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Biomass production and protein content of azolla cultured under K enriched water

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Abstract: **An ever-increasing cost of chemical fertilizers and widening gap between demand and supply necessitated the utilization of biofertilizers as partial substitute to the chemical fertilizers. Azolla has a strong capacity of absorbing potassium from water within the range of 1-50 ppm and the K content of azolla increased in logarithmic form and used for its biomass production (Kannaiyan, 1990). *Azollae filiculoides* grown with 2 agriculturally important potassic fertilizers (Potassium Chloride, Potassium shulphate) as main plot in eight concentrations (0, 2, 5, 10, 20, 30, 40 and 50 ppm of K) as sub-plots in split plot design replicated thrice. Potassium sulphate was superior and significantly produced 3.59 g g^{-1} of biomass of azolla. With respect to concentration, 40 ppm of K recorded highest biomass of 6.08 g g^{-1} on 30^{th} day of incubation. Though the biomass production was more during 30^{th} day of incubation and the growth rate was more on 15^{th} day of incubation. The K fertilizer such as KCl and K_2SO_4 significantly influenced the protein content in azolla during all the days of incubation. The K_2SO_4 with 40 ppm concentration was significantly superior in maintaining higher protein content in azolla during all the days of incubation followed by 30 ppm of K_2SO_4 at all the stages of incubation except 90^{th} day of incubation.**

1. INTRODUCTION

The *Azolla* fern doubles its biomass in 2–5 days under ideal environmental conditions. It can supply more than half of the required nitrogen to the rice crop and besides providing nitrogen it is beneficial in wetland rice fields for bringing number of changes which includes preventing rise in pH, reducing water temperature, curbing NH_3 volatilization, suppressing weeds and mosquito proliferation (Pabby *et al*. 2004). The integrated use of organic and inorganic fertilizers is desirable to sustain crop yields and maintenance of soil health (Prasanna *et al*. 2008). With ever-increasing cost of chemical fertilizers and widening gap between demand and supply, utilization of biofertilizers can partially substitute the chemical fertilizers. The response of Azolla to potassic fertilization is most effective in stimulating the growth (Kannaiyan, 1990). Azolla has a strong capacity of absorbing potassium from water within the range of 1-50 ppm and the K content of azolla increased in logarithmic form and used for its biomass production. In view of the continuing increase in cost and scarcity of mineral fertilizers resulting from the use of high-cost fossil energy, there is renewed interest in organic recycling and biological nitrogen-fixation to improve soil fertility and productivity.

2. MATERIALS AND METHODS

An incubation experiment was conducted by growing *Azollae filiculoides* with 2 agriculturally important potassic fertilizers (Potassium Chloride, Potassium shulphate) as main plot in eight concentrations (0, 2, 5, 10, 20, 30, 40 and 50 ppm of K) as sub-plots laid down in split plot design replicated thrice. The Azolla fern was grown in a tray with a dimension of 23 x 15 x 6 cm^3 filled with 1.5 litres of potassic solutions. All fern grown in a plastic tray was collected, washed with distilled water, drained and weighed and expressed in grams. The relative growth rate (g g^{-1} day^{-1} – gram per gram of Azolla per day) and doubling time were computed from the fresh weight of the Azolla fern collected on each sampling by following mathematical models (France and Thornley, 1984). The data were analysed statistically as per the procedures given by Panse and Sukhatme (1985) and the treatments were compared based on the critical difference for its effectiveness.

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3. RESULTS AND DISCUSSION

Biomass production:

Biomass production of azolla as influenced by the main effect of K fertilizers and its concentrations is presented in Table 1. Both fertilizer and concentration significantly influenced the biomass production of azolla during all the days of incubation. Among the K fertilizers, the effect of potassium sulphate on biomass production of azolla was superior and significantly registered the higher mean biomass of 3.59 g g^{-1}. The observed higher growth of azolla on potassium sulphate might be due to the presence of sulphur as residue in the particular fertilizer considered as essential and enhanced the growth of azolla (Singh *et al*., 1997). The biomass production of azolla was increased with the advancement of period of sampling during early stages of incubation up to 30^{th} days after incubation and maintained almost constant growth during the later stages of incubation. The highest biomass of 5.38 and 5.56 g g^{-1} was recorded on 30^{th} day after incubation by potassium chloride and potassium sulphate respectively. Irrespective type of K fertilizer, the highest mean biomass growth of 5.47 g g^{-1} was also registered on 30^{th} day after incubation. The *Azolla* plant had covered the total surface area (100 m^2) of the pond at 20 days after the introduction of 1 kg of fresh *Azolla* (Shiomi and Kitoh, 2001). The biomass analysis shows that the great nitrogen, phosphorus, potassium and organic content could favor the use of *Azolla* as a biofertiliser (Costa *et al*., 1999).

Table 1. Biomass growth (g g^{-1}) of Azolla as influenced by the main effect of K fertilizer and their concentration

Treatment	**Period of incubation, Days**						**Mean**
	7	**15**	**30**	**60**	**90**	**120**	
Fertilizer							
F_1 (KCl)	2.32	3.41	5.38	3.45	3.45	2.99	**3.50**
F_2 (K_2SO_4)	2.44	3.49	5.56	3.51	3.50	3.05	**3.59**
Mean	**2.38**	**3.45**	**5.47**	**3.48**	**3.48**	**3.02**	
CD (0.05)	0.17	0.09	0.07	0.05	0.02	0.03	
Concentration							
C_1 (0 ppm)	2.13	3.20	4.76	2.98	2.92	2.49	**3.08**
C_2 (2 ppm)	2.27	3.33	5.22	2.97	3.04	2.69	**3.25**
C_3 (5 ppm)	2.33	3.39	5.55	3.03	3.21	2.84	**3.39**
C_4 (10 ppm)	2.40	3.46	5.59	3.72	3.74	3.20	**3.68**
C_5 (20 ppm)	2.48	3.55	5.42	3.48	3.43	2.99	**3.56**
C_6 (30 ppm)	2.50	3.58	5.66	3.94	3.89	3.38	**3.82**
C_7 (40 ppm)	2.55	3.64	6.08	4.19	4.06	3.55	**4.01**
C_8 (50 ppm)	2.40	3.45	5.51	3.54	3.54	3.05	**3.58**
Mean	**2.13**	**3.20**	**4.76**	**2.98**	**2.92**	**2.49**	**3.08**
CD (0.05)	0.19	0.04	0.04	0.04	0.04	0.06	

The various concentration ranging from 0 ppm to 50 ppm of K significantly influenced the biomass production of azolla during all the periods of incubation. The highest mean biomass of 4.76 g g^{-1} was recorded during 30^{th} day of incubation and the lowest of 2.13 g g^{-1} was observed on 7^{th} day of incubation irrespective of the concentration of K fertilizer. Nordiah *et al*. (2012) confirmed that water sources with different nutrient contents increased in biomass of azolla although fluctuated with time. The highest biomass production of 6.08 g g^{-1} was recorded by 40 ppm of K solution on 30^{th} day of incubation and the lowest of 2.13 g g^{-1} was registered by 0 ppm of K on 7^{th} day of incubation. Among the various concentration, the 40 ppm of K significantly produced more biomass during all the days of incubation and in an average 4.01 g g^{-1} was produced by the same concentration.. The utilization rate of Azolla potassium by early and late rice was 14.1% and 17.6% respectively (Zhang *et al*., 1991). Optimum Azolla growth with full nitrogenase activity occurs when the concentration levels for P. K, Mg and Ca nutrients are 0.03, 0.6, 0.5 and 0.5 m mol l^{-1} respectively (Lumpkin, 1987 and Yatazawa *et at*., 1980).

Table 2. Biomass growth (g g^{-1}) of Azolla as influenced by the interaction effect of K fertilizer with their concentration

Treatment	Period of incubation, Days						Mean
	7	15	30	60	90	120	
F_1C_1	2.13	3.20	4.80	2.95	2.77	2.22	**3.01**
F_1C_2	2.25	3.30	4.81	2.76	2.90	2.50	**3.09**
F_1C_3	2.30	3.37	5.53	2.91	3.22	2.75	**3.35**
F_1C_4	2.35	3.43	5.59	4.09	4.12	3.40	**3.83**
F_1C_5	2.38	3.49	5.12	3.24	3.19	2.80	**3.37**
F_1C_6	2.40	3.52	5.47	4.02	3.91	3.50	**3.80**
F_1C_7	2.45	3.56	6.29	4.17	4.05	3.75	**4.05**
F_1C_8	2.30	3.40	5.42	3.45	3.45	3.00	**3.50**
F_2C_1	2.13	3.20	4.72	3.01	3.07	2.75	**3.15**
F_2C_2	2.28	3.35	5.63	3.17	3.18	2.87	**3.41**
F_2C_3	2.36	3.40	5.57	3.14	3.20	2.92	**3.43**
F_2C_4	2.45	3.48	5.59	3.35	3.35	3.00	**3.54**
F_2C_5	2.57	3.60	5.71	3.72	3.67	3.17	**3.74**
F_2C_6	2.60	3.64	5.84	3.86	3.86	3.25	**3.84**
F_2C_7	2.64	3.72	5.86	4.21	4.07	3.35	**3.98**
F_2C_8	2.50	3.50	5.59	3.63	3.63	3.10	**3.66**
Mean	**2.38**	**3.45**	**5.47**	**3.48**	**3.48**	**3.02**	
CD (0.05)							
F at C	0.288	0.097	0.077	0.067	0.053	0.077	
C at F	0.26	0.06	0.05	0.06	0.05	0.78	

The interaction between K fertilizers (KCl and K_2SO_4) and their concentration on biomass production of azolla is presented in Table 2. The interactive effect of K fertilizer and its various concentrations significantly and positively influenced the biomass production during all the periods of incubation. Irrespective of the concentration of K fertilizer, the highest mean biomass of 5.47 g g^{-1} was recorded during 30^{th} day of incubation and the lowest of 2.38 g g^{-1} was observed during 7^{th} day of incubation. Irrespective of the period of incubation, the highest mean biomass growth of 4.05 g g^{-1} was registered by 40 ppm of KCl followed by 40 ppm of K_2SO_4 (3.98 g g^{-1}) and the lowest mean biomass growth was observed under 0 ppm of both the fertilizers. Among the various concentration of KCl, the 40 ppm significantly produced more biomass during all the days of incubation. The interactive effect of K_2SO_4 and its various concentrations significantly influenced the biomass production during all the periods of incubation and the highest mean biomass of 5.86 g g^{-1} was recorded during 30^{th} day of incubation and the lowest of 2.13 g g^{-1} was observed during 7^{th} day of incubation. Though the biomass production was more during 30^{th} day of incubation, the growth rate was more on 15^{th} day of incubation (Fig 1 & 2). Among the various concentration of K_2SO_4, the 40 ppm significantly produced more biomass during all the days of incubation and in an average 3.98 g g^{-1} was produced by the same concentration irrespective of the period of sampling. The growth and nitrogen-fixing capacity of *Azalia imbricata* were studied with special reference to the effects of the mineral nutrient status in the medium. The threshold levels of P, K, Mg, and Ca in the medium for *Azalia* growth were *ca.* 0.03, 0.4, 0.4, and 0.5 mmol l^{-1}, respectively. Full development of nitrogenase activity was not realized at concentrations below 0.03, 0.6, 0.5, and 0.5 mmol·l^{-1}, respectively (Yatazawa *et al*., 1980).

Protein content:

The protein content in azolla significantly influenced by the both main and interaction effect of fertilizer and its concentration during all the periods of incubation under K enriched water (Table 3 and 4). In general the protein content was increased with the advancement of period of incubation during initial stages up to 30^{th} day and decreased at the later stages of incubation. The protein content of azolla on 7^{th} day of incubation in K enriched water was 126.6 µg g^{-1} and the highest content of 250 µg g^{-1} was registered during 30^{th} day of incubation. The both K fertilizer such as KCl and K_2SO_4 significantly influenced the protein content in azolla during all the days of incubation. Among the K fertilizer, the K_2SO_4 always registered significantly higher protein content in all the days of incubation (Table 3). The highest protein content of 274.2 µg g^{-1} was observed during 30th day of incubation by K_2SO_4. However, the highest mean protein content of 215.9 µg g^{-1} was recorded by the same fertilizer followed by KCl with the protein content of 158.1 µg g^{-1}. Biomass content of protein and fiber weren't very different regardless of where the plants were grown). Crude protein values were even greater than those observed in some other fodder plants (CEIP, 1980) like maize (Costa *et al*., 1999).

Table 3. Protein content (µg g^{-1}) in Azolla as influenced by the main effect of fertilizer and their concentration

Treatment	Period of incubation, Days						Mean
	7	15	30	60	90	120	
Fertilizer							
F_1 (KCl)	112.8^b	183.1^b	225.8^b	161.8^b	150.6^b	114.8^b	**158.1**
F_2 (K_2SO_4)	140.4^a	311.1^a	274.2^a	241.3^a	177.1^a	151.1^a	**215.9**
Mean	**126.6**	**247.1**	**250.0**	**201.5**	**163.8**	**132.9**	
CD (0.05)	3.7	1.6	1.5	0.8	1.4	1.1	
Concentration							
C_1 (0 ppm)	97.0^g	193.6^h	174.2^h	158.9^h	124.4^g	89.0^h	**139.5**
C_2 (2 ppm)	111.5^f	208.9^g	212.9^g	171.4^g	138.3^f	103.7^g	**157.7**
C_3 (5 ppm)	115.6^e	245.9^f	234.5^f	191.9^f	147.5^e	116.0^f	**175.2**
C_4 (10 ppm)	124.5^d	249.2^e	238.2^e	202.8^e	169.9^d	138.8^e	**187.2**
C_5 (20 ppm)	133.6^c	258.4^d	263.6^d	213.3^c	173.4^c	148.6^c	**198.5**
C_6 (30 ppm)	142.7^b	268.9^b	289.5^b	223.9^b	180.6^b	155.6^b	**210.2**
C_7 (40 ppm)	156.0^a	291.8^a	318.6^a	238.5^a	202.4^a	166.5^a	**229.0**
C_8 (50 ppm)	132.3^c	260.6^c	268.8^c	211.5^d	174.2^c	145.5^d	**198.8**
Mean	**126.6**	**247.1**	**250.0**	**201.5**	**163.8**	**132.9**	
CD (0.05)	2.0	1.7	1.9	0.7	1.5	1.2	

The main effect of concentration significantly influenced the protein content of azolla during all the days of incubation. Irrespective of the days of incubation, the highest mean protein content of 229 µg g^{-1} was registered by 40 ppm of K solution followed by 30 ppm of K solution (210.2 µg g^{-1}). During all the days of incubation the 40 ppm of K solution maintained higher protein content in azolla. The maximum protein content of 318.6 µg g^{-1} was observed under same concentration during 30th day of incubation followed by 30 ppm of K solution during the same period of incubation. The highest concentration of 50 ppm K positioned in the third place during the initial stages and the fourth position in the advanced stages of incubation. However, the azolla grown in 0 ppm (water) had the lowest protein content during all the days of incubation. The high level of crude protein of *Azolla* and higher lysine concentration indicated that *Azolla* plants may be a good source of fish feed and significantly higher content of dehydro-ascorbic acid (1,909 mg kg^{-1} dry weight) than that of L-ascorbic acid (10 mg kg^{-1} dry weight) in *Azolla* grown for 3 day was observed (Shiomi and Kitoh, 2001). Though the biomass production was more during 30th day of incubation and the growth rate was more on 15th day of incubation.

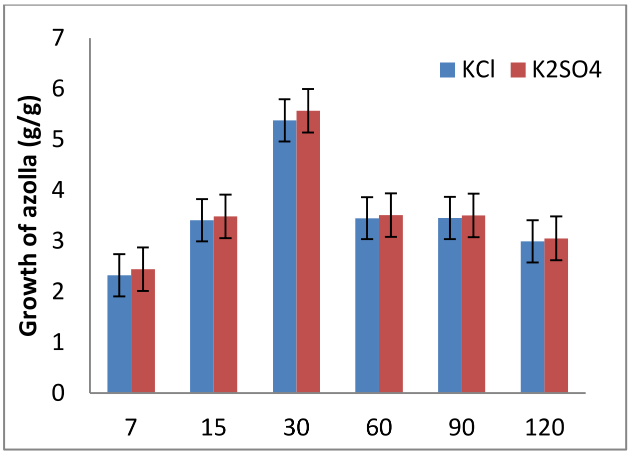

Fig 1. Biomass production of azolla (g g^{-1}) as influenced by KCl and K_2SO_4 at different period of incubation.

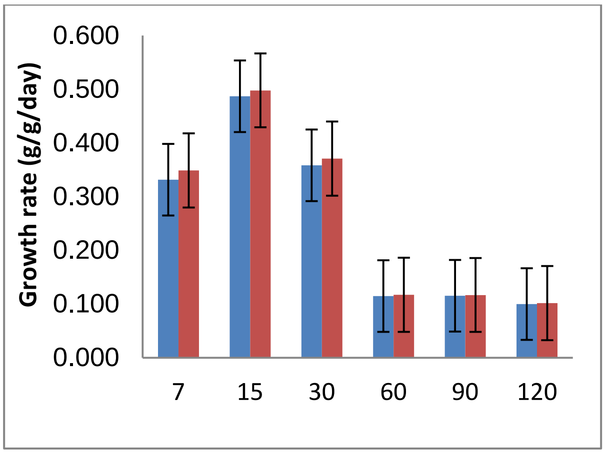

Fig 2. Growth rate of azolla (g $g^{-1}day^{-1}$) as influenced by KCl and K_2SO_4 at different period of incubation.

Table 4. Protein content (µg g^{-1}) in Azolla as influenced by the interaction effect of fertilizer and their concentration

Treatment	Period of incubation, Days						Mean
	7	15	30	60	90	120	
F_1C_1	90.1^k	139.8^o	175.6^m	121.8^n	105.8^m	65.1^n	**116.4**
F_1C_2	98.2^j	158.4^n	192.0^l	141.5^m	126.8^l	74.2^m	**131.9**
F_1C_3	100.3^j	176.8^m	222.9^k	153.8^l	129.7^k	89.6^l	**145.5**
F_1C_4	116.4^h	179.2^l	225.8^j	155.8^k	156.7^h	133.4^j	**161.2**
F_1C_5	118.5^{gh}	192.6^k	236.5^h	170.6^i	159.5^g	137.5^h	**169.2**
F_1C_6	125.6^f	203.4^j	247.0^g	181.5^h	165.3^f	140.6^g	**177.2**
F_1C_7	132.9^e	224.2^i	269.3^e	201.6^f	200.5^b	142.5^f	**195.2**
F_1C_8	120.4^g	190.7^k	237.3^h	167.4^j	160.4^g	135.1^i	**168.6**
F_2C_1	103.9^i	247.3^h	172.7^n	195.9^g	143.0^j	112.9^k	**162.6**
F_2C_2	124.7^f	259.3^g	233.7^i	201.2^f	149.7^i	133.1^j	**183.6**
F_2C_3	130.8^e	314.9^f	246.0^g	230.0^e	165.2^f	142.3^f	**204.9**
F_2C_4	132.5^e	319.1^e	250.5^f	249.8^d	183.1^e	144.2^e	**213.2**
F_2C_5	148.7^c	324.2^d	290.6^d	256.0^c	187.3^d	159.7^c	**227.8**
F_2C_6	159.7^b	334.3^b	332.0^b	266.2^b	195.9^c	170.6^b	**243.1**
F_2C_7	179.1^a	359.4^a	367.9^a	275.4^a	204.2^a	190.4^a	**262.7**
F_2C_8	144.1^d	330.5^c	300.3^c	255.5^c	188.0^d	155.8^d	**229.0**
Mean	**126.6**	**247.1**	**250.0**	**201.5**	**163.8**	**132.9**	
CD (0.05)							
F at C	4.3	2.6	2.8	1.3	2.4	1.8	
C at F	2.9	2.3	2.7	1.1	2.2	1.6	

Among the interaction effect between K fertilizer and its concentration, the K_2SO_4 with 40 ppm concentration was significantly superior in maintaining higher protein content in azolla during all the days of incubation followed by 30 ppm of K_2SO_4 at all the stages of incubation except 90^{th} day of incubation during which the 40 ppm of KCl had maintained higher protein content next to 40 ppm of K_2SO_4. Irrespective of the period of incubation, the 40 ppm of K_2SO_4 recorded higher protein content of 262.7 µg g^{-1} which was 8 % more than the 30 ppm and 14 % higher than 50 ppm of K_2SO_4.

4. CONCLUSION

The potassium sulphate fertilizer was superior and significantly produced higher biomass of azolla. With respect to concentration, 40 ppm of K recorded more biomass on 30^{th} day of incubation. Though the biomass production was more during 30^{th} day of incubation, the growth rate was more on 15^{th} day of incubation. The K fertilizer such as KCl and K_2SO_4 significantly influenced the protein content in azolla during all the days of incubation. The K_2SO_4 with 40 ppm concentration was significantly superior in maintaining higher protein content in azolla during all the days of incubation.

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