Soil Fertility as Influenced by K Enriched *Azolla*

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Authors' contributions

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ABSTRACT

Biological Nitrogen Fixation (BNF) is a major source of fixed N for plant life and to sustain production and productivity of agricultural crops. An incubation experiment was conducted by growing *Azollae filiculoides* with 2 agriculturally important potassic fertilizers (Potassium Chloride, Potassium shulphate) as main plot in seven concentrations (0, 5, 10, 20, 30, 40 and 50 ppm of K) as sub-plots laid down in split plot design replicated three times. The collected *Azolla* was incorporated with soil at 10 t/ha and maintained at two moisture condition such as 60 and 100 percent and assessed soil fertility by estimating various available plant nutrients and organic carbon status..The mean organic carbon content of the soil was 0.657 and 0.525% at 60 and 100% moisture contents respectively. The available N content ranged from 216.2 to 327.3 and 191.1 to 285.3 kg/ha from 0 to 40 ppm of K concentration at 60 and 100% moisture respectively because the *Azolla* had a high N content which released into the soil after decomposition. *Azolla* also contributed to the supply of phosphorus, potassium, sulfur, zinc, iron and molybdenum in sufficient amounts in addition to other micronutrients besides addition of nitrogen. Among the various concentration, 40 and 50 ppm K were significantly maintained higher and equal soil available P status of 75.17 and 77.33 kg / ha

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respectively. The fertilizer, K_2SO_4 with 30, 40 and 50 ppm at 60% moisture content and 40 and 50 ppm of K_2SO_4 and 40 ppm of KCl produced statistically higher and equal available K at 100% moisture. The soil biological health, mineralization and consequent increase in nutrient status by the application of K enriched *Azolla* was more under 60% soil moisture content than fully saturated soil.

Keywords: Biofertilizer; Azolla; K-enrichment; soil fertility; organic carbon and available NPK.

1. INTRODUCTION

Intensive crop production is the demand of time to feed the vast growing population in India. This has created a pressure to use more chemical fertilizer. Use of chemical fertilizer injudiciously without organic manure has created many soil health problems like low fertilizer use efficiency, poor soil physical condition, reduced water holding capacity, degraded rhizospharic properties, and low fertility [1]. Rice crops remove around 16-17 kg N for the production of each ton of paddy [2]. Most of the rice soils of the world are deficient in N, so, fertilizer N applications are required to meet its N demand [3]. Generally, urea is applied as the N source for rice production [4]. But the efficiency of added urea-N is very low, due to denitrification, NH_3 volatilization and leaching [3]. Therefore, alternate sources of N has to be evolved to supply crop demanded N with less or no environmental pollution. The demand of fertilizers and manures is increasing at the present scenarios to maximize the crop production. Biological Nitrogen Fixation (BNF) is a major source of fixed N for plant life and to sustain production and productivity of agricultural crops. Estimate of global terrestrial showed that, the BNF ranged from 100 to 290 million tonnes of N/year. Of this, 40–48 million tonnes is estimated to be biologically fixed N in agricultural crops and fields. BNF is one of the natural sources of nitrogen for rice and *Azolla*-cyanobacteria biomass has been identified as potential source of nitrogen. The integrated nutrient management is to maintain or adjust plant nutrient supply to achieve a given level of crop production by optimizing the benefits from all possible sources of plant nutrients [5]. Organic manures are considered to play a significant role in nutrient contribution. The use of organic fertilizer is a way to improve soil fertility. *Azolla* can be used as organic fertilizer [6]. Subedi and Shrestha [7] explained that, *Azolla* does not only increase the productivity of rice but also improve the long-term soil fertility. All other biofertilizers simply solubilize or mobilize the nutrients that are already present in soils. Whereas the *Azolla* is unique in the sense that it acts as host to the N-fixing cyanobacteria after which it is used virtually as a green manure. In the process, it adds not only the biologically fixed N but also the other nutrients absorbed from the soil and present in its biomass. Against the total anticipated biofertilizers demand of 1 million tonne in the country, the current supply position is very low (<10 000 tonnes). The present investigation studied soil fertility improvement by *Azolla* grown under varied K fertilizer commonly used in agriculture.

2. MATERIALS AND METHODS

2.1 Study Site

An incubation experiment was conducted by growing *Azollae filiculoides* with 2 agriculturally important potassic fertilizers (Potassium Chloride, Potassium shulphate) as main plot in seven concentrations (0, 5, 10, 20, 30, 40 and 50 ppm of K) as sub-plots laid down in split plot design replicated three times. The experimental site was Rice Research Station, Ambasamudram located between 8°42' N and 77°28' E with an altitude of 64.8 m above mean sea level. The mean annual rainfall received was 913 mm. The experimental soil was acidic in reaction (pH 5.84) and free from salinity (0.08 dS m^{-1}) with sandy clay in texture. The organic carbon content was 0.56%. The soil was low in available nitrogen (210 kg ha^{-1}), high in available P (24.5 kg ha^{-1}) and medium in available K (150 kg ha^{-1}). One gram of *Azolla* fern was grown in a tray with a dimension of 23 x 15x 6 cm^3 filled with 1.5 litres of potassic solutions (Plate 1) and the fern was collected on 7th, 15th, 30th, 60th, 90th and 120th day after incubation / culturing, rinsed with distilled water and analysed for various biometric and biochemical parameters.

The collected *Azolla* was pooled and incorporated with soil at 10 t/ha and maintained at two moisture condition such as 60 and 100 percent. The *Azolla* incorporated soil was collected after 30 days of incubation and processed and analyzed for various available plant nutrients and organic carbon status. Organic carbon present in soil /oxidized by chromic acid ($K_2Cr_2O_7$) in the presence of conc. H_2SO_4. Potassium dichromate on reaction with

Plate 1. View of incubation experiment

H_2SO_4 provided nascent oxygen which combined with carbon to form CO_2. The excess chromic acid left unused by the organic matter was determined by back titration with 0.5 N ferrous sulphate or ferrous ammonium sulphate using diphenylamine indicator [8]. Available nitrogen in the soil was estimated by alkaline permanganate method [9]. Available phosphorus extracted with 0.03 N NH_4F and 0.025 N HCl. The amount of P extracted was treated with ammonium molybdate and antimony potassium tartarate and developed colour with ascorbic acid. The intensity of blue colour /was determined colorimetrically at 660nm [10]. The soil was leached with neutral normal ammonium acetate and the K^+ ions in the exchange sites were replaced by NH_4^+ ions. The K^+ ions in solution was then determined with the flame photometer [11].

The soil samples were collected from the incubation bottle after 90 days of incubation were shade dried, processed and sieved through 0.5 and 2 mm sieve for estimating organic carbon and available NPK respectively.

2.2 Statistical Analysis

The data were analysed statistically using computer software [12]. Differences among the mean values of the treatment were compared by the LSD test when the F test from the analysis of variance was significant at the p= 0.005 level.

3. RESULTS

3.1 Organic Carbon

The effect of K fertilizer, its concentration and their interaction on the organic carbon content of *Azolla* incorporated into the soil at 60% and 100% moisture content is presented in Table 1 and 2. The concentration of K fertilizer alone influenced the organic carbon content of the *Azolla* incorporated at both 60% and 100% moisture content. The mean organic carbon content of the soil was 0.657 and 0.525% by K enriched *Azolla* at 60 and 100% moisture contents respectively. *Azolla* enriched with 40ppm K solution recorded significantly higher soil organic carbon content of 0.763 and 0.623% respectively at 60 and 100% soil moisture content (Table 1) which was 42 and 56% more than the *Azolla* grown under 0ppm K. However, it was on par with 50 and 30 ppm of K solution in both the moisture-maintained soil. The 20 ppm K registered 0.697 and 0.567 % of organic carbon content in soil maintained at 60 and 100% moisture content which were 9 to 10 % less than the highest organic carbon maintained by 40 ppm of K.

3.2 Available N

The main effect of *Azolla* grown under K fertilizer and its concentration alone significantly influenced the available N content at both 60 and 100% moisture content. On an average 291.9 and 256.9 kg ha^{-1} of available N was maintained by the incorporation of K enriched *Azolla* in soil maintained at 60 and 100% moisture content respectively (Table 1). Among the K fertilizer, K_2SO_4 significantly maintained higher available N status of 297.2 and 262.2 kg ha^{-1} which was 4 to 5% higher than the KCl. The KCl fertilizer registered 286.6 and 251.6 kg ha^{-1} of soil available nitrogen content at 60 and 100%

moisture content respectively. The available N content ranged from 216.2 to 327.3 and 191.1 to 285.3 kgha^{-1} from 0 to 40 ppm of K concentration at 60 and 100% moisture content respectively. Though, the 40 and 50 ppm of K were on par with each other, they however, maintained significantly higher available N status of 327 and 285 kg ha^{-1} at 60 and 100% moisture content respectively and it was 4.0% higher than 30ppm of K enriched *Azolla*.

3.3 Available P

The main and interaction of fertilizer and their concentration significantly influenced the available P status of *Azolla* incorporated soil maintained at 100% moisture content where as the main effect was alone significantly influenced the available P content at 60% moisture condition. On an average, the K enriched *Azolla* registered 59.63 and 42.13 kgha^{-1} of available P at 60 and 100% soil moisture respectively. Among the fertilizers used, the K$_2$SO$_4$ maintained higher available P content of 61.71 and 44.21 kgha^{-1} in the soil incorporated with K enriched

Azolla at 60 and 100% moisture content respectively followed by KCl which registered the available P content of 57.54 and 40.04 kgha^{-1} (Table 1). Among the various concentration, 40 and 50 ppm K was significantly maintained higher and equal soil available P status of 75.17 and 77.33 kgha^{-1} respectively which was 8.6% more than the 30ppm K (71.17 kgha^{-1}) at 60 % moisture content.
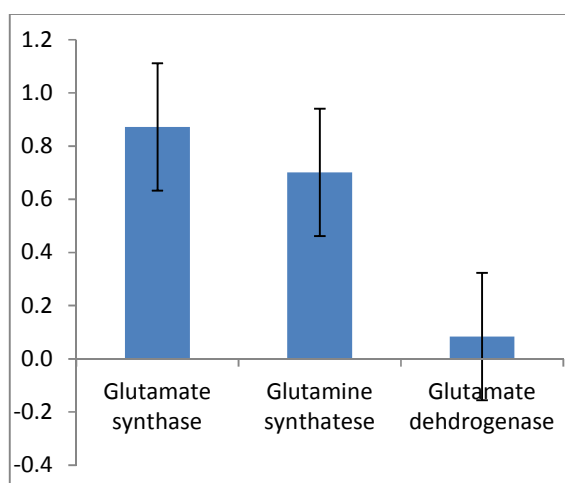
But in 100% soil moisture content at 30, 40 and 50 ppm, K was significantly maintained higher and on par soil available P status followed by 20 ppm K. The lowest available P of 34.5 and 22.5 kgha^{-1} was registered by soil incorporated with *Azolla* enriched with 0ppm of K at 60 and 100% moisture content. Under the interaction between K fertilizer and its concentration, K$_2$SO$_4$ at 50 and 30 ppm were significantly superior in maintaining higher available P status at 100 % moisture content followed by 40 ppm K$_2$SO$_4$ and it was on par with 50 ppm of KCl (Table 2). However, the 0 ppm of both the fertilizer produced lowest available P content (21.33 and 23.67 kg ha^{-1}) of *Azolla* incorporated soil.

Table 1. Soil fertility of K enriched *Azolla* as influenced by main effect of fertilizer and concentration at 60 and 100% moisture content

Soil fertility parameters	Organic carbon (%)		Available N (kgha^{-1})		Available P (kgha^{-1})		Available K (kgha^{-1})	
Moisture	60%	100%	60%	100%	60%	100%	60%	100%
Fertilizer								
F$_1$ (KCl)	0.644	0.513	286.6[b]	251.6[b]	57.54[b]	40.04[b]	205.1[b]	160.9[b]
F$_2$ (K$_2$SO$_4$)	0.670	0.538	297.2[a]	262.2[a]	61.71[a]	44.21[a]	215.5[a]	171.3[a]
Mean	**0.657**	**0.525**	**291.9**	**256.9**	**59.63**	**42.13**	**210.3**	**166.1**
SEd	0.006	0.006	1.17	1.16	0.85	0.85	1.02	1.02
CD (0.05)	NS	NS	5.02	5.0	3.68	3.68	4.40	4.4
Concentration								
C1 (0 ppm)	0.518[f]	0.398[e]	216.2[g]	191.2[g]	34.50[g]	22.50[f]	157.8[g]	125.8[f]
C2 (2 ppm)	0.552[e]	0.422[e]	266.8[f]	236.8[f]	44.50[f]	29.50[e]	172.5[f]	135.5[e]
C3 (5 ppm)	0.587[d]	0.457[d]	280.0[e]	250.0[e]	51.67[e]	36.67[d]	190.2[e]	153.2[d]
C4 (10 ppm)	0.632[c]	0.502[c]	296.2[d]	259.2[d]	57.83[d]	39.83[c]	204.5[d]	162.5[c]
C5 (20 ppm)	0.697[b]	0.567[b]	307.5[c]	270.5[c]	64.83[c]	46.83[b]	219.3[c]	177.3[b]
C6 (30 ppm)	0.755[a]	0.622[a]	314.0[b]	277.0[b]	71.17[b]	53.17[a]	238.7[b]	189.7[a]
C7 (40 ppm)	0.763[a]	0.623[a]	327.3[a]	285.3[a]	75.17[a]	53.17[a]	250.0[a]	195.0[a]
C8 (50 ppm)	0.753[a]	0.613[a]	327.2[a]	285.2[a]	77.33[a]	55.33[a]	249.5[a]	189.5[a]
Mean	**0.657**	**0.525**	**291.9**	**256.9**	**59.63**	**42.13**	**210.3**	**166.1**
SEd	0.011	0.013	2.96	3.0	1.08	1.07	2.89	2.89
CD (0.05)	0.023	0.235	6.06	6.1	2.20	2.19	5.93	5.92

Table 2. Soil fertility of K enriched *Azolla* as influenced by interaction effect between fertilizer Vs. concentration at 60 and 100% moisture content

Soil fertility parameters	Organic carbon (%)		Available N (kgha^{-1})		Available P (kgha^{-1})		Available K (kgha^{-1})	
Moisture	60%	100%	60%	100%	60%	100%	60%	100%
F$_1$C$_1$	0.510	0.390	214.3	189.3	33.33^j	21.33^j	156.3^f	124.3^h
F$_1$C$_2$	0.543	0.413	263.7	233.7	44.00^i	29.00^i	171.3^e	134.3^f
F$_1$C$_3$	0.577	0.447	278.0	248.0	51.67^h	36.67^h	187.3^d	150.3^e
F$_1$C$_4$	0.617	0.487	287.0	250.0	56.67^g	38.67^{gh}	195.3^d	153.3^e
F$_1$C$_5$	0.670	0.540	302.7	265.7	61.00^f	43.00^f	207.0^c	165.0^d
F$_1$C$_6$	0.727	0.593	307.3	270.3	65.00^e	47.00^e	226.0^b	177.0^c
F$_1$C$_7$	0.757	0.617	319.7	277.7	73.00^c	51.00^d	249.3^a	194.3^{ab}
F$_1$C$_8$	0.753	0.613	320.3	278.3	75.67^{bc}	53.67^{cd}	248.3^a	188.3^b
F$_2$C$_1$	0.527	0.407	218.0	193.0	35.67^j	23.67^j	159.3^f	127.3^h
F$_2$C$_2$	0.560	0.430	270.0	240.0	45.00^i	30.00^i	173.7^e	136.7^f
F$_2$C$_3$	0.597	0.467	282.0	252.0	51.67^h	36.67^h	193.0^d	156.0^e
F$_2$C$_4$	0.647	0.517	305.3	268.3	59.00^{fg}	41.00^{fg}	213.7^c	171.7^{cd}
F$_2$C$_5$	0.723	0.593	312.3	275.3	68.67^d	50.67^d	231.7^b	189.7^b
F$_2$C$_6$	0.783	0.650	320.7	283.7	77.33^{ab}	59.33^a	251.3^a	202.3^a
F$_2$C$_7$	0.770	0.630	335.0	293.0	77.33^{ab}	55.33^{bc}	250.7^a	195.7^{ab}
F$_2$C$_8$	0.753	0.613	334.0	292.0	79.00^a	57.00^{ab}	250.7^a	190.7^b
Mean	0.657	0.525	291.9	256.9	59.63	42.13	210.3	166.1
SEd								
F at C	0.016	0.017	4.08	4.09	1.66	1.65	3.96	3.96
C at F	0.016	0.016	4.18	4.19	1.52	1.51	4.09	4.09
CD (0.05)								
F at C	NS	NS	NS	NS	4.39	4.39	8.71	3.9
C at F	NS	NS	NS	NS	3.11	3.11	8.38	4.1

Fig. 1. Nitrogen assimilating enzymes as influenced by K fertilizer
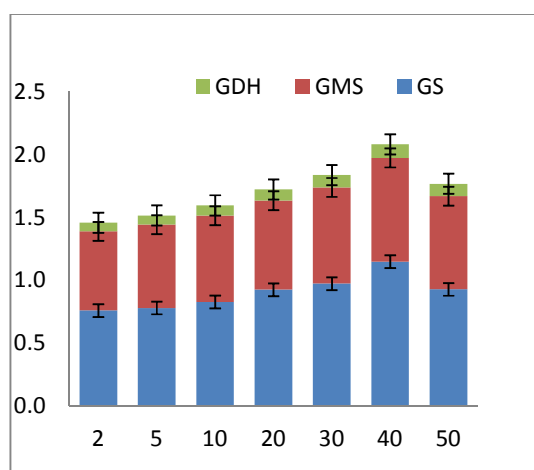
Fig. 2. Nitrogen fixing enzyme as influenced by concentration of fertilizers

3.4 Available K

The available K content of soil incorporated with K enriched *Azolla* was significantly influenced by the main and interaction effect of K fertilizer and its concentration. Irrespective of the fertilizer and their concentration about 210.3 and 166.1 kgha^{-1} of available K was maintained by the

incorporation of K enriched *Azolla* in soil at 60 and 100% moisture respectively (Table 1). Among the K fertilizer, K_2SO_4 was superior in maintaining available K content (215.5 and 171.3 kgha^{-1}) in *Azolla* incorporated soil which was 5 - 7% more than the KCl (205.1 and 160.9 kgha^{-1}). With respect to concentration of K, 40 and 50 ppm of K at 60% moisture and 30.40 and 50 ppm of K at 100% moisture registered significantly higher and equal available K in *Azolla* incorporated soil followed by 30 and 20 ppm of K at 60 and 100% moisture respectively. The *Azolla* grown under 0 ppm of K registered the lowest available K content of 157.8 and 125.8 kgha^{-1}at 60 and 100% moisture content respectively. Under the interaction between K fertilizer and its concentration, K_2SO_4 with 30, 40 and 50 ppm at 60% moisture content and 40 and 50 ppm of K_2SO_4 and 40 ppm of KCl produced statistically higher and equal available K in *Azolla* incorporated soil maintained at 60 and 100% moisture respectively (Table 2). The 0 ppm of both the fertilizer registered the lowest available K content (159.3 and 156.3 kgha^{-1}) in soil incorporated with *Azolla* grown under these concentrations at both moisture content.

4. DISCUSSION

Soil fertility is influenced by the humic substances formed during the decomposition of *Azolla* [13]. Incorporation of *Azolla* enhanced the soil nutrients availability by their biological activity. The decomposed organic matter from *Azolla* biomass played an active role in the development of microbial population. Similarly, [14] showed the increased of cellulolytic and urea hydrolyzing activities in addition to significant increase in the population of heterotrophic bacteria by the added *Azolla*. Soil incorporation of *Azolla* also increased urease and phosphatase activity [15]. *Azolla* contains macro, secondary and micronutrients that is important for quality rice production [16]. Sutanto [17] stated that, the use of 7.5 ton ha^{-1} *Azolla* to paddy field increased soil organic matter (C-organic) 0.09 times of control (without *Azolla*). Syamsiyah and Sunarmint [6] proved that, application of *Azolla* at 2 ton ha^{-1} could increase the organic matter up to 3.69% compare to the field without *Azolla*. The increasing of organic C is caused by the high content of organic C in *Azolla*. The incorporated *Azolla* into soil would soon be mineralized. Watanabe et al. [18] stated that 90% of *Azolla* was decomposed in 4 weeks and releases humic substances in to the soil. The increase in grain yield might be due to build up of soil organic

carbon and more nitrogen through the integrated use of NPK and green manuring with *Azolla*.

Biological Nitrogen Fixation (BNF) is a major source of fixed N for plant life and to sustain production and productivity of agricultural crops. Estimate of global terrestrial showed that the BNF ranged from 100 to 290 million tonnes of N/ year. Of this, 40–48 million tonnes is estimated to be biologically fixed in agricultural crops and fields. BNF is one of the natural sources of nitrogen for rice and *Azolla*-cyanobacteria biomass has been identified as potential source of nitrogen. The glutamate synthase enzyme dominated in ammonia assimilation followed by glutamine synthates and glutamate dehydrogenase in *Azolla* (Fig, 1).The activity of all the three enzymes were more at the 40 ppm of K followed by 30 and 50 ppm of K. Incorporation of 40 ppm K either as KCl and K_2SO_4 incubated *Azolla* enhanced ammonia assimilation and improved soil fertility (Fig. 2) on 30 days which may help to reduce nitrogen demand for rice crop [19].

According to Roy [20], incorporation of 6 t of *Azolla* ha^{-1}, equivalent to 36 kg of N ha^{-1} before planting and incorporation of 1 ton ha^{-1}*Azolla*, equivalent to 24 kg N ha^{-1} after 3-4 days of planting. Fogg et al. [21] have found that *Azolla* and cyanobacteria bio-fertilizer can add as much as 30-120 kg N ha^{-1} per crop. Many researchers have considered cyanobacteria as apromising source of nitrogen in tropical rice soils. Valiente et al. [22] investigated the potential contribution of N_2 fixation by indigenous cyanobacteria to rice soil with increasing amount of fertilizers. Ventura and Watanabel [23] concluded that about 50% of the N in *Azolla* was mineralized after 2 weeks of incubation with more than 3% N content. Use of *Azolla* as green manuring and as intercrop proved beneficial and significantly improved P content. The highest available phosphorus (29.6 kg ha^{-1}) was recorded with 100% NPK + green manuring of *Azolla* [16]. *Azolla* also contributes to the supply of Phosphorus, Potassium, Sulfur, Zinc, Iron and Molybdenum in sufficient amounts in addition to other micronutrients besides addition of Nitrogen. Similarly, the soil biological health due to application of *Azolla* has resulted in improving mineralization and consequent increase in the soil microbial status [24].

5. CONCLUSION

Soil biological health, mineralization and consequent increase in nutrient status by the

application of K enriched *Azolla* was more under 60% soil moisture content than fully saturated soil. Further, enrichment of *Azolla* with 40 ppm of K_2SO_4 enhanced nutrient content in *Azolla* and simultaneously in soil.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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