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Effect of phosphatic fertilizer on growth and yield of *Azolla microphylla*

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Abstract
Azolla is small aquatic fern which has symbiotic association with nitrogen fixing *Anabaena azollae*. It is used as the green manuring crop for rice cultivation. An experiment entitled "Role of phosphatic fertilizer on growth and yield of *Azolla microphylla*" was undertaken at Plant Pathology Section, College of Agriculture, Nagpur during the year 2022-2023. The experiment was carried out in Completely Randomized Design (CRD) in ten treatments with three replication and ten treatments The observations such as fresh and dry weight of *Azolla*, chlorophyll content, heterocyst per cent, root length, frond size and nitrogen and phosphorus content were estimated at 15, 30, 45 and 60 DAI. It was revealed from the FRP tank (30x70x30 cm^2) experimentation, that among the Phosphatic fertilizers SSP (Single Super Phosphate) at 100 g/tank was found significantly superior over all other split doses phosphatic fertilizers. Significantly higher fresh weight of *Azolla* was obtained by the treatment T_1 (SSP 100 g/tank) 528.46 g/tank at 60 DAI and dry weight (42.27g/tank). Root length was significantly affected by SSP, DCP, TCP over uninoculated control. Application of SSP at 100 g/tank increased the root length 8.0 cm, 8.5 cm, 8.9 cm and 9.2 cm at 15, 30, 45 and 60 DAI. Similar observation of SSP at 100 g/tank treatment enhanced frond length 1.5cm,1.8 cm,2.1 cm and 2.5 cm at 15, 30, 45 and 60 DAI respectively. It was recorded from the data that the treatment addition of SSP at 100 g/tank was best in increasing all the parameters significantly and can be used for the multiplication of *Azolla* in the FRB tank.

Keywords: *Azolla microphylla*, phosphatic fertilizers, heterocyst

1. Introduction
The word *Azolla* has a Greek origin. It results from the agglutination of two words, Azo and Ollyo, which means killed by drought, these plants are very sensitive to water deficit. *Azolla* commonly known as mosquito fern, duckweed fern, fairy moss, and water fern, is a genus of small aquatic fern. The term *Azolla* was first coined by Lamarck in 1783 [12]. Studies on *Azolla* have generated tremendous interest in the scientific community because of nitrogen fixing ability of this ferns. The capacity of Nitrogen fixation from the atmosphere is due to symbiotic association with *Anabaena azollae* and the presence of nitrogenase enzyme in the heterocyst. The *Anabaena azollae* is located within the cavities of dorsal lobes of *Azolla* fronds. *Azolla* commonly known as mosquito fern, duckweed fern, fairy moss, and water fern, is a genus of small aquatic ferns with worldwide distribution. *Azolla* is found in both temperate and tropical regions. The *Azolla* plants are triangular or polygonal in shape. It is free floating and aquatic but can grow on moist soil as long as the moisture persists in the soil. The symbiotic Blue green algae is confined to the dorsal lobe (Peters and Mayne, 1974) [15]. The ventral lobe which helps in floating due to its convex surface touching water has a few stomata and Trichomen (Eames, 1936) [5]. The cost of phosphatic fertilizer increasing day by day. With a view the reduce the cost on P fertilizer and utilize indigenous P sources in agriculture. Therefore an investigation was carried out to evaluate with different level. This study was mainly aims on "Role of phosphatic fertilizer on growth and yield of *Azolla microphylla.*

2. Materials and Methods
A pot experiment was conducted in the Net house of Plant Pathology Section, College of Agriculture Nagpur during the year of 2022-2023. The experiment was carried out in completely randomized design (CRD) in ten treatment with three replications. The

treatments were viz., Single Super Phosphate (SSP), Dicalcium phosphate (DCP), Tricalcium phosphate (TCP) by using 100 g/tank, 150 g/tank and 200 g/tank each. 30 FRB tanks of similar size (130X70X30 cm^2) were taken up for the experimental study. 3/4th part of tank was filled up by water. *Azolla microphylla* was inoculated in the tank at the rate of 50 g/tank. For the experimental studies Single Super Phosphate was added in the first, second and third tank @ 100,150,200 g Dicalcium Phosphate @ 100,150,200 g dose each and Tricalcium Phosphate was applied @ 100,150,200 g in each tank. Uninoculated treatment served as control. During the experiment the pH of medium was maintained at 6.5. *Azolla* were blotted dry to record the fresh weight then the plants were dried at 60^0C to record the dry weight at various interval. Total chlorophyll content (mg g^{-1}) of the dried leaves was estimated by colorimetric method as suggested by Subudhi and Watanabe (1981) [17]. Heterocyst percentage was determined and calculated according to Konde and Kannaiyan (2000) [10] by using the following formula.

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

The nitrogen content in *Azolla* was analysed 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].

3. Results and Discussion

Fresh weight of *Azolla*

It was observed from the data presented in Table 1 that there were significant differences on fresh weight of Azolla over uninoculated codnrol. Significantly higher fresh weight of Azolla was recorded with Treatment T_1 (SSP 100 g/tank) at 15 DAI. The next best treatment was T_4 (335.33 g/tank) followed by T_3 treatment (319.66 g/tank). At 30, 45 and 60 DAI, the difference were found to be significant over uninoculated control. The data shows that there was increasing in fresh weight from 15 DAI till 60 DAI linearly. The increase in fresh weight might to be due to easily availability of phosphatic inorganic fertilizers which enhanced the growth attributing characters and finally aids higher fresh weight of *Azolla.* The growth of the present investigation are in harmony with those reported by Watanabe *et al*. (1988) [19] and Mangaraj (1997) [13]. Uninoculated treatment T_{10} recorded lowest fresh weight of *Azolla* at all the intervals.

Dry weight of *Azolla*

It was clearly observed from the data (Table 1) that there was significant increase in dry weight of *Azolla*. Highest dry weight was recorded by T_1 treatment (42.27 g/tank) and it was significantly superior over all other treatments except T2 treatment (32.13g/tank). Rest of the treatment were at par with each other. T_2 treatment 29.60 g/tank, 30.01 g/tank, 32.13 g/tank. The data clearly shows that there was increase in dry weight from 15 DAI to 60 DAI linearly. Application of phosphate increase the biomass in all the treatments and these observations are in conformity with the reports of Subudhi and Watanabe (1981) [17], Watanabe (1988) [19] and Shah *et al.* (1989) [18] reported the phosphatic requirement for the growth of *Azolla* N fixation. Kashuri and Watanabe (1998) [19] showed that the phosphorus was necessary for better growth in increasing dry matter of *Azolla.*

Chlorophyll content

At 15 DAI, maximum chlorophyll content was noticed in T_1 treatment (0.68 mg/g) and was found significantly superior over all other treatments. Treatment T_{10} recorded 0.49 mg/g chlorophyll content. At 30, 45 and 60 DAI there was increase in chlorophyll content in all the treatments. At 30, 45 and 60 DAI, maximum chlorophyll content was recorded by the T_1 treatment (0.80 mg/g, 0.99 mg/g and 1.21 mg/g) followed by T_2 treatment 0.71 mg/g, 0.89 mg/g and 1.06 mg/g at 30, 45 and 60 DAI. Similar observations have been made by Ghate (2013) [6] who observed increase in chlorophyll content due to application of phosphorus.

Heterocyst per cent

Maximum heterocyst percentage was recorded with T_1 treatment (18.1 per cent) and it was found significantly superior over all other treatments followed by T_2 treatment (15.70 per cent) at 15 DAI. At 30, 45 and 60 DAI the treatment T_1 recorded the highest heterocyst percentage 19.01, 20.20 and 21.90 per cent respectively and it was found significantly superior over all other treatment except T_2 recording 17.14, 18.23 and 20.10 per cent at 30, 45 and 60 DAI. There were linearly increase in heterocyst percentage at 30 DAI onward till 60 DAI. The increase in heterocyst at all the split doses of phosphorous may be due to more availability of nutrients. Environmental factors such as sunlight and temperature might be one of the reason in increasing heterocyst percentage. Sood *et al*., (2005) examined requirement of phosphorous for the growth of *Azolla*. Uheda and Maejima (2009) [20] and Maejima *et al*., (1980) [21] and Konde *et al*., (2000) [10] studied the heterocyst relation with nitrogen fixation.

Nitrogen content

Application of SSP treatment (100 g conc.) recorded maximum nitrogen per cent 3.98 at 15 DAI and it was at par with all other treatments. Similar trend was noticed at 30 DAI. However, at 45 and 60 DAI the treatment T_1 recorded 4.42 per cent N content at 45 DAI and 4.76 per cent N content at 60 DAI and it was significantly superior over all other treatments. Split doses of phosphorous at 100 g/tank was significant as compared to 150 g/tank of sources of phosphate application. The results are supported by the findings of Dawar and Singh (2003) [4] and Kollah *et al*., (2015) [9] who reported influence of phosphorus application enhanced the nitrogen content in *Azolla.*

Phosphate content

The treatment T_1 recorded maximum P content 0.45 per cent at 15 DAI, 0.49 per cent at 30 DAI, 0.54 per cent at 60 DAI respectively. It was at par with all other treatments except T_2 treatment at all the intervals. The next best treatment was T_2 (SSP 150 g/tank) followed by T_4 (DCP 100 g/tank. Recent studies on phosphorus application seems to be more important for *Azolla* multiplication and the present results are in conformity with the reports of Subudhi *et al*., (1981) [17]. Rajput and Verma (1999) [16], Costa *et al*., (1999) [3] and Pattanayak *et al*., (2001) [14] studied the application of phosphorus for better growth of *Azolla* in field condition.

Frond size

Application of single super phosphate at 100 g/tank

treatment recorded the maximum in size of frond 1.51 cm^2 and was found significantly higher over all other treatment at 15 DAI except treatment T_2. However, there were significant increase in frond size in the same treatment recording 1.82 cm^2 at 30 DAI, 2.17 cm^2 at 45 DAI, 2.53 cm^2 at 60 DAI respectively. The increase in frond size might be attributed due to more availability of nutrients and application of split doses of phosphorus. Subudhi and Watanabe (1980) [17] reported the requirement of phosphorus for growth of *Azolla*.

Root length

At 15 DAI maximum root length was registered by the treatment T_1 (8.0 cm/tank) and it was significantly superior over all other treatments. However, at 30 DAI the same treatment T_1 was at par with T_2 and T_4 treatment recording 8.3 and 8.0 cm/tank, but at 45 and 60 DAI. The treatment T_1 (8.9 and 9.2 cm/tank) were at par with at with all other treatments but was significantly superior over T_8, T_9 and T_{10} treatment recording 7.0, 6.5 and 6.3 cm/tank root length. There was increase in root length from 15 DAI till 60 DAI linearly. Many researchers viz., Pattanayak *et al*., (2001) [14], Arora and Saxena (2005) [2] and Hamid *et al*., (2007) [7] showed that the phosphorus deficiency affected the *Azolla pinnata* growth and at could grow at all the P levels application.

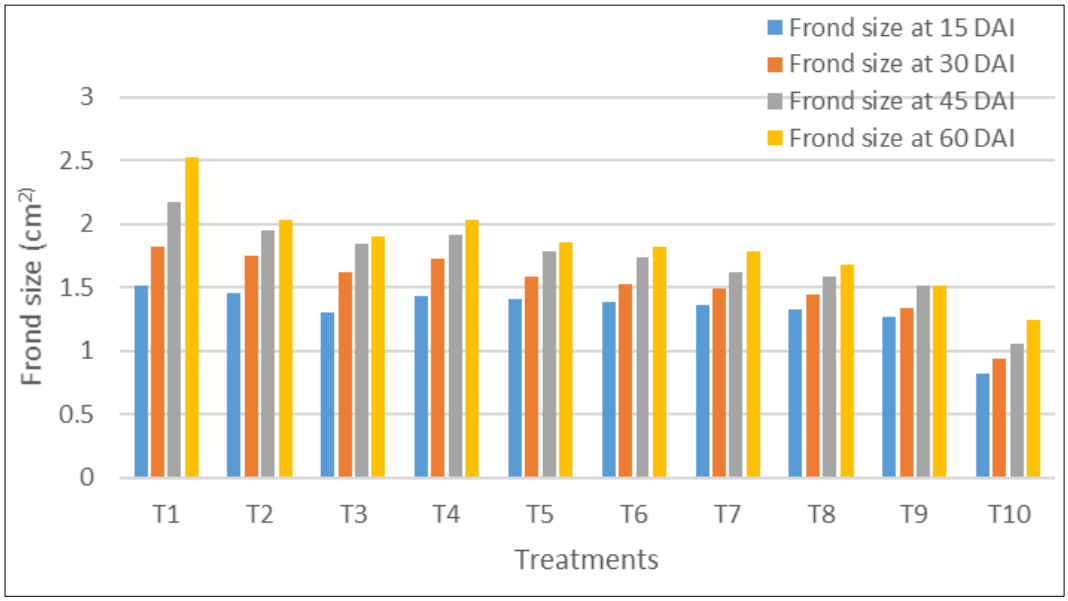

Fig 1: Effect of different treatments on frond size of *Azolla microphylla* at various stages

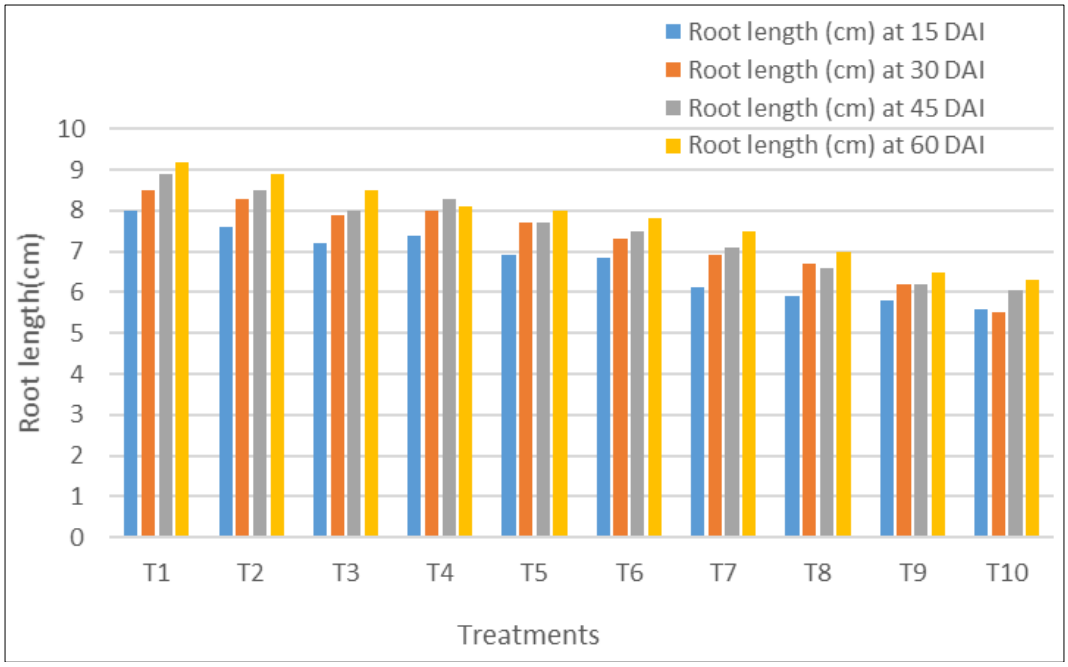

Fig 2: Effect of different treatments on root length of *Azolla microphylla* at various stages

Table 1: Effect of different treatments on growth parameters on *Azolla microphylla* at various stages

Treatments	Fresh weight (g/tank)				Dry weight (g/tank)				Chlorophyll (mg/g)				Heterocyst percentage			
	DAI				DAI				DAI				DAI			
	15	30	45	60	15	30	45	60	15	30	45	60	15	30	45	60
T_1- SSP 100 g	382.16	428.33	486.66	528.46	30.12	34.75	36.13	42.27	0.68	0.80	0.99	1.21	18.10	19.01	20.20	21.90
T_2- SSP 150 g	349.00	370.00	391.33	401.66	26.23	29.60	30.01	32.13	0.59	0.71	0.89	1.06	16.31	17.14	18.23	20.10
T_3- SSP 200 g	319.66	335.33	345.33	357.46	23.14	26.83	27.18	28.59	0.47	0.59	0.73	0.79	15.56	16.06	16.66	17.76
T_4-DSP 100 g	335.33	344.00	354.00	365.76	24.12	27.50	28.4	29.26	0.57	0.61	0.85	0.85	15.70	16.47	17.03	18.51
T_5-DSP 150 g	252.33	269.00	286.66	352.73	18.22	21.50	24.66	28.21	0.47	0.57	0.68	0.76	14.63	15.23	15.96	16.88
T_6-DSP 200 g	242.00	268.33	282.33	334.20	17.98	21.48	23.80	26.73	0.43	0.53	0.63	0.69	14.61	14.94	15.16	16.00
T_7- TCP 100 g	235.66	252.66	262.66	310.66	16.99	20.26	22.42	24.85	0.40	0.50	0.59	0.60	13.73	13.91	14.18	14.89
T_8- TCP 150 g	227.66	247.00	250.33	271.36	16.41	19.58	20.08	21.70	0.32	0.48	0.55	0.55	12.16	12.62	13.72	14.00
T_9- TCP 200 g	224.26	238.83	245.5	260.46	16.12	19.10	20.00	20.83	0.28	0.45	0.49	0.45	12.02	12.50	12.71	13.03
T_{10}-Control	182.00	215.66	242.66	252.40	13.95	17.47	18.32	20.33	0.49	0.41	0.31	0.20	11.60	11.68	11.75	11.60
S.E. (±m)	5.94	4.73	6.80	17.98	1.95	3.51	2.73	2.78	0.01	0.01	0.02	0.02	0.32	0.55	0.85	0.74
C.D (P= 0.01)	23.93	19.06	27.73	72.36	7.87	14.14	11.02	11.19	0.04	0.04	0.09	0.10	1.32	2.22	3.43	3.01

Table 2: Effect of different treatments on growth parameters on *Azolla microphylla* at various stages

Treatments	Nitrogen content (%)				Phosphate content (%)				Frond size (cm^2)/pl				Root length (cm) /pl			
	DAI				DAI				DAI				DAI			
	15	30	45	60	15	30	45	60	15	30	45	60	15	30	45	60
T_1- SSP 100 g	3.98	4.00	4.42	4.76	0.45	0.49	0.54	0.57	1.51	1.82	2.17	2.53	8.00	8.50	8.90	9.20
T_2- SSP 150 g	3.90	3.95	4.22	4.48	0.48	0.47	0.51	0.55	1.46	1.75	1.95	2.03	7.60	8.30	8.50	8.90
T_3- SSP 200 g	3.77	3.80	4.06	4.18	0.40	0.43	0.47	0.51	1.30	1.62	1.84	1.90	7.20	7.90	8.00	8.50
T_4-DSP 100 g	3.85	3.91	4.12	4.29	0.42	0.45	0.50	0.52	1.43	1.73	1.91	2.03	7.40	8.00	8.30	8.10
T_5-DSP 150 g	3.72	3.76	3.83	3.98	0.39	0.42	0.47	0.51	1.41	1.58	1.78	1.86	6.90	7.70	7.70	8.00
T_6-DSP 200 g	3.64	3.70	3.78	3.87	0.39	0.41	0.45	0.50	1.38	1.52	1.74	1.82	6.85	7.30	7.50	7.80
T_7- TCP 100 g	3.58	3.67	3.71	3.80	0.37	0.40	0.43	0.47	1.36	1.49	1.62	1.79	6.13	6.90	7.10	7.50
T_8- TCP 150 g	3.50	3.58	3.67	3.75	0.36	0.38	0.41	0.46	1.32	1.44	1.58	1.68	5.90	6.70	6.60	7.00
T_9- TCP 200 g	3.53	3.35	3.55	3.64	0.35	0.36	0.38	0.44	1.27	1.34	1.51	1.51	5.80	6.20	6.20	6.50
T_{10}-Control	2.28	2.26	2.24	2.22	0.27	0.25	0.23	0.20	0.82	0.94	1.05	1.24	5.60	5.50	6.05	6.30
S.E. (±m)	0.12	0.11	0.03	0.03	0.03	0.05	0.04	0.05	0.01	0.01	0.01	0.02	0.15	0.14	0.45	0.53
C.D (P= 0.01)	0.50	0.44	0.13	0.14	0.13	0.22	0.19	0.23	0.06	0.05	0.04	0.10	0.64	0.57	1.82	2.14

4. Conclusion

The present study concludes that the application of single super phosphate (SSP) at 100 g/tank (T1) significantly enhanced the growth and yield attributes of Azolla at all stages of observation. Treatment T1 recorded the highest fresh and dry weight, chlorophyll content, heterocyst percentage, nitrogen, and phosphorus content, as well as maximum frond size and root length. The increase in growth parameters can be attributed to the enhanced availability of phosphorus, which promotes nitrogen fixation and overall plant development. The findings are consistent with previous studies, highlighting the critical role of phosphorus in Azolla cultivation for sustainable agricultural practices.

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