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Effects of *Azolla* and inorganic nitrogen application on growth and yield of rice in mwea irrigation scheme

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Abstract

Use of inorganic fertilizers constitutes 20% of the rice production cost in Mwea. *Azolla* fern, which grows in Mwea Irrigation paddies, has the potential to supplement the nitrogen requirement, thus reducing the fertilizer costs. A field experiment was conducted in Mwea Irrigation Scheme during 2015 long and short rains to determine the effect of *Azolla* incorporation and inorganic nitrogen on growth and yield of rice. The treatments comprised three nitrogen fertilizer levels (0, 30 and 60kg N ha^{-1}) and three *Azolla* biomass levels (0, 7.5 and 15tons ha^{-1}) laid out in a randomized complete block design with a split-plot arrangement. Data on plant height and number of tillers per plant were collected at 21, 35, 45, and 65 and 75 days after transplanting rice while yield and yield components were determined at 120 days after transplanting rice. Soil was analysed for N, P and K, before and after termination of the experiment. Data were subjected to analysis of variance using SAS and means separated using the least significant difference test at $p \leq 0.05$. *Azolla* incorporation significantly increased residual soil phosphorus, grain weight, % grain filling and grain yield. Inorganic nitrogen significantly increased plant height, tiller numbers, neck node, and panicle length, number of panicle m^{-2} and grain yield. Grain yield increase from *Azolla* treatment ranged from 5 to 42% compared to that of inorganic nitrogen which ranged from 18 to 36%. Application of 15t ha^{-1} of *Azolla* biomass recorded the highest yield, however, it was not significantly different from that of 7.5t ha^{-1}.

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Introduction

In Mwea Irrigation Scheme, paddy rice cultivation is intensively done within 8,000 ha of arable land (Rice MAPP, 2012). In this area, soil fertility is a challenge especially due to N limitation. Low nitrogen fertility in paddies is attributed to over exploitation of soils through continuous cropping and export of nutrients through removal of rice straw as hay without commensurate replacement in form of organic manure and inorganic fertilizers. This phenomenon of removal of straw for fodder is reported to impoverish the soil through depletion of K and Si (Dobermann, 2002). Studies by Rice MAPP (2012) indicated that the cost of adding inorganic fertilizers to rice fields constituted 20% of the total cost of rice production in Mwea Irrigation Scheme.

Nitrogen is the most limiting factor in crop production and it is essential for growth, protein manufacture and yield (Blumenthal *et al.*, 2008). It is an important nutrient required in large quantities for growth of rice (De Datta, 1991). Wanjogu *et al.* (1995) recommended 60-80kg ha^{-1} N to a rice crop in Mwea Irrigation Scheme. Continous use of inorganic sources of nitrogen is a major source of contamination and pollution of aquatic environments (Choudhury, 2005). Besides, the costs of these fertilizers are high. Thus, use of bio-fertilizers such as *Azolla* is a good alternative to inorganic fertilizers.

Azolla fern is prevalent worldwide and has been used over centuries for fertilization in paddies as a source of nitrogen. However, its use reduced with the manufacture of inorganic fertilizers after the industrial revolution (Carrapico *et al.*, 2002). The fern has a symbiotic association with cyanobacteria, through which it fixes nitrogen released to rice crop in paddies (Bocchi *et al.*, 2010; Carrapico, 2002). *Azolla anabaena* association can fix 3-7kg N ha^{-1} daily of which more than 50% is made available within the first 6-8 weeks of crop growth, when incorporated in the soil (Watanabe, 1997). According to Mlingham *et al.* (2014), *Azolla* decomposes rapidly and releases 60-80% of its tissue nitrogen within two months of incorporation into, the soil, making it important for use as a bio-fertilizer for rice crop growth**.** *Azolla* has been shown to contain 4-5% N, 1-1.5% P and 2-3% K on a dry weight basis (Watanabe *et al.*, 1983), and fixes nitrogen at a rate higher than legumes (Wagner, 1997). It is capable of replacing 50% of inorganic nitrogen requirement (Bochi, 2010). According to IRRI (1997), it can provide up to 40kg N/ha in two weeks. When *Azolla* biomass is incorporated in the soil, it provides N, P and K nutrients whose residual effects benefit crops grown in the subsequent season (Ferentinos, 2002).

Studies on *Azolla* bio-fertilizer use have shown that it increases yield equivalent to application of 60-80kg N/ha, enhances rapid mineralization of nitrogen in the soil, improves soil physical and chemical condition and increases soil organic matter (Subedi *et al.*, 2015). Consequently, it improves productivity and crop performance (Alim, 2012). Setiawati *et al.* (2018) reported increase in the available soil P, plant P content and tiller numbers with use of composted *Azolla* powder. Watanabe (1997) reported that *Azolla* use increased straw and grain yield by more than 17%. In Ahero, *Azolla* with supplemental inorganic nitrogen gave significant increase in paddy yields although biomass production was reported to be uneconomical (AIR, report no 44, 1987).

The species of *Azolla* used I this trial was the native *Azolla nilotica* (AIR, report no 45, 1987). However, new invasive species have been reported (Hill, 2004). Considering that different species have varied nitrogen contribution and that current *Azolla* biomass levels in flooded paddies at peak times is up to 50%, a positive effect can be obtained from *Azolla* use. The use of *Azolla* as an organic source of nitrogen therefore has the potential to reduce the fertilizer cost of rice production**.** Thus, integration of *Azolla* which has nitrogen fixation ability and is available in Mwea paddies has the potential to improve soil fertility, increase rice productivity and reduce the cost of production. This study evaluated the effects of application of *Azolla* bio-fertilizer and inorganic nitrogen on growth and yield of rice in paddy fields of Mwea Irrigation Scheme.

Materials and methods

Site description

The experiment was undertaken in Mwea Irrigation Scheme, within Mwea Irrigation and Agricultural Development Centre (MIAD) at A and D line research plots, during the period of 2015 and 2016 short rains. The average temperature for the period was 23ºC with relative humidity of 78% (Table 1). The experimental plots had black cotton soils (vertisols), and with a pH average of 6.0. The average and maximum temperatures were higher during growth and active tillering but lower during heading and maturity for second season than first season (Fig 1).

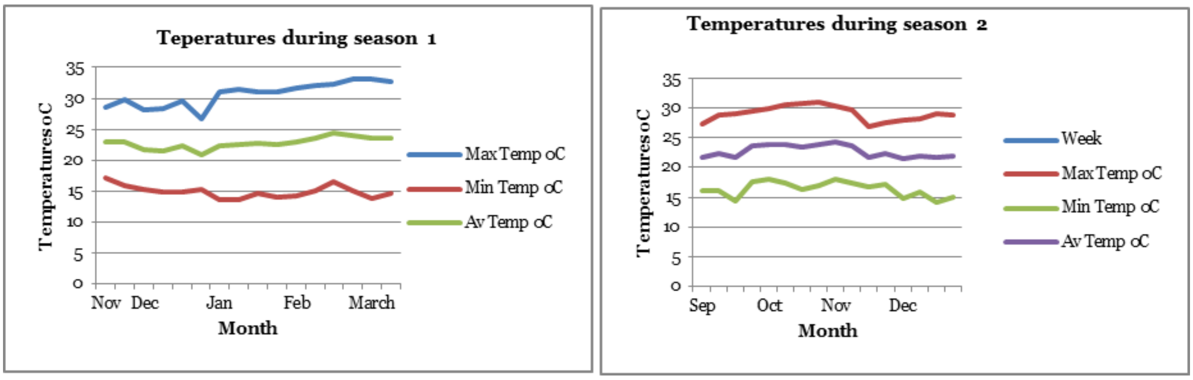

Fig 1. Temperatures during the experimental period of 2015 and 2016.

Experimental design

The treatments in this study comprised three nitrogen levels of 0, 30 and 60kg N ha^{-1} and three levels of *Azolla* fresh biomass of 0, 7.5 and 15tons ha^{-1}. The treatments were laid out in a randomized complete block design with a split plot arrangement. The nitrogen was assigned to the main plots while *Azolla* was assigned to the sub-plots. Phosphorus and Potassium were applied at standard rates of 50kg P_2O_5 ha^{-1} and 50kg K_2O ha^{-1} respectively, to all plots by broadcasting at transplanting. Triple superphosphate and muriate of potash were used as the sources of P and K respectively. Each main plot was separated by a 500 gauge polythene sheet to prevent lateral nutrient mobility. Basmati 370 was the test variety. It was grown at a spacing of 30 x 15cm, using one seedling per hill and transplanted 21 days after sowing. Weed control was done by manual weeding, three times at 14, 30 and 45 days after transplanting. Pests (stem borer and leaf folders) and disease (blast and brown spot) control were done by spraying with Cyclone and Cabendazim at 21 and 35 Days after transplanting, respectively.

Data collection

Data collected included plant height, tiller numbers, yield and yield components (panicle number, spikelets per panicle, grain weight and% filled grains). Ten hills per plot, excluding two border rows, were sampled to determine plant height and tiller numbers at 21, 30, 42, 55 and 75 days after transplanting, which corresponded with rooting, tillering, maximum tillering, panicle initiation and heading stages, respectively.

Plant height was measured incm from ground level to the vertical tip of the flag leaf using a measuring ruler. Tiller numbers were obtained by physically counting all the plants per hill in each of the tagged hills. Productive panicles were also counted from 10 hills and number of spikelets in each panicle determined. Grain yield was determined by harvesting plants from 1 m^2 in each plot. Ten hills from each plot were harvested, panicles counted and threshed. Unfilled grains were separated using water of specific gravity 1.01 and filled grains counted by an electronic multi-auto grain counter (Everwell corporation-Tokyo Japan) to determine the number of spikelets per panicle. Grain weight (g) was obtained by weighing 1000 filled grains using an electronic scale and adjusting the weight to 14% moisture content. Percentage of ripened grain (%) was obtained after separating filled and empty grains using a salt solution of specific gravity 1.01. Weather data was also recorded for the period. Soils were analyzed for pH, N, P and K before and after terminating the experiment.

Data analysis

Data was summarized in excel package and subjected to analysis of variance using SAS. Post hoc analysis (where there was significance) was carried out using the least significant difference test at p≤.05.

Results

Effect of Azolla incorporation and nutrient fertilizer on balance nutrient status in rice fields

The soil nutrient status at the end of the experiment is shown in Table 1. During the first season, application of inorganic nitrogen fertilizer had a significant effect on residual soil nitrogen and Ec but not on pH, N, P, K and organic carbon. Nitrogen fertilizer application had no effect on pH, N, P, K, organic carbon and Ec during the second season. In the first season, application of 30kg N ha^{-1} and 60kg N ha^{-1} significantly increased residual %N, relative to the no-fertilizer control. *Azolla* incorporation significantly increased the residual P content in the soil in both seasons but it had no effect on residual pH, N, K and Ec. Inorganic fertilizer application significantly affected N and Ec only in the first season. In the second season N fertilizer applied had no significant effect on all the said parameters. There was no significant interaction between inorganic nitrogen and *Azolla* incorporation with respect to all measured soil attributes.

Table 1. Balance nutrient status of *Azolla* and inorganic N in the soil during 2015 long and short rains.

	pH	% N	P (ppm)	K (meq/l)	% organic C	Ec (ds/m)
0kg ha^{-1} N	5.26	0.12	10.11	0.10	1.40	21.20
30kg ha^{-1} N	5.24	0.14	9.44	0.09	1.55	21.90
60kg ha^{-1} N	5.27	0.14	9.56	0.09	1.55	18.00
Mean	5.3	0.1	9.7	0.1	1.5	20.4
P-value	0.87	0.02	0.61	0.21	0.39	0.03
LSD (0.05)	NS	0.01	NS	NS	NS	2.90
CV (%)	2.6	8.9	17.1	38.5	16.0	14.0
0t ha^{-1} *Azolla*	5.19	0.13	8.22	0.08	1.45	20.00
7.5t ha^{-1} *Azolla*	5.31	0.13	10.11	0.09	1.56	20.00
15t ha^{-1} *Azolla*	5.30	0.13	10.78	0.09	1.49	20.90
P-value	0.23	0.65	0.01	0.83	0.62	0.16
LSD (0.05)	NS	NS	1.66	NS	NS	NS
AxN P-value	0.66	0.61	0.47	0.57	0.42	0.05
LSD (0.05)	NS	NS	NS	NS	NS	2.90
	Season 2					
	pH	% N	P (ppm)	K (meq/l)	% Organic C	Ec (ds/m)
0kg ha^{-1} N	4.89	0.14	9.33	0.25	1.78	50.00
30kg ha^{-1} N	4.99	0.15	10.00	0.22	1.75	45.00
60kg ha^{-1} N	4.85	0.17	9.67	0.223	1.72	44.00
Mean	4.90	0.15	9.67	0.23	1.75	46.00
P-value	0.33	0.32	0.60	0.59	0.87	0.79
LSD (0.05)	NS	NS	NS	NS	NS	NS
CV (%)	4.1	9.5	14.27	32.0	11.0	43.0
0t ha^{-1} *Azolla*	4.96	0.14	8.33	0.24	1.82	48.00
7.5t ha^{-1} *Azolla*	4.81	0.15	10.11	0.21	1.72	38.00
15t ha^{-1} *Azolla*	4.97	0.16	10.57	0.25	1.71	53.00
P-value	0.19	0.07	0.01	0.37	0.49	0.32
LSD (0.05)	NS	NS	1.38	NS	NS	NS

NS: Not significant, Means followed by the same letter within the same treatment group are statistically the same

Effect of Azolla and inorganic N on plant height and tiller numbers

During both seasons, application of 60kg N ha^{-1} had significantly more tillers per plant than either 30kg N ha^{-1} or the no-fetilizer. The effect of application of 30kg N ha^{-1} was not significantly different from that of control on tillers per plant during 45, 60 and 75 DAT's. There was no significant interaction between inorganic nitrogen and *Azolla* with respect to the number of tillers per plant. During the first season, application of 15t ha^{-1} *Azolla* gave significantly more tillers per plant than application of 7.5t ha^{-1} *Azolla* or control. In the second season, application of *Azolla* had no significant effect on tillers per plant.

Table 2. Effects of Inorganic N and *Azolla* on plant height and tillers during long and short rains of 2015.

Treatment	Season 1					
	Plant height (cm)			Tillers numbers m^{-2}		
	45DAT	60DAT	75DAT	45DAT	60DAT	75DAT
0kg N ha^{-1}	70.7	93.2	117.2	632.9	450.9	438.5
30kg N ha^{-1}	71.5	94.6	117.3	668.8	535	509.5
60kg N ha^{-1}	75.2	100.7	120.3	827.5	655	625.1
Mean	72.5	96.2	118.1	709.7	547	524
P- value	0.050	0.012	0.160	0.009	0.004	0.002
LSD (0.05)	3.8	4.84	NS	122.6	84.1	93.14
CV (%)	5.4	5.02	3.2	17.3	15.4	17.8
0t ha^{-1} *Azolla*	73.1	95.9	117.2	630.5	506	462.9
7.5t ha^{-1} *Azolla*	72.3	95.8	118.8	663.3	507	493.4
15t ha^{-1} *Azolla*	72.5	97.9	118.7	835.3	628	616.8
Mean	72.6	96.5	118.2	709.7	547.0	524.4
P- value	0.810	0.570	0.490	0.006	0.010	0.007
LSD (0.05)	NS	NS	NS	122.6	84.1	93.14
CV (%)	5.4	5.0	3.2	17.3	15.4	17.8
	Season 2					
0kg ha^{-1}	53.7	71.4	115.3	370	388	359.9
30kg N ha^{-1}	57.5	77.3	120.1	395	411.5	384
60kg N ha^{-1}	59.1	84.9	124.9	458	481	450.4
Mean	56.7	77.6	119.6	408	427	398
P- value	0.02	0.0001	0.025	0.003	0.002	0.002
LSD (0.05)	3.6	4.71	6.6	46.1	45.7	39
CV (%)	6.3	6.1	5.2	11.4	10.7	9.8
0t ha^{-1} *Azolla*	57.6	77.3	120.7	386	426.3	384.8
7.5t ha^{-1} *Azolla*	57	79.95	120.2	402	428	395
15t ha^{-1} *Azolla*	55.6	76.43	118.9	435.4	426	
Mean	56.7	77.9	119.9	407.8	426.8	390.0
P- value	0.51	0.28	0.78	0.11	0.99	0.11
LSD (0.05)	NS	NS	NS	NS	NS	NS
CV (%)	6.3	6.1	5.2	11.4	10.7	9.8

NS: Not significant, Means followed by the same letter within the same treatment group are statistically the same

Effects of Azolla and inorganic nitrogen and on yield and yield components

Application of inorganic N fertilizer significantly affected the length of neck node in the first but not in the second season (Table 3). There also no significant interaction effect of inorganic N and *Azolla* on length of neck node. However, application of 30kg N ha^{-1} gave significantly longer rice plant neck node than 60kg N ha^{-1} and the no-fertilizer control. *Azolla* application had no significant effect on neck node.

Application of inorganic N, *Azolla* incorporation, and the interaction between inorganic fertilizer and *Azolla* had no significant effect on panicle length and number of spikelets panicle^{-1} in both seasons. Inorganic N fertilizer application significantly increased the number of panicle m^{-2} in both seasons. Application of 60kg N ha^{-1} had significantly higher panicle m^{-2} than 30kg N ha^{-1}. During both first and second season, inorganic fertilizer application had no significant effect on grain weight. However, *Azolla* had significant effect on grain weight during the second season. Application of 15t ha^{-1} *Azolla* gave significantly higher grain weight than control but it was similar in grain yield to 7.5t ha^{-1} *Azolla* application. The interaction between inorganic N fertilizer and *Azolla* incorporation had no significant effect on grain weight in both seasons. During both first and second season, inorganic nitrogen significantly reduced% grain filling. Application of 60kg N ha^{-1} gave significantly lower% grain filling than either 30kg N ha^{-1} and no-fertilizer treatment in the first season, but not significantly different from application of 30kg N ha^{-1} in the second season. *Azolla* application did not affect% grain filling. The effect of interaction between *Azolla* and inorganic nitrogen on% grain filling was not significant.

Inorganic nitrogen fertilizer application significantly increased grain yields during both seasons.

During the first season, application of 60kg N ha^{-1} gave significantly higher grain yields than 30kg N ha^{-1} or control treatment. During the second season, 60kg N ha^{-1} had significantly higher yields than control but similar to 30kg N ha^{-1} treatment. *Azolla* incorporation significantly increased grain yield during both seasons. Application of 15t ha^{-1} *Azolla* had significantly higher grain yield than control but had similar grain yield to that of 7.5t ha^{-1} *Azolla* application. The effect of interaction between *Azolla* and inorganic nitrogen was not significant.

Table 3. Effect of inorganic N and *Azolla* on yield and yield components during the short and long rains of 2015.

Treatment	Season 1						
	Neck Node (cm)	Panicle length	Spikelets / panicle	Panicle m^{-2}	Grain weight	% filled grains	Yield (t/ha)
0kg ha^{-1} N	3.4	26.1	78.0	352.1	0.0213	0.79	3.5
30kg ha^{-1} N	3.7	25.8	82.7	413.0	0.0215	0.74	4.2
60kg ha^{-1} N	3.46	25.9	80.3	480.7	0.0211	0.69	4.9
Mean	3.5	25.9	80.6	415.3	0.0213	0.74	4.2
P- value	0.006	0.620	0.101	0.001	0.697	0.014	0.001
LSD (0.05)	0.14	NS	NS	34.50	NS	0.06	0.39
CV (%)	4.0	2.9	6.5	8.2	4.3	8.3	9.6
0t ha^{-1} *Azolla*	3.5	25.9	78.7	397.5	0.0212	0.73	3.9
7.5t ha^{-1} *Azolla*	3.6	25.9	82.7	419.0	0.0215	0.74	4.3
15t ha^{-1} *Azolla*	3.5	26.0	80.4	429.3	0.0213	0.75	4.4
Mean	3.5	25.9	80.6	415.3	0.0213	0.74	4.2
P- value	0.320	0.990	0.304	0.206	0.800	0.880	0.039
LSD (0.05)	NS	NS	NS	NS	NS	NS	0.34
	Season 2						
0kg ha^{-1} N	3.7	24.6	72.3	351.0	0.0229	0.86	4.8
30kg ha^{-1} N	3.79	25.3	74.8	401.7	0.0227	0.85	6.0
60kg ha^{-1} N	3.64	24.9	82.7	447.7	0.0223	0.77	6.5
Mean	3.7	24.9	74.6	400.0	0.0226	0.83	5.7
P-value	0.500	0.060	0.050	0.001	0.063	0.007	0.0001
LSD(0.05)	NS	NS	8.7	24.13	NS	0.043	0.61
CV (%)	7.04	2.60	11.20	7.60	3.4	5.2	10.6
0t ha^{-1} *Azolla*	3.7	24.8	68.2	401.4	22.0	0.82	5.2
7.5t ha^{-1} *Azolla*	3.75	25.09	78.8	392.3	23.0	0.83	5.8
15t ha^{-1} *Azolla*	3.65	24.9	76.7	406.7	23.0	0.84	6.0
Mean	3.7	24.9	74.6	400.0	0.0226	0.83	5.7
P-value	0.7	0.45	0.59	0.6	0.048	0.58	0.039
LSD (0.05)	NS	NS	NS	NS	0.83	NS	0.61

NS: Not significant, Means followed by the same letter within the same treatment group are statistically the same, Grain weight=weight of 1000 grains

Discussion

Azolla application significantly enhanced soil phosphorus content, while inorganic N fertilizer enhanced soil N content. *Azolla* tissue contained 1-1.5% P with a low C: N ratio, which makes its mineralization faster than other crops. This fast mineralization rate had an effect on enhancing the total soil P content. Subedi *et al. (*2013) reported similar findings. Inorganic N fertilizer (sulphate of ammonia) used in the study had high% of readily available N (21%). Comparatively, *Azolla* has lower N amounts (3.5%), whose release upon decomposition is also slow. Consequently, Inorganic N source was capable of improving the soil N status better than *Azolla*. The effect of inorganic N changing the soil N status has been previously reported by Cheng *et al.*, (2014). Awodun (2008) also reported the effect of *Azolla* increasing soil N, P and K status.

Azolla application increased grain weight and yield and tiller numbers. The tissue N content of *Azolla* had about 4% N, which upon decomposition is released for growth and development of crops (Ganeshamurthy *et al.*, 2006). However, the effect of *Azolla,* compared to inorganic fertilizer, benefited later stages of rice crop development. This suggests a comparatively slow release of nutrient N. The comparatively slow release suggests that *Azolla* incorporation should be done early before transplanting for it to benefit the vegetative stage of rice crop. Reported findings of Ito *et al.* (1985) showed a slow release with 60% of *Azolla* tissue N available within 20 days of incorporation.

The significant effect of *Azolla* on both growth and yield components during the second season compared to only yield components in the first season may be partly attributed to environmental temperatures. The second season had comparatively higher average temperatures (30ºC) than the first season (29ºC) and hence the effect experienced on yield and yield components. Previous studies by Qui *et al.* (2012) also showed that higher temperatures enhanced organic matter breakdown. Results therefore suggest that benefits of *Azolla* are more pronounced under high temperatures. The effect of application of 15t ha^{-1} *Azolla* was similar to that of 7.5t ha^{-1}. This suggests that 7.5t ha^{-1} *Azolla* is an optimum level beyond which it is uneconomical and has no significant increase in yields. This is in conformity with the recommended application of about 7.5t ha^{-1} organic manure (Tanaka, 1969).

Conclusion

Azolla application is beneficial to a rice crop, increases soil P status and enhances grain yield and yield components. Inorganic fertilizer releases N faster, increases residual soil N and benefits both growth and reproductive stages of rice crop.

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