/g at 15, 30, 45 and 60 DAI. This may be due to the increase in plant density which leads to dilution of chlorophyll pigments. However, minimum chlorophyll content was recorded in uninoculated control T10 recording 0.51, 0.45, 0.41 and 0.31 mg/g at 15, 30, 45 and 60 DAI respectively. It was evident from the table 1 that there was a gradual increase in chlorophyll content from 15 DAI to 45 DAI. Ismail and Mohamed (2010) [7] reported increase of photosynthetic pigments with increasing PO3-concentration. The increase in cholorphyll content in *Azolla* due to application of vermicompost, cowdung manure and compost have been reported by Potdukhe *et al*. (2020) [15]. Heterocyst percentage was recorded at 15, 30, 45 and 60 DAI and results indicated significant differences over uninoculated control. The data clearly indicates the treatment T_6 recorded highest heterocyst percentage as compared to all other treatments (22.61, 24.14, 24.87 and 25.45 per cent at 15, 30, 45 and 60 DAI) followed by treatment T_5. This may be due to availability of more soluble nutrients which was easily assimilated by the symbionts. Similar type of results was reported by the Mangaraj amd Maurya (1997) [13] and Maejima *et al.* (2003) [12] who reported seasonal variation in heterocyst percentage in application of nutrients. With respect to phosphorus and nitrogen content there were significant differences due to various treatments over uninoculated treatment (Table 3). Higher P and N content was found in Treatment T_6. The phosphorus content ranged from 0.27 per cent to 0.56 per cent at 15 DAI, 0.28 to 0.61 per cent at 30 DAI, 0.25 to 0.68 per cent at 45 DAI and 0.22 to 0.74 per cent at 60 DAI. These findings are in conformity with the reports of Potdukhe *et al.* (2020) [15] who reported higher P content in *Azolla*. Variation in availability of P content have been reported by Hamid *et al*. (2007) [6]. While nitrogen content ranged from 2.27 to 4.12 per cent at 15 DAI, 2.31 to 4.30 per cent at 30 DAI, 2.24 to 4.60 per cent at 45 DAI and 2.24 to 5.04 per cent at 60 DAI. Kolla *et al*. (2015) [11] reported potential of cynobacterial symbionts varied between 30-60kgN/ha. Gatole (2019) [4] found increase in N and P content due to application of organic matter. Frond size of *Azolla* was recorded at 15, 30, 45 and 60 DAI. The results indicated significant differences over uninoculated treatment. Maximum frond size of 1.67 cm^2 at 15 DAI, 1.90 cm^2 at 30 DAI, 2.17 cm^2 at 45 DAI and 2.27 cm^2 at 60 DAI was recorded under T6 treatment. The increase in frond size may be due to the high nitrogen content in the plant cell which promotes vegetative growth. Similarly, root length of

Azolla was recorded at 15, 30, 45 and 60 DAI. The results indicated significant differences over uninoculated treatment T_{10}. Significantly higher root length was obtained by T_6 treatment (1.91, 3.14, 3.17 and 3.23 cm at 15, 30, 45 and 60 DAI respectively) followed by T_5 treatment (1.82, 2.80, 2.94 and 3.17 cm at 15, 30, 45 and 60 DAI respectively). This might be due to high levels of phosphorus concentration in the organic matter. Thus, from the present experiment it is inferred that application of compost @2kg/tank amplified the fresh and dry weight of *Azolla*, chlorophyll content, heterocyst percentage, N and P content and frond size at various intervals. However, root length was enhanced by Treatment T_9 at various intervals.

Table 2: Effect of organic matter on various parameters of *Azolla microphylla*.

Treatments	Fresh weight (g/tank)				Dry weight (g/tank)				Chlorophyll content (mg/g)				Heterocyst per cent			
	15 DAI	30 DAI	45 DAI	60 DAI	15 DAI	30 DAI	45 DAI	60 DAI	15 DAI	30 DAI	45 DAI	60 DAI	15 DAI	30 DAI	45 DAI	60 DAI
T_1 Vermicompost (1 kg)	132.83	220.17	282.33	374.83	6.18	10.16	13.05	17.47	1.38	1.55	1.51	1.42	16.53	17.31	17.61	18.07
T_2Vermicompost (1.5 kg)	127.83	207.50	256.83	341.00	5.94	9.79	11.98	15.92	1.32	1.48	1.44	1.39	16.02	16.87	17.27	17.63
T_3 Vermicompost (2 kg)	108.00	177.33	245.67	325.33	5.02	8.34	11.34	15.17	1.29	1.39	1.36	1.31	13.01	13.70	14.25	14.54
T_4 Compost (1 kg)	149.67	287.50	367.83	427.83	6.95	13.38	17.21	21.96	1.50	1.70	1.62	1.57	18.08	19.99	20.22	20.83
T_5 Compost (1.5 kg)	150.00	301.00	382.00	491.67	6.98	14.00	17.80	22.91	1.52	1.72	1.65	1.58	19.26	21.27	21.59	22.03
T_6 Compost (2 kg)	161.17	314.50	400.33	511.17	7.31	14.57	18.54	23.46	1.57	1.77	1.68	1.62	22.61	24.14	24.87	25.45
T_7 FYM (1 kg)	136.50	227.83	325.50	430.67	6.40	9.36	14.94	19.88	1.41	1.59	1.54	1.48	16.76	17.52	17.91	18.33
T_8 FYM (1.5 kg)	125.33	201.00	274.33	381.33	5.84	10.56	12.83	17.63	1.34	1.49	1.46	1.40	16.14	16.96	17.36	17.81
T_9 FYM (2 kg)	112.83	184.50	254.50	331.50	5.36	8.58	11.58	15.47	1.30	1.41	1.37	1.33	13.37	13.75	14.38	14.78
T_{10} Uninoculated control	68.17	73.33	78.83	82.17	3.14	3.52	3.67	3.88	0.51	0.45	0.41	0.31	7.20	7.33	7.46	7.79
SE ± (m)	4.87	15.85	17.24	18.18	0.25	0.06	0.79	0.81	0.01	0.01	0.01	0.02	0.16	0.14	0.12	0.05
C.D. (P=0.01)	19.58	63.79	69.39	73.16	1.00	2.60	3.22	3.26	0.06	0.05	0.06	0.08	0.66	0.55	0.46	0.21

Table 3: Effect of organic matter on N and P contents, frond size and root length of *Azolla microphylla*.

Treatments	Nitrogen content (per cent)				Phosphorous content (per cent)				Frond size plant $^{-1}$ (cm)				Root length plant $^{-1}$ (cm)			
	15 DAI	30 DAI	45 DAI	60 DAI	15 DAI	30 DAI	45 DAI	60 DAI	15 DAI	30 DAI	45 DAI	60 DAI	15 DAI	30 DAI	45 DAI	60 DAI
T_1 Vermicompost (1 kg)	3.72	3.81	3.90	4.21	0.47	0.52	0.58	0.63	1.33	1.60	1.70	1.80	1.52	1.83	2.23	2.50
T_2 Vermicompost(1.5kg)	3.64	3.70	3.84	4.11	0.45	0.50	0.56	0.61	1.27	1.50	1.60	1.67	1.64	2.21	2.40	2.60
T_3 Vermicompost (2 kg)	3.44	3.51	3.60	3.91	0.43	0.47	0.54	0.58	1.20	1.40	1.50	1.57	1.70	2.47	2.50	2.63
T_4 Compost (1 kg)	3.94	4.02	4.44	4.80	0.51	0.56	0.62	0.70	1.43	1.77	1.87	2.00	1.43	1.70	2.10	2.23
T_5 Compost (1.5 kg)	4.01	4.22	4.53	4.86	0.54	0.58	0.64	0.72	1.50	1.80	1.93	2.07	1.50	1.82	2.17	2.47
T_6 Compost (2 kg)	4.12	4.30	4.60	5.04	0.56	0.61	0.68	0.74	1.67	1.90	2.17	2.27	1.61	1.96	2.37	2.57
T_7 FYM (1 kg)	3.79	3.88	4.07	4.28	0.48	0.53	0.59	0.65	1.37	1.67	1.77	1.83	1.74	2.51	2.81	3.00
T_8 FYM (1.5 kg)	3.68	3.74	3.86	4.15	0.46	0.51	0.57	0.62	1.30	1.53	1.67	1.70	1.82	2.80	2.94	3.17
T_9 FYM (2 kg)	3.46	3.54	3.63	4.08	0.44	0.49	0.55	0.59	1.27	1.47	1.57	1.60	1.91	3.14	3.17	3.23
T_{10} Uninoculated control	2.27	2.31	2.24	2.24	0.27	0.28	0.25	0.22	0.70	1.20	1.40	1.33	1.10	1.40	1.40	1.50
SE ± (m)	0.02	0.03	0.04	0.03	0.02	0.03	0.02	0.01	0.07	0.11	0.09	0.08	0.11	0.16	0.22	0.21
C.D. (P=0.01)	0.10	0.13	0.16	0.14	0.09	0.11	0.10	0.06	0.29	0.45	0.37	0.34	0.47	0.67	0.91	0.83

4. Conclusion

The present study concludes that the application of different levels of organic matter significantly influenced the growth, yield, and physiological parameters of Azolla. Among the treatments, T6 (compost @ 2 kg/tank) consistently recorded superior performance, yielding the highest fresh and dry biomass, chlorophyll content, heterocyst percentage, nitrogen, and phosphorus content, as well as frond size across all observation intervals. The enhanced growth attributes can be attributed to improved nutrient availability, leading to better nutrient assimilation and symbiotic efficiency. The findings are in agreement with previous research, demonstrating the potential of organic amendments in boosting Azolla production. Therefore, compost application at optimal levels can be recommended for sustainable Azolla cultivation and nutrient management

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