Original Research Article

Soil ~~F~~fertility as influenced by incorporation of K enriched ~~a~~Azolla

Abstract

Use of chemical fertilizer injudiciously without organic manure has created many soil health problems. Therefore, alternate sources of N has to be evolved to supply crop demanded N with less or no environmental pollution. Biological Nitrogen Fixation (BNF) is a major source of fixed N for plant life and to sustain production and productivity of agricultural crops. 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. 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 ~~thrice~~ 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. Soil fertility ~~is~~ was influenced by the ~~humic~~/humid substances formed during the decomposition of Azolla. 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. ~~Application of 10 tons/ha of manure were able to increase soil organic C by 24.4% compared to control (Syamsiyah *et al*. 2015)~~. 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 ~~has~~/had a high N content ~~and~~ released into the soil after decomposition. Azolla also contribute~~s~~d to the supply of ~~P~~phosphorus, ~~P~~potassium, ~~S~~sulfur, ~~Z~~zinc, ~~I~~iron and ~~M~~molybdenum in sufficient amounts in addition to

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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 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 in azolla incorporated soil maintained 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: between 4-8 words

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 (Awodun, 2008). Rice crops remove around 16-17 kg N for the production of each ton of paddy cultivation (Sahrawat, 2000). Most of the rice soils of the world are deficient in N, so, fertilizer N applications are required to meet its N demand. Generally, urea is applied as the N source for rice production. But the efficiency of added urea-N is very low, due to denitrification, NH_3 volatilization and leaching (De Datta and Buresh 1989). 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 days 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 in agricultural crops and fields. BNF is one of the natural

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1. One to two lines of introduction to the topic
2. Aim/objective of the study
3. Place and duration of the study
4. Methodology adopted
5. Results; summary with main points
6. Conclusion

Note: All the above should be written with not more than 300 words as stated in the Journal's authors guide

Also; Keywords between 4 to 8 words should be written

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Comment [EA5]: Important point but with no reference 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 (Subba Rao, 2005). 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 (Syamsiyah *et al*., 2015). Subedi and Shrestha (2015) 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 ~~thrice~~ three times. 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 and the fern was collected on 7^{th}, 15^{th}, 30^{th}, 60^{th}, 90^{th} and 120^{th} day after incubation / culturing, rinsed with distilled water and analysed for various biometric and biochemical parameters.

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Comment [EA6]: Basic information about the study/experimental site, meteorological and soil information

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The collected ~~a~~Azolla was incorporated with soil ~~@~~at 10 t~~–~~/ha and maintained at two moisture condition such as 60 and 100 percent. The ~~a~~Azolla incorporated soil was collected after 30 days of incubation, processed and analyzed for various available plant nutrients and organic carbon status. Organic carbon present in soil ~~is~~/was oxidi~~s~~ed/oxidized by chromic acid ($K_2Cr_2O_7$) in the presence of conc. H_2SO_4. Potassium dichromate on reaction with H_2SO_4 provide~~s~~d nascent oxygen which combine~~s~~d with carbon ~~and~~ to form~~s~~ CO_2. The excess chromic acid left unused by the organic matter ~~is~~/was determined by back titration with 0.5 N ferrous sulphate or ferrous ammonium sulphate using diphenylamine indicator (Walkley and Black, 1934). Available nitrogen in the soil ~~is~~ was estimated by alkaline permanganate method (Subbiah and Asija, 1956). Available phosphorus extracted with 0.03 N NH_4F and 0.025 N HCl. The amount of P extracted ~~is~~ was treated with ammonium molybdate and antimony potassium tartarate and developed colour with ascorbic acid. The intentensity of blue colour ~~is~~/was determined colorimetrically at 660 nm (Bray *et al*., 1945). The soil ~~is~~ was leached with neutral normal ammonium acetate and the K^+ ions in the exchange sites ~~are~~ were replaced by NH_4^+ ions. The K^+ ions in solution ~~is~~ was then determined with the flame photometer (Standford and English (1949).

Data Collection

Statistical Analysis

3. Results

3.1. Organic carbon

The effect of K fertilizer, its concentration and their interaction on the organic carbon content of ~~a~~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 ~~a~~Azolla incorporated ~~soil incubated~~ at both 60 % and 100 % moisture ~~status~~ content.

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Comment [EA10]: How was the data analysed and with what statistical tool?

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The mean organic carbon content of the soil was 0.657 and 0.525-% by K enriched ~~a~~Azolla at 60 and 100–% –moisture contents respectively. Azolla enriched with 40–ppm K solution recorded significantly ~~superior and~~ higher soil organic carbon content of 0.763 and 0.623-% respectively at 60 and 100 % soil moisture ~~status~~ content (Table 1) which was 42 and 56-% more than the ~~control~~ ~~(a~~Azolla grown under 0-ppm K~~)~~. However, it was on par with 50 and 30-ppm of K solution in both the ~~moisture maintained~~ 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 ~~a~~Azolla grown under K fertilizer and its concentration alone significantly influenced the available N content at both 60 and 100–% moisture ~~status~~ content. On an average 291.9 and 256.9 ~~kg / ha~~ kgha^{-1} of available N was maintained by the incorporation of K enriched ~~a~~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~~ kgha^{-1} ~~and it~~ which was 4 to 5–% higher than the KCl which registered 286.6 and 251.6 ~~kg / ha~~ kgha^{-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 ~~kg / ha~~ 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 kgha^{-1} at 60 and 100 % moisture ~~status~~ content respectively and it was 4.0% ~~percent~~ higher than 30-ppm of K enriched ~~a~~Azolla.

3.3. Available P

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The main and interaction of fertilizer and their concentration significantly influenced the available P status of ~~a~~Azolla incorporated into the 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 ~~a~~Azolla registered 59.63 and 42.13 ~~kg / ha~~ kgha^{-1} of available P at 60 and 100% soil moisture respectively. Among the fertilizers used, the K_2SO_4 ~~was superior and~~ maintained higher available P content of 61.71 and 44.21 ~~kg / ha~~ kgha^{-1} in the soil incorporated with K enriched ~~a~~Azolla at 60 and 100 % moisture content respectively followed by KCl which registered the available P content of 57.54 and 40.04 ~~kg / ha~~ kgha^{-1} (Table 1). Among the various concentration, 40 and 50-ppm K ~~were~~ was significantly maintained higher and equal soil available P status of 75.17 and 77.33 ~~kg / ha~~ kgha^{-1} respectively which was 8.6% ~~percent~~ more than the 30-ppm K (71.17 ~~kg / ha~~ kgha^{-1}) at 60% moisture content.

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

Soil Fertility Parameters	Organic Carbon (%)		Available N (~~Kg/ha~~ kgha^{-1})		Available P (~~Kg/ha~~ kgha^{-1})		Available K (~~Kg/ha~~ 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_2SO_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}

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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 condition content

Soil Fertility Parameters	Organic Carbon (%)		Available N (Kg/ha kgha^{-1})		Available P (Kg/ha kgha^{-1})		Available K (Kg/ha kgha^{-1})	
Moisture	60 %	100 %	60 %	100 %	60 %	100 %	60 %	100 %
F_1C_1	0.510	0.390	214.3	189.3	33.33^{j}	21.33^{j}	156.3^{f}	124.3^{h}
F_1C_2	0.543	0.413	263.7	233.7	44.00^{i}	29.00^{i}	171.3^{e}	134.3^{f}
F_1C_3	0.577	0.447	278.0	248.0	51.67^{h}	36.67^{h}	187.3^{d}	150.3^{e}
F_1C_4	0.617	0.487	287.0	250.0	56.67^{g}	38.67^{gh}	195.3^{d}	153.3^{e}
F_1C_5	0.670	0.540	302.7	265.7	61.00^{f}	43.00^{f}	207.0^{c}	165.0^{d}
F_1C_6	0.727	0.593	307.3	270.3	65.00^{e}	47.00^{e}	226.0^{b}	177.0^{c}
F_1C_7	0.757	0.617	319.7	277.7	73.00^{c}	51.00^{d}	249.3^{a}	194.3^{ab}
F_1C_8	0.753	0.613	320.3	278.3	75.67^{bc}	53.67^{cd}	248.3^{a}	188.3^{b}
F_2C_1	0.527	0.407	218.0	193.0	35.67^{j}	23.67^{j}	159.3^{f}	127.3^{h}
F_2C_2	0.560	0.430	270.0	240.0	45.00^{i}	30.00^{i}	173.7^{e}	136.7^{f}
F_2C_3	0.597	0.467	282.0	252.0	51.67^{h}	36.67^{h}	193.0^{d}	156.0^{e}
F_2C_4	0.647	0.517	305.3	268.3	59.00^{fg}	41.00^{fg}	213.7^{c}	171.7^{cd}
F_2C_5	0.723	0.593	312.3	275.3	68.67^{d}	50.67^{d}	231.7^{b}	189.7^{b}
F_2C_6	0.783	0.650	320.7	283.7	77.33^{ab}	59.33^{a}	251.3^{a}	202.3^{a}
F_2C_7	0.770	0.630	335.0	293.0	77.33^{ab}	55.33^{bc}	250.7^{a}	195.7^{ab}
F_2C_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

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available P at 60 and 100 % soil moisture respectively. Among the fertilizers used, the K_2SO_4 was superior and maintained higher available P content of 61.71 and 44.21 kg / ha in the soil incorporated with K enriched azolla at 60 and 100 % moisture respectively followed by KCl which registered the available P content of 57.54 and 40.04 kg / ha (Table 1). 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 respectively which was 8.6 percent more than the 30 ppm K (71.17 kg / ha) at 60 % moisture content.~~

But in 100~~-~~% soil moisture ~~condition~~ content~~,~~ at 30, 40 and 50~~-~~ppm, K ~~were~~ 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 ~~kg / ha~~ kgha^{-1} was registered by soil incorporated with ~~a~~Azolla enriched with 0~~-~~ppm of K at 60 and 100~~-~~% moisture ~~condition~~ content. Under the interaction between K fertilizer and its concentration, K_2SO_4 at 50 ~~-~~and 30 ppm were significantly superior in maintaining higher available P status at 100 % moisture content followed by 40 ppm K_2SO_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~~ kgha^{-1}) of ~~a~~Azolla incorporated soil.

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3.4. Available K

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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 ~~kg / ha~~ kgha^{-1} of available K was maintained by the incorporation of K enriched ~~a~~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 ~~kg / ha~~ kgha^{-1}) in ~~a~~Azolla incorporated soil which was 5 -7 %~~per cent~~ more than the KCl (205.1 and 160.9 ~~kg / ha~~ 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 ~~a~~Azolla incorporated soil followed by 30 and 20 ppm of K at 60 and 100 % moisture respectively. The ~~a~~Azolla grown under 0 ppm of K registered the lowest available K content of 157.8 ~~-~~and 125.8 ~~kg / ha~~ kgha^{-1} at 60 and

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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 ~~a~~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 ~~kg / ha~~ kgha^{-1}) in soil incorporated with ~~a~~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 (Bhardwaj and Gaur 1970). Incorporation of Azolla enhanced the soil nutrients availability by their biological activity. The decomposed organic matter from Azolla biomass plays~~s~~ed an active role in the development of microbial population. Similarly, Kannaiyan and Subramani (1992) 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 (Thanikachalam *et al*. 1984). Azolla contains macro, secondary and micronutrients that is important for quality rice production (Kumar and Shahi, 2016). Sutanto (2002) 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 *et al*. (2015) proved that, application of Azolla ~~@~~ at 2 ~~tons/ha~~ 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*, (1991) 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.

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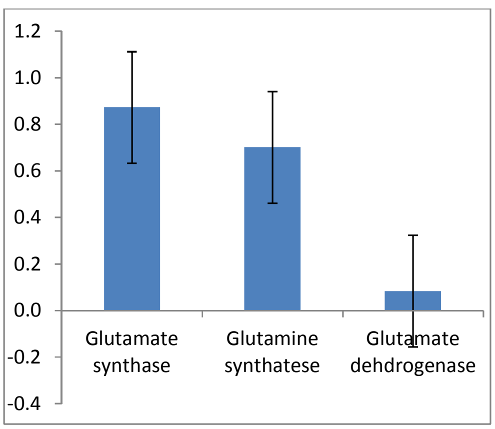
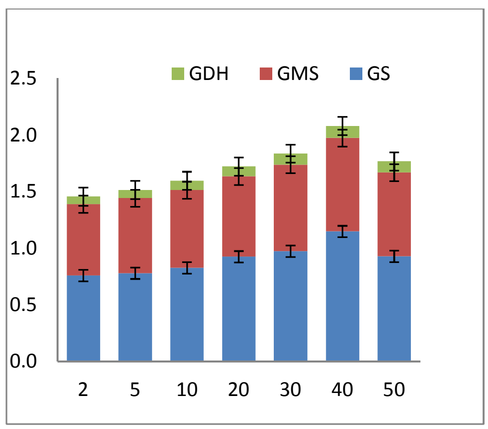
Biological Nitrogen Fixation (BNF) is a major source of fixed N for plant life and to sustain production and productivity of agricultural crops. Estimate of global terrestrial showed that the BNF ranged from 100 to 290 million tonnes of N / year. Of this, 40–48 million tonnes is estimated to be biologically 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 ~~a~~Azolla enhanced ammonia assimilation and improved soil fertility (Fig. 2) on 30 days which may help to reduce nitrogen demand for rice crop (Muruganayaki, 2017).



~~Fig~~ Figure 1. Nitrogen assimilating enzymes as influenced by K fertilizer



~~Fig~~ Figure 2. Nitrogen fixing enzyme as influenced by concentration of fertilizers

According to Roy, (1981), incorporation of 6 t of ~~*Azolla*/ha~~ *Azolla* ha^{-1}, equivalent to 36 kg of ~~N/ha~~ N ha^{-1} before planting and incorporation of 1 ~~t/ha~~ ton ha^{-1} *Azolla*, equivalent to 24 kg ~~N/ha~~ N ha^{-1} after 3-4 days of planting. Fogg *et al*. (1973) have found that *Azolla* and cyanobacteria bio-fertilizer can add as much as 30-120 kg ~~N / ha~~ N ha^{-1} per crop. Many

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researchers have considered cyanobacteria as a promising source of nitrogen in tropical rice soils. (Valiente *et al.*, 1998) investigated the potential contribution of N_2 fixation by indigenous cyanobacteria to rice soil with increasing amount of fertilizers. Ventura *et al.* (2012) concluded that about 50% of the N in Azolla was mineralized after 2 weeks of incubation with more than 3% N content. Use of aAzolla as green manuring and as intercrop proved beneficial and significant result over control in respect of P content. The highest available phosphorus (29.6 kg ha^{-1}) was recorded with 100% NPK + green manuring of aAzolla (Kumar and Shahi, 2016). 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 (Yadav *et al*. 2014).

5. Conclusion

Soil biological health, mineralization and nutrient status by the application of K enriched Azolla was more under 60 % soil moisture content than fully saturated soil. Further, enrichment of aAzolla with 40 ppm of K_2SO_4 enhanced nutrient content in aAzolla and subsequently in soil.

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