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UTILIZATION OF DIFFERENT ORGANIC MATTER IN THE PRODUCTION OF *AZOLLA MICROPHYLLA*

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ABSTRACT

***Azolla* is small aquatic fern which has symbiotic association with nitrogen fixing *Anabaena azollae*. An experiment was carried out in CRD with nine treatments in three replications during the year 2018-2019. In each pot two kg organic matter in 15 lit of water added. The observations such as fresh and dry weight, chlorophyll content and heterocyst percentage, phosphorus and nitrogen content and weather parameters in *Azolla* were recorded at 30,60,90 and 120 days after inoculation. The results shows that there were significant differences on the above parameters over uninoculated soil treatment. In the experiment in which, maximum fresh weight of *Azolla* was obtained in the treatment of T_5 (Compost) 318.84 g pot^{-1} at 30 DAI and 470.89 g pot^{-1} at 60 DAI. Similarly dry weight of *Azolla* was also recorded with T_5 recording 14.86 g pot^{-1} at 30 DAI and 22.60 g^{-1}pot at 60 DAI, and 23.46 and 23.23 g pot^{-1} at 90 and 120 DAI respectively. As regard chlorophyll content treatment T_5 (compost) registered 1.61 mg g^{-1} at 30 DAI and 1.76 mg g^{-1} at 60 DAI. Chlorophyll was decreased in 90 and 120 days. Heterocyst percentage was found to be increased as application of compost manure at all the intervals significantly. It was revealed from the data that the compost manure treatment proved to be best in increasing all the parameters followed by FYM treatment on growth of *Azolla*.**

(Key words: *Azolla microphylla*, organic matter, heterocyst, weather parameters)

INTRODUCTION

Azolla is a genus of aquatic ferns and small leafed floating plants, native to the tropics, subtropics, and warm temperature regions of Africa, Asia, and the Americas (Costa *et al.*,2009).The genus of *Azolla* is very sensitive to lack of water in aquatic ecosystems such as stagnant waters, ponds, ditches, canals or paddy fields.The earliest mention of the plant seems to be in ancient Chinese dictionary that appeared about 2000 years ago. *Azolla* was used in about the 11th century in Vietnam. By 1980 renewed interest in this symbiotic association was shown by the demand for a less fossil energy-dependent agriculture technology (Shi *et al.,* 1988). The application of *Azolla* has been reported to increase rice yield by 0.4-1.5 t ha^{-1} over the control, in most of the experimental sites in China, Vietnam, India, Thailand, Philippines and USA. (Kikuchi *et al*.,1984). The benefit of enriching soil organic matter status by incorporated *Azolla* biomass has also been reported by Singh and Singh,1995). The use of *Azolla* as a biofertilizer for irrigated rice cultivation has already been found successful in many countries of the world (Lumpkin and Plucknett,1987). The Nitrogen fixation potential of the *Azolla-anabaena* system has been estimated to be 1.1 kg N ha^{-1} day^{-1} and one crop of *Azolla* provided 20-40 kg N ha^{-1} to the rice crop in about 20-25 days (Watanabe *et al*., 1977).

MATERIALS AND METHODS

The inoculum consisted of blotted fern *Azolla* fronds 2freshly collected from mother culture maintained in tap water. Growth observations were recorded from fresh biomass and dry weight of the fronds.

Growth of *Azolla* in tank

Growth of Azolla was studied in different organic matter viz., vermicompost, cow dung, goat manure, sheep manure, compost, FYM, poultry manure, soil, and nutrient combination like P+K+Zn (0.6 mg cm-^{2}) in the cement tank of size (80 × 34 × 45 cm) and the tap water is used to carry out the experiment. Tap water was filled 3/4 capacity of pot 80 lit water tank^{-1}. Required quantities of organic matter was added as (15 liter water + 2 kg) organic matter or soil does is same like organic matter and required quantity of nutrients as per treatment details were applied and 0.6 mg P_2O_5 cm-^{2} through SSP, 0.6 mg K_2O cm-^{2} through potassium sulphate, and 0.6 mg Zn cm-^{2} through $ZnSo_4$ were added.

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Treatments details

T_1-Vermicompost, T_2 - Cow dung, T_3- Goat manure, T_4 - Sheep manure, T_5 – Compost, T_6 –FYM, T_7 -Poultry manure, T_8 - Soil, and T_9- P + K +Zn. The experiment was conducted in Completely Randomized Design with nine treatments in three replications. *Azolla* were blotted dry to record the fresh weight then the plants were dried at 60^0C 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). The nitrogen content in leaves was determined by Microkjeldhal's method given by Ali and Watanabe (1986). The phosphorus content on leaves was estimated by Vandmolybdate method as suggested by Jackson (1967).

Heterocyst percentage was calculated by using the following formula.

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

RESULTS AND DISCUSSION

Effect of organic matter on growth of *Azolla*

The data pertating to the influence of different organic matter on fresh weight of *Azolla* at 30, 60, 90 and 120 DAI are presented in table 1. The results presented in table 1 indicated that, all the treatments were found significantly superior over application of soil treatment. Maximum fresh weight of *Azolla* was obtained with T_5 treatment (Compost) i.e. (318.84 g pot^{-1} at 30 DAI , 470.89 g pot^{-1} at 60 DAI, 488.85 g pot^{-1} at 90 DAI and 528.07 g pot^{-1} at 120 DAI (respectively) which was followed by treatment T_6 (FYM) i.e. (227.51 g^{-1} pot at 30 DAI, 431.09 g pot^{-1} at 60 DAI, 459.86 g^{-1} pot at 90 DAI and 477.08 g pot^{-1} at 120 DAI). Treatment T_5 found significantly superior over all other treatments except T_6.The fresh weight was found to be decreasing both in all days i.e. 58.38 and 179.66 g pot^{-1} at 30 DAI and 60 DAI and 333.53 and 393.16 g pot^{-1} at 90 DAI and 120 DAI respectively.

It is evident from the table 1 that there were significant differences on fresh weight of *Azolla* over uninoculated soil treatment at 30 DAI. Treatment T_2 (Compost) produced the highest fresh weight (220.03 g pot^{-1}) as compared to individual application of organic matter. Further increase in fresh weight was noticed by the treatment T_6 (FYM) 227.51 g pot^{-1}. However, significant rise in fresh weight was recorded by the treatment T_5 (Compost) 318.84 g $^{-1}$pot and was significantly superior over all other treatments. At 60 DAI in experiment there was rise in *Azolla* production in all the treatments. Treatment T8 (Soil) alone recorded 132.22 g pot^{-1} *Azolla* production. Application of poultry manure T_7 (Poultry manure) treatment 179.66 g^{-1}pot reduced fresh weight as compared to other treatment. the reduced in fresh weight might be due to chemical composition in poultry manure which might have reduced the fresh weight treatment T_5 (Compost) recorded significantly higher fresh weight 488.85 g pot^{-1} at 90 DAI and 528.07 g pot^{-1} at120 DAI of *Azolla.* Andreeilee *et al.* (2015) reported the effect of organic matter increased the biomass production of Azolla. Application of organic matter in the form of cow faces and cattle urine with application of 30 kg N ha^{-1} increased N content of paddy crop.

Effect of organic matter on dry weight of *Azolla*

It is evident from data presented in table 2 that at 30 DAI maximum dry weight of *Azolla* recorded by the treatment T_5 (compost) i.e.14.86 g pot^{-1} and was significantly superior over all other treatments followed by treatment T_6 (FYM) 10.89 g pot^{-1} and in T_2 (Cow dung) 10.56 g pot^{-1} treatment. Lowest fresh weight of *Azolla* was recorded in T_8 (Soil) treatment 2.49 g pot^{-1}. These might be due to lowest nutrient availability for growth of *Azolla*. However, at 60 DAI, significant differences were noticed over uninoculated soil treatment but there was progressively increase on dry weight of *Azolla*. Maximum dry weight of *Azolla* was recorded of the treatment T_5 (Cow dung) 22.60 g pot^{-1}), which was found significantly superior over all other treatments followed by FYM (19.53 g pot^{-1}) and Cow dung (15.83 g pot^{-1}). Treatments T_2 (Cow dung), T_3 (Goat Manure) and T_4 (Sheep manure) were found at par with each other. Similar trend was carried at 90 DAI and 120 DAI with significant rise in dry weight of Azolla in all the nutrient over uninocuated soil treatment. At 120 DAI compost treatment (T_5) alone produced 23.23 g pot^{-1} *Azolla* followed by FYM treatment i.e. 22.25 g pot^{-1} and goat manure treatment 21.08 g pot^{-1}, such differences may be carried because of variation in the nutritional value of organic matters which directly provide supplement intake of macro and micro nutrients in so that the plants may grow more optimally. Similar findings have been reported by Veerabahu (2015), who tested *A. microphylla* and found that paddy crop fertilized with cow dung increased the growth of *Azolla* at pH 6.00 to 7.00.

Effect of organic matter on chlorophyll content of *Azolla*

The data in table 3 shows that there were significant differences on chlorophyll content at 30,60,90 and 120 DAI over uninoculated soil treatment. Chlorophyll content was ranging from 1.27 to 1.61 mg g^{-1} at 30 DAI, 1.33 to 1.76 mg g^{-1} at 60 DAI, 1.29 to 1.68 mg g^{-1} at 90 DAI and 1.27 to 1.63 mg g^{-1} at 120 DAI. Treatment T_8 (Soil) recorded 1.27 and 1.33 mg g^{-1} at 30 and 60 D AI and 1.32 and 1.30 mg g^{-1} chlorophyll content at 90 and 120 DAI respectively. However, there was gradual increase in chlorophyll content in all the treatments. Maximum chlorophyll content was recorded by the T_2 (Cow dung) i.e.1.61 and 1.76 mg g^{-1} in 30 and 60 days and 1.68 and 1.63 mg g^{-1} in 90 and 120 DAI respectively. Treatment T_5 (Compost) was found significantly superior over all other treatments except T_6 (FYM) i.e. 1.57 and 1.70 mg g^{-1} in 30 and 60 days and 1.63 and 1.58 mg g^{-1} in 90 and 120 days. Dawar and Singh (2002) and Pabby *et al.*(2003) studied the chlorophyll content in

A. microphylla when the paddy was fertilized by soil based culture. It is clearly seen from the data that there was decreasing the chlorophyll content from 90 to 120 DAI in all the treatments. This might be due to utilization of all the organic matter present in the medium.

Effect of organic matter on Heterocyst

The data furnished in table 4 regarding heterocyst percentage indicates a significant change due to various treatments over uninoculated soil treatment. At 30 DAI, the percentage of heterocyst was ranging from 16.85 to 21.25 % and 17.05 to 22.02 at 90 DAI, 17.42 to 22.21% at 60 DAI and 16.96 to 21.74% at 120 days, respectively. Treatment T_5 (Cow dung) had the highest heterocyst percentage (21.54%) at 30 DAI and 22.21% at 60 DAI and 22.02% at 90 DAI and 21.74% at 120 DAI) followed by T_6 treatment (FYM) at different intervals.

Minimum heterocyst percentage recorded as 16.85, 17.42, 17.05 and 16.96 at 30, 60, 90 and 120 DAI, respectively in the treatment T_8 (Soil) followed by T_9 (P+K+Zn) i.e.17.37%, 18.22%, 18.19% and 18.13% at 30,60,90 and 120 DAI respectively. This might be due to P deficiency in the medium. Maximum heterocyst percentage was recorded at 30 and 60 DAI in all treatments and later on decreasing rate at 90 and 120 DAI. Mangaraj *et al.* (1997) found decrease in fern biomass and N content when crop was fertilized with high dose of phosphorus. Variation in heterocyst due to light intensity and mature leaves have been reported by Maejima *et al*. (2011).

The data presented in table 2 indicates significant differences over uninoculated soil treatment. Highest nitrogen content was found in treatment T_5 (Cow dung) i.e. 4.12% at 30 DAI, 5.05% at 60 DAI, 5.03% at 90 DAI and 4.61% at 120 DAI. which was followed by T_6 (Soil) while P content was also found highest in treatment T_5 (Cow dung) 0.62% at 30 DAI, 0.75 % at 60 DAI, 0.72% at 90 DAI and 0.67% at 120 DAI). Nitrogen content affects the growth of *Azolla*. In present investigation minimum N content was recorded in T_8 treatment (Soil). Mian and Azmal (1989) and, Watanabe and Ramirez (1989) studied the effect of P application on paddy which affected N fixation by *A. pinnata.*

Variation in supply of P and other micronutrients has been reported on the nutrient content of *A. pinnata* by Yadav (2014). All the treatments were found superior over soil treatment. Application of compost manure exhibited the highest N and P content. Minimum P and N content was noticed in T_8 treatment (Soil) 0.44 to 0.49% P and 2.90 to 3.07 % N in 30 and 60 DAI and 0.46 to 0.45% P and 3.01 to 2.94 % N at a 90 and 120 DAI. Watanabe *et al*. (1988) reported that the growth of *Azolla* was found decreasing when the medium was P deficient. These finding corroborates with the reports of Khosravi *et al.* (2005), who recorded the toxic effect of Pb, Cd, Ni and Zn on *Azolla filiuloides* but in present investigation *A. microphylla* was used. However, the species of *Azolla* also play an important role in the micronutrient response.

There was significant correlation between morning and evening relative humidity. Treatments T_7 (Poultry manure) and T_8 (Soil) had significant correlation 0.993 each in morning and evening relative humidity followed by T_9 treatment (P+K+Zn) 0.981. However, morning relative humidity treatment T1 (vermin compost) had significantly higher correlation 0.904. Other parameters viz., maximum temperature, minimum temperature, sunshine hours and light intensity played negative correlation with all the treatments understudy. These results are in line with reports of Indira *et al.* (2014), who reported optimum requirement of temperature, light intensity and relative humidity for vegetative mass yield of *Azolla.* Thus, it is inferred from the present investigation that the application of organic matter viz., cow dung or compost or FYM positively influenced the growth of *Azolla*.

Table 1. Effect of various treatments on fresh and dry weight and chlorophyll content of *Azolla*

Treatments	Fresh wt. (g pot^{-1})				Dry wt. (g pot^{-1})				Chlorophyll content (mg g^{-1})			
	30 DAI	60 DAI	90 DAI	120 DAI	30 DAI	60 DAI	90 DAI	120 DAI	30 DAI	60 DAI	90 DAI	120 DAI
T_{1}- Vermicompost	80.89	193.96	395.88	420.85	3.76	9.05	19.00	19.09	1.38	1.47	1.43	1.39
T_{2}- Cow dung	220.03	359.90	449.14	464.99	10.56	15.83	20.91	21.03	1.48	1.65	1.57	1.51
T_{3}-Goat manure	152.56	327.32	418.59	452.90	6.90	15.23	18.97	21.08	1.41	1.49	1.47	1.42
T_{4}- Sheep manure	209.26	311.49	446.00	461.17	9.48	14.46	20.88	20.94	1.42	1.51	1.48	1.46
T_{5} -Compost	318.84	470.89	488.85	528.07	14.86	22.60	23.46	23.23	1.61	1.76	1.68	1.63
T_{6}- FYM	227.51	431.09	459.86	477.08	10.89	19.53	21.44	22.25	1.57	1.70	1.63	1.58
T_{7}- Poultry Manure	58.38	179.66	333.53	393.16	2.72	8.38	14.67	17.73	1.29	1.42	1.36	1.32
T_{8}- Soil	53.28	132.22	233.28	272.08	2.49	6.17	11.19	13.05	1.27	1.33	1.29	1.27
T_{9}- P+K+Zn	80.66	138.82	269.01	305.24	3.54	6.48	11.83	13.79	1.33	1.39	1.36	1.35
SE ± (m)	5.92	16.88	17.02	18.26	0.28	0.70	0.89	0.85	0.01	0.04	0.01	0.01
CD (P=0.01)	24.09	68.71	69.29	74.36	1.16	2.86	3.63	3.46	0.05	0.16	0.05	0.05

Table 2. Effect of different treatments on heterocyst, phosphorus and nitrogen content in Azolla

Treatments	Heterocyst per cent				Phosphorus per cent				Nitrogen per cent			
	30 DAI	60 DAI	90 DAI	120 DAI	30 DAI	60 DAI	90 DAI	120 DAI	30 DAI	60 DAI	90 DAI	120 DAI
T_{1}- Vermicompost	18.45	19.29	19.06	18.61	0.47	0.57	0.54	0.51	3.53	3.68	3.60	3.55
T_{2}- Cow dung	20.01	20.44	20.04	20.09	0.56	0.69	0.66	0.60	4.02	4.79	4.62	4.42
T_{3}-Goat manure	18.77	19.46	19.10	18.92	0.49	0.63	0.60	0.54	3.84	4.38	4.33	3.90
T_{4}- Sheep manure	19.75	20.25	19.22	19.06	0.54	0.67	0.64	0.57	3.93	4.54	4.41	4.36
T_{5} -Compost	21.25	22.21	22.02	21.74	0.62	0.75	0.72	0.67	4.12	5.05	5.03	4.61
T_{6}- FYM	20.16	21.33	20.27	20.21	0.58	0.73	0.69	0.63	4.04	4.85	4.65	4.54
T_{7}- Poultry Manure	17.76	18.24	18.08	17.79	0.45	0.54	0.50	0.47	2.94	3.11	3.06	3.00
T_{8}- Soil	16.85	17.42	17.05	16.96	0.44	0.49	0.46	0.45	2.90	3.07	3.01	2.94
T_{9}- P+K+Zn	17.37	18.22	18.19	18.13	0.50	0.62	0.58	0.54	4.02	4.12	4.09	4.05
SE ± (m)	0.27	0.11	0.21	0.13	0.01	0.01	0.01	0.01	0.08	0.06	0.01	0.03
CD (P=0.01)	0.78	0.48	0.89	0.55	0.05	0.05	0.05	0.05	0.36	0.25	0.05	0.12

Table 3. Correlation on effect of different weather parameters on yield of *Azolla*

Treatments	Max. Temp	Min. Temp	R.H. Morning	R.H. Evening	Sunshine hour	Light intensity
T1 - Vermicompost	-0.960	-0.989	0.975**	0.904**	-0.175	-0.977
T2 - Cow dung	-0.945	-0.994	0.965**	0.812**	-0.407	-0.954
T3 - Goat manure	-0.926	-0.990	0.969*	0.782*	-0.439	-0.936
T4 - Sheep manure	-0.945	-0.997	0.976**	0.886**	-0.242	-0.978
T5 - Compost	-0.844	-0.942	0.927**	0.642*	-0.608	-0.852
T6 - FYM	-0.861	-0.932	0.892**	0.653*	-0.632	-0.861
T7 - Poultrymanure	-0.930	-0.990	0.993*	0.853**	-0.237	-0.951
T8 - Soil	-0.930	-0.990	0.993*	0.854**	-0.235	-0.952
T9 - P+K+Zn	-0.945	-0.976	0.981**	0.890**	-0.129	-0.955

**. Correlation is significant at the 0.01 level

*. Correlation is significant at the 0.05 level

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