EFFECTS OF *AZOLLA* BIOMASS GROWTH ON FLOOD WATER TEMPERATURE AND pH, TILLERING AND YIELD OF PADDY RICE†

[EFECTO DEL CRECIMIENTO DE LA BIOMASA DE *AZOLLA* EN LA TEMPERATURA DEL AGUA DE INUNDACIÓN Y pH, MACOLLAMIENTO Y RENDIMIENTO DE ARROZ]

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SUMMARY

Azolla is a floating pteridophyte, which forms an association with an endosymbiont, the nitrogen-fixing cyanobacterium *Anabaena azollae*. In Mwea Irrigation Scheme paddies, *Azolla* biomass coverage ranges from 25 to 50 % with a high biomass growth rate and this has caused a challenge in its management. Knowledge on the effect of *Azolla* biomass on rice tillering, root development and grain yield are important in order to exploit its potential benefits in Mwea Irrigation Scheme, where nearly 60 % of the total rice in Kenya is produced. The effect of *Azolla* on tillering, plant biomass, adventitious roots development, basal internode growth and yield of rice, was studied during 2015/2016 short and long rains. *Azolla* was inoculated in 30 cm x 30 cm plastic pots planted with rice variety Basmati 370. Controls where no *Azolla* was inoculated were included. Tiller number, plant height, flood water temperature and pH were determined at 21, 32, 42, 60 and 75 days after transplanting (DAT). At 120 DAT, grain yield and yield components were determined. Length of basal node and adventitious root were measured and dry biomass weights of, root and above ground biomass determined. Water temperature in *Azolla* inoculated pots were significantly lower than in controls by 0.8 °C and 1.3 °C in the second and first seasons respectively with a daily fluctuation of 2 °C. The pH of flood water in potted rice inoculated with *Azolla* was significantly lower than in control. *Azolla* inoculated potted rice had significantly higher number of tillers/m^2, spikelets per panicle^{-1}, panicle size m^{-2}, 1000 grain weight and plant biomass than in non-inoculated controls. Adventitious roots were significantly longer and more per unit area in all treatments than in controls. The basal stem node length was significantly shorter while root biomass was less for the *Azolla* inoculated potted rice than in controls. In all treatments, *Azolla* doubled its biomass within an average of 5 days.

Key words: *Azolla;* biomass; inoculated; Rice; tillering.

RESUMEN

Azolla es un pteridófito flotante, que forma una asociación con un endosimbionte, la cianobacteria fijadora de nitrógeno *Anabaena azollae*. En los arrozales del esquema de irrigación de Mwea, la cobertura de biomasa de *Azolla* oscila entre el 25 y el 50% con una alta tasa de crecimiento de la biomasa y esto causa un desafío en su gestión. El conocimiento sobre el efecto de la biomasa de *Azolla* en el macollamiento del arroz, el desarrollo de las raíces y el rendimiento de grano es importante para aprovechar sus beneficios potenciales en el Esquema de Riego Mwea, donde se produce casi el 60 % del arroz total en Kenia. El efecto de *Azolla* sobre la macolla, la biomasa de las plantas, el desarrollo de raíces adventicias, el crecimiento de los entrenudos basales y el rendimiento del arroz se estudió durante las lluvias cortas y largas de 2015/2016. *Azolla* se inoculó en macetas de plástico de 30 cm x 30 cm sembradas con arroz variedad Basmati 370. Se incluyeron controles donde no se inoculó *Azolla*. El número de plantas, la altura de la planta, la temperatura del agua de inundación y el pH se determinaron a los 21, 32, 42, 60 y 75 días después del trasplante (DAT). A 120 DAT, se determinaron el rendimiento de grano y los componentes de rendimiento. Se midió la longitud del nodo basal

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y la raíz adventicia y se determinaron los pesos de biomasa seca, la biomasa de la raíz y del suelo. La temperatura del agua en las macetas inoculadas de *Azolla* fue significativamente más baja que en los controles en 0.8 °C y 1.3 °C en la segunda y primera temporada, respectivamente, con una fluctuación diaria de 2 °C. El pH del agua de inundación en arroz en macetas inoculado con *Azolla* fue significativamente menor que en el control. El arroz en maceta inoculado con *Azolla* tenía un número significativamente mayor de macollas/m^2, espiguillas por panícula^{-1}, tamaño de panícula m^{-2}, peso de 1000 granos y biomasa vegetal que en los controles no inoculados. Las raíces adventicias fueron significativamente más largas y más por unidad de área en todos los tratamientos que en los controles. La longitud basal del nudo del tallo fue significativamente más corta, mientras que la biomasa de la raíz fue menor para el arroz en maceta inoculado con *Azolla* que en los controles. En todos los tratamientos, *Azolla* duplicó su biomasa en un promedio de 5 días.

Palabras clave: *Azolla*; biomasa inoculado Arroz; macollamiento.

INTRODUCTION

Azolla is an aquatic fern mainly found in tropical and temperate paddies and forms a symbiotic association with nitrogen fixing blue-green algae, *Anabaena azollae*. In Mwea Irrigation Scheme paddies, *Azolla* biomass coverage ranges from 25% to 50% at peak times. It has a fast biomass doubling rate and this has caused a challenge in its management making the farmers to have a negative perception about it. The fast multiplication rate of *Azolla* makes it a noxious weed (Ivens, 1989).

According to Campbell (2011), it doubles its biomass within 7-10 days under high nutrient levels and temperatures above 18 °C. Kitoh et al. (1993) reported a doubling rate of 2-3 days under laboratory condition, using polluted pond water while Hussner (2010) reported a doubling rate of 3-10 days. In Mwea Irrigation Scheme, Kenya, the doubling rate was found to be 4-6 days under average temperatures of 24 °C, relative humidity of 75% and pH of 6.85. *Azolla* biomass growth rate is affected by the environmental conditions and increases with light intensity and temperature (Wagner, 1997). The fast multiplication rate can lead to coverage of as high as 100 %, with a 30 cm thick mat which can block light, prevent photosynthesis and seriously affect oxygen diffusion (Landsdown, 2015). The biomass coverage shields light and hinders heat uptake from the environment thus reducing flood water temperatures. Fosu-Mensah et al. (2015), reported a reduction in flood water temperatures by 1.9-2.0 °C.

Studies have also shown that a full cover of *Azolla* significantly increases tiller count and grain yield but reduces flood water pH by 1.4 - 2.2 (de Macale and Vlek, 2004). In Kenya, studies on *Azolla* in Ahero research station have been done by incorporating it in paddies. However, physio-chemical characteristics and rice growth have not been conducted. The objective of this study was to determine the effects of *Azolla* biomass growth on flood water temperature and pH, tillering, adventitious root development and yield of paddy rice. The results of this experiment are therefore expected to enlighten farmers on the increased nitrogen efficiency uptake, increased tillering and grain weight of paddy rice.

MATERIALS AND METHODS

Experimental site

The study was conducted in Mwea Irrigation Scheme, at Mwea Irrigation and Agricultural Development Centre (MIAD), in Kirinyaga County located at an altitude of 1159 metres above sea level. Mwea lies within Agro-ecological zones LM3 and LM4 (marginal cotton zones). The climate is tropical with equatorial and medium high altitude characteristics and receives an average of 930 mm of rainfall out of which 510 mm is during the long rainy season, with 66 % reliability. The mean temperature is 22 ^oC with a minimum of 17^oC and a maximum of 28 ^oC. The soils are predominantly imperfectly drained vertisols (LB 8).

Experimental design and treatments

Treatments comprised *Azolla* inoculated and non-inoculated plots (control), laid out in a random complete block design, with three replications. In the *Azolla* treated pots, 40 g of fresh *Azolla* biomass (adequate to attain a total surface coverage of 500g/0.9 m^2 within 20 days) was inoculated in plastic pots, each measuring 30 cm x 30 cm, filled to 20 cm mark with top soil of Mwea rice growing area. Non treated control pots were not inoculated with *Azolla* biomass. Nitrogen was applied at the recommended Mwea levels (Wanjogu *et al*., 1992) of 60 kg N ha^{-1} in three splits: at transplanting, 21 days after transplanting and 55 days after transplanting. Phosphorus (P_2O_5) and potassium (K_2O) were supplied also at recommended Mwea levels of 50 kg ha^{-1}, each as triple super phosphate and muriate of potash respectively to all plots at seedling transplanting time. Rice variety Basmati 370 obtained from MIAD Seed Production Unit, and *Azolla* collected from the drainage canal within MIAD fields, were used for the trial. Two rice seedlings each were transplanted per pot and weed control was done manually at 21 and 35 days after transplanting. Water levels in the pots were maintained at 2.5-5 cm above soil level throughout the growing season.

About 5 g of fresh *Azolla* biomass was also inoculated in 7 plastic pots flooded with 3 litres of canal water obtained from MIAD irrigation canal (replenished every 3 days) and left to grow for 10 days to determine the doubling time. Temperatures and relative humidity for the growth period were recorded and final *Azolla* fresh biomass weight taken on the 10^{th} day after inoculation. *Azolla* doubling time was determined based on the formula DT = t/r (Kitoh and Shiomi, 1991) where: DT is doubling time, t = duration of growth; r = log wt. /w0 / 0.301; wt. = weight of *Azolla* at time t; w0 = weight of *Azolla* at zero time.

Data collection

Flood water temperatures were recorded at 9 am and 3 pm on 21, 35, 45 and 60 days after transplanting, by partial immersion of an ordinary mercury thermometer. Three measurements were taken from different points in each pot at each recording time. About 50 mls of flood water was drawn using a glass beaker on 21, 31, 45 and 60 days and pH determined using glass electrode method (Jackson, 1958). Ten hills per treatment were sampled to determine plant height and tiller numbers at 21, 30, 42, and 55 and 75 days after transplanting, corresponding to rooting, tillering, maximum tillering, panicle initiation and heading stages respectively.

Plant height was determined by measuring from ground level to the vertical tip of flag leaf, tillers numbers were obtained by physically counting tillers in each hill and averaging for 10 hills. Productive panicles were counted from 10 hills and number of spikelets in each panicle determined. Grain yield was determined by harvesting plants from 10 hills per treatment, threshing and weighing grains at adjusted moisture content of 14%. Unfilled grains were separated using a salt solution of specific gravity 1.01 and filled grains counted using an electronic multi-auto grain counter (Everwell corporation-Tokyo Japan) and the number of spikelets per panicle determined. Grain weight (g) was determined by weighing 1000 filled grains (using an electronic grain counter) and adjusting the weight to 14% moisture content. At 120 days after transplanting (maturity time), plants were excavated using a panga then uprooted by hand, root biomass separated from the shoot and both oven dried to constant weight at 100 ^{0}C for 28 hrs (Faichney and White, 1983). Root and shoot dry weights were then obtained using a scale (Salter Bracknell digital scale). Basal node length was determined from 5 plants per hill, for 10 hills, using a measuring rule. The numbers of adventitious roots per node were obtained by physically counting the roots with the aid of a hand lens (Eschenbach, Germany).

Data analysis

Data collected were subjected to analysis of variance using Statistical Analysis System SAS (version 9.0) statistical application software. Post hoc analysis (where there was significance) was carried out using the least significant difference test at $p \leq 0.05$.

RESULTS

Daily temperatures

The average temperatures for season 1 ranged between 21.6 and 24.0^{0}C, increasing steadily during heading (shown by red arrow). During season II, the weekly average temperatures were higher but gradually decreasing at heading time. The average temperatures during rice crop growth stage for the first season were lower than the second season. At reproductive stage, average temperatures for the second season were however, higher than in the first season.

***Azolla* biomass doubling rate**

The doubling rate of *Azolla* biomass ranged from 4.0 to 5.7 days in the first season and 4.4 to 5.9 days in the second season (Table 2).

Linear regression relationship between doubling time and average temperatures

The correlation coefficient between doubling time and temperature during the first season (r=0.18) was stronger than in the second season (r=0.07) as shown in Fig 1.2a and 1.2b.

Effect of *Azolla* biomass on flood water temperature and temperature fluctuations

During the first season, flood water temperature readings for *Azolla* inoculated samples recorded a p-value of 0.021, 0.030 and 0.035 at 21, 32 and 42 DATs respectively, which were significantly lower than that of the control (Table 3). In the second season, there was also significant reduction in flood temperatures in *Azolla* inoculated pots at 32 and 60 DATs (Table 3). The daily temperature fluctuations were significantly higher for the *Azolla* inoculated pots than non inoculated pots at all sampling points in the first season and at 32 and 60 DAT in the second season. In *Azolla* inocluated pots, flood water temperatures fluctuated from 1.5 to 5.6 ^{o}C and 1.1 to 3.9 ^{o}C for the first and second seasons respectively (Table 4).

Table 1. Average temperatures of irrigation water and relative humidity during 2017 at MIAD centre

							Heading time					
Season 1	Jan				Feb				Mar			
	w1	w2	w3	w4	w1	w2	w3	w4	w1	w2	w3	w4
Max temps	26.9	19.0	28.2	29.7	29.2	30.7	31.6	30.2	28.7	29.3	30.0	29.6
Min temps	14.8	25.9	15.7	14.7	15.2	16.3	16.4	17.8	18.3	18.9	19.6	17.7
Av temps	20.8	22.4	21.9	22.2	22.2	23.5	24.0	24.0	23.5	24.1	24.8	23.7
RH%	88.0	68.0	67.3	59.0	60.2	55.8	54.0	61.2	71.0	64.1	62.9	67.5
Season 2	April				May				Jun			
	w1	w2	w3	w4	w1	w2	w3	w4	w1	w2	w3	w4
Max temps	26.7	27.2	27.4	25.9	25.6	25.9	27.0	27.0	26.5	26.0	26.7	24.0
Min temps	18.6	17.5	18.9	18.7	18.7	18.7	16.8	18.8	16.3	16.3	17.2	16.2
Av temps	22.7	22.3	23.1	22.3	22.1	22.3	21.9	22.9	21.4	21.2	22.0	20.1
RH%	77.0	75.0	76.2	78.3	81.1	79.8	73.5	69.7	69.2	71.4	67.6	74.3

W1= week 1

Table 2. *Azolla* biomass doubling rate at MIAD centre, Mwea, during the year 2016

S/No	Temp ^{0}C	RH %	Weight t0 (g)	Weight t10 (g)	Doubling time (days)
Season 1					
1	25.3	77.0	5.0	20.0	5.0
2	24.8	76.0	5.0	16.9	5.7
3	24.4	75.0	5.0	20.1	5.0
4	21	71.0	5.0	28.7	4.0
5	23.3	69.0	5.0	20.3	4.9
6	23.8	74.0	5.0	21.3	4.8
7	25.8	76.0	5.0	27.0	4.1
Average	**24.1**	**74.0**	**5.0**	**22.0**	**4.8**
Season 2					
1	24.4	84.0	5.0	20.0	5.0
2	25.0	91.0	5.0	17.8	5.5
3	22.5	89.0	5.0	24.0	4.4
4	23.4	79.0	5.0	17.5	5.5
5	23.5	85.0	5.0	22.3	4.6
6	23.2	77.0	5.0	16.1	5.9
7	23.9	92.0	5.0	18.8	5.2
Average	**23.7**	**85.3**	**5.0**	**19.5**	**5.2**

Weight t_0=initial fresh biomass weight; weight t_{10}= biomass weight on 10th day; temp=Av temp; RH=relative humidity.

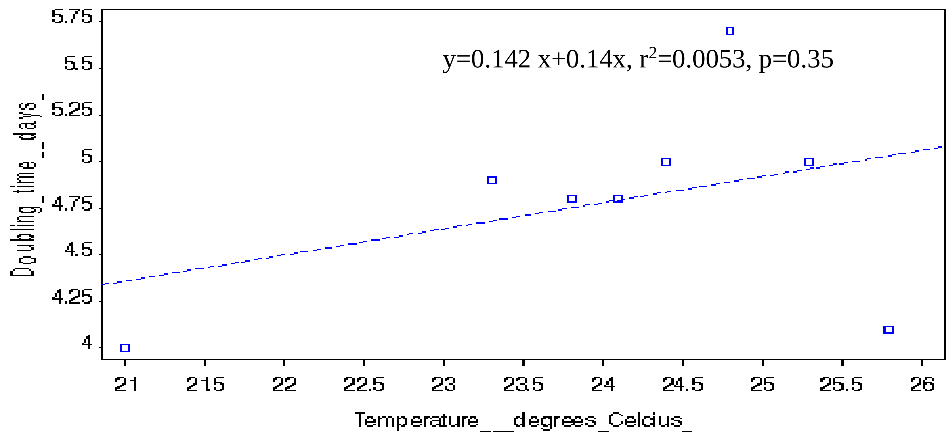

Fig 1.2a: Linear relationship between doubling time and average temperatures during the first season

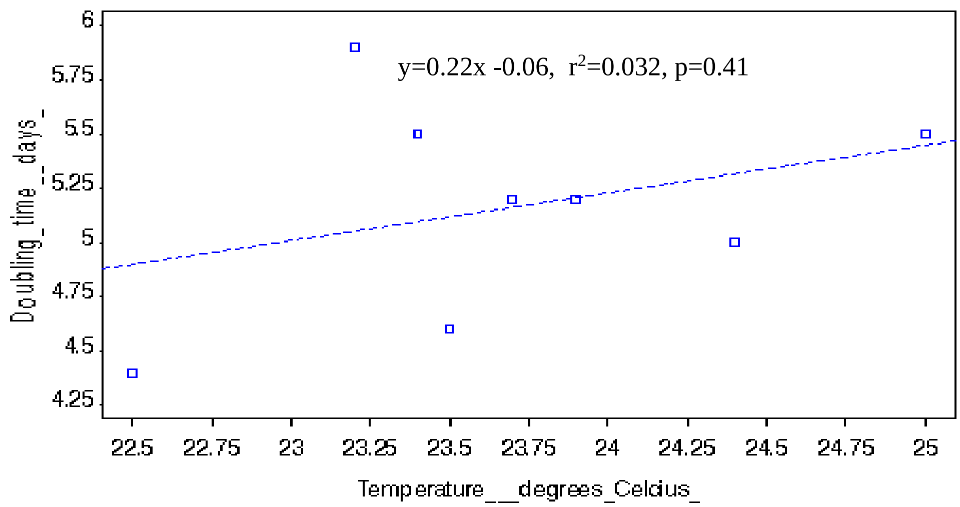

Fig 1.2b: Linear relationship between doubling time and average temperatures during the second season

Effect of *Azolla* biomass cover on flood water pH

Flood water pH was significantly lower in *Azolla* inoculated pots than the controls at all sampling points, in the two seasons of research. The pH differences between *Azolla* inoculated pots and the control ranged from 0.7 to 1.7 in the first season and 0.6 to 0.9 in the second season (Table 5).

Effect of *Azolla* growth on adventitious roots of rice plants

During both seasons, plants in *Azolla* inoculated pots showed significantly more adventitious roots per node, more and longer roots/mm^2 than non-inoculated control plants. *Azolla* inoculation increased adventitious roots per node and roots/m^2, compared to non-inoculated control (Table 6 and Figure 2). In *Azolla* inoculated pots, rice plants basal internodes were significantly shorter than non-inoculated plants.

Effect of *Azolla* biomass on root length, root biomass and plant biomass

During the two seasons under consideration, roots were significantly shorter in *Azolla* inoculated pots than in non-inoculated control pots (Table 7). *Azolla* inoculation had a significantly shorter root length than in the control. Also, *Azolla* inoculation significantly increased root biomass and plant biomass compared to non-inoculated control. Tiller numbers and plant height for the *Azolla* inoculated pots also significantly increased.

Effect of *Azolla* on yield components and grain yield

The effect of *Azolla* inoculation on panicle length, spikelets/panicle, number of panicles, grain weight, percentage grain filling and yield, is shown in Table 8. *Azolla* inoculation, with a p-value of 0.140 and 0.52 had no significant effect on panicle length during season one and two respectively. Number of spikelets per panicle was significantly higher in the *Azolla* inoculated pots than in non-inoculated control pots, during the second season. However, there were no significant differences during the first season between the *Azolla* inoculated and non-inoculated control plots in this parameter. *Azolla* inoculated pots showed higher number of panicle/m^2 and 1000-grain weight than non-inoculated control during the first season. The second season showed no significant differences with respect to the number of panicles/m^2 and 1000-grain weight. Although there were no significant differences in percentage grain filling and grain yields during both seasons, grain yields varied from 4.4-5.65 t/ha and 2.3 to 2.8 t/ha during the first and second season respectively.

Table 3. Effect of *Azolla* biomass on flood water temperatures (°C) in Mwea, during the year 2016

	21 DAT	32 DAT	42 DAT	60 DAT
Season 1				
No *Azolla* inoculation	28.0	26.2	23.4	25.5
Azolla inoculation	25.6	25.4	22.2	24.7
Mean	26.8	25.8	22.8	25.1
Standard Error	±0.25	±0.01	±0.17	±0.27
P-value	0.021	0.030	0.035	0.184
LSD (0.05)	1.54	0.6	1.06	NS
CV (%)	1.6	0.7	1.3	1.9
Season 2				
No *Azolla* inoculation	18.7	21.6	24.3	24.8
Azolla inoculation	18.7	20.6	23.7	23.1
Mean	18.7	21.1	23.9	23.9
Standard error	±0.18	±0.15	±0.69	±0.20
LSD (0.05)	NS	0.8	NS	1.6
CV (%)	1.7	1.2	3.4	1.9

No Azolla= control; DAT= Days after transplanting

Table 4. Effect of *Azolla* biomass on flood water temperatures fluctuation (^{0}C) in Mwea during the year 2016

	21 DAT	**32 DAT**	**42 DAT**	**60 DAT**
Season 1				
No *Azolla* inoculation	2.23	0.71	1.95	1.6
Azolla inoculation	5.61	1.5	2.8	3.3
Mean	3.92	1.107	2.3	2.47
Standard error	±0.42	±0.99	±0.18	±0.22
LSD (0.05)	2.56	0.6	1.1	1.31
CV (%)	18.6	15	13.2	15.2
Season 2				
No *Azolla* inoculation	2.01	0.33	0.57	1.8
Azolla inoculation	1.56	1.78	1.06	3.92
Mean	1.78	0.68	0.81	2.85
Standard error	±0.21	±0.15	±0.14	±0.17
LSD (0.05)	NS	0.71	NS	1.06
CV (%)	20.6	29.6	30.2	10.6

No Azolla= control; DAT= Days after transplanting

Table 5. Effect of *Azolla* on flood water pH in Mwea during the year 2016.

	21 DAT	**32 DAT**	**42 DAT**
Season 1			
No *Azolla* inoculation	8.5	9.3	8.2
Azolla inoculation	7.8	7.6	7.2
Mean	8.2	8.5	7.7
Standard error	±0.02	±0.12	±0.13
P-value	0.001	0.009	0.036
LSD (0.05)	0.11	0.73	0.82
CV (%)	0.4	2.4	3.0
Season 2			
No *Azolla* inoculation	9.0	8.1	8.3
Azolla inoculation	8.1	7.5	7.4
Mean	8.6	7.84	7.9
Standard error	±0.07	±0.13	±1.0
P-value	0.040	0.0403	.0420
LSD (0.05)	0.822	0.609	0.795
Cv (%)	2.74	2.21	2.9

DAT= days after transplanting.

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Table 6: Effect of *Azolla* on adventitious roots and basal internode length of rice plants in Mwea during the year 2016.

	Number of roots per node	Number of roots per mm^2	Root length (cm)	Basal internode length (cm)
Season 1				
No *Azolla* inoculation	9.3	3.3	2.84	7.5
Azolla inoculation	13.0	16	7.24	4.45
Mean	11.20	9.70	5.40	5.98
Standard error	±0.47	±0.94	±0.514	±0.20
P-value	0.028	0.011	0.003	0.001
LSD (0.05)	2.8	5.7	3.13	1.0
CV (%)	7.3	6.9	17.7	5
Season 2				
No *Azolla* inoculation	8.0	4.0	3.2	4.5
Azolla inoculation	16.0	9.7	7.6	2.7
Mean	12.0	6.8	5.4	3.65
Standard error	±1.08	±0.23	±0.52	±0.19
P-value	0.03	0.003	0.003	0.002
LSD (0.05)	6.6	1.4	3.15	1.2
CV (%)	15.6	5.9	16.6	9

DISCUSSION

Azolla double its biomass within 5.5 to 6.7 days. The rate was faster during the first season than the second. The doubling rate was within the range of 3-10 days reported by Hussner (2010). The relatively faster doubling rate during the first season than in the second season can be attributed to higher temperatures, which may have enhanced enzymatic, photosynthetic and stomatal conductance thus increasing growth rate (Sage and Kubien, 2007). *Azolla* has been reported to tolerate a wider range of temperatures but with an optimum of between 18-27 °C (theazollafoundation.org). Temperatures average of 22±0.21 °C in Mwea Irrigation scheme is therefore conducive for *Azolla* biomass accumulation, which may be beneficial for increased soil nutrient status. However, findings in Mwea Irrigation scheme in 2016, showed a reducing trend of biomass levels attributed to water shortage.

Azolla non-inoculated **Non treated** — *Azolla* inoculated **Treated** — *Azolla* inoculated **Treated** — non-inoculated **Non treated**

Figure 2. Effect of *Azolla* biomass growth on basal node and adventitious root growth

Azolla biomass growth reduced flood water temperatures and increased daily water temperature fluctuations by an average of 1.1 ± 0.31 °C and 2 ± 0.24 °C respectively. The reduction in flood water temperature can be attributed to the presence of *Azolla* coverage on flood water surface which shielded flood water from solar radiation. Temperatures in the water therefore remained lower than that of the control. The evapo-transpiration effect of the plant biomass, which draws heat energy for evaporation from the water body, also increased cooling of the water body. The temperature reduction effect was higher in the first than in the second season. This may be attributed to higher average temperatures and lower relative humidity in the first season than in the second, which enhanced evapotranspiration and thus increased the flood water temperature. Fosu-Mensah et al. (2015) reported a reduction in flood water temperatures of between 1.9 and 2.0 °C under *Azolla* cover. The author attributed this to prevention of rapid heating of the flood water by *Azolla* canopy.

Table 7. Effects of *Azolla* on rice plant root length, root biomass, plant biomass, tiller numbers and plant height in Mwea during the year 2016.

	Root length (cm)	Root biomass per hill (g)	Plant biomass per hill (g)	Tiller numbers	Plant height (cm)
Season 1					
No *Azolla* inoculation	46.5	14.0	71.7	247.0	156.8
*Azolla i*noculation	38.6	15.9	96.1	253.0	160.4
Means	42.5	15.0	83.9	250.4	158.6
Standard error	±1.04	±5.7	3.3	±5.0	±0.51
P-value	0.032	0.031	0.032	0.440	0.037
LSD (0.05)	6.3	1.5	19.2	NS	3.1
CV (%)	4.2	6.5	6.5	3.4	0.6
Season 2					
No *Azolla* inoculation	45.0	12.6	16.5	142.3	118.9
Azolla inoculation	37.3	16.5	38.1	148.3	123.9
Means	41.2	14.6	27.3	145	121.0
Standard error	±1.28	±0.57	±1.00	±0.66	±2.2
P-value	0.040	0.024	0.038	0.023	0.247
LSD (0.05)	6.8	6.1	3.4	13.4	NS
CV (%)	4.8	4.1	6.7	0.8	3.1

Table 8. Effect of *Azolla* biomass growth on yield components and grain yield in Mwea during the year 2016

	Panicle length (cm)	Spikelets /panicle	Panicles /m^2	Grain weight (g)	Filled grains (%)	Yield (t/ha)
Season 1						
No *Azolla* inoculation	30.0	95.7	518.5	21.6	64.0	4.3
Azolla inoculation	29.5	96.5	556.0	22.1	70.3	5.65
Means	29.7	96.1	537.0	21.9	67.0	5.0
Standard error	±0.14	±8.4	±35.2	±0.06	±0.04	±0.93
P-Value	0.140	0.950	0.053	0.036	0.420	0.144
LSD (0.05)	NS	NS	213	0.4	NS	NS
CV (%)	0.8	15.0	11.3	0.5	11.0	14.1
Season 2						
No *Azolla* inoculation	25.9	82.6	211.0	23.0	64.0	2.3
Azolla inoculation	25.78	88	192.0	24.0	65.0	2.8
Means	25.8	85.3	201.0	23.0	64.5	2.5
Standard error	±0.13	±0.43	±5.51	±0.35	±0.02	±0.009
P-Value	0.52	0.01	0.14	0.37	0.89	0.072
LSD (0.05)	NS	2.61	NS	NS	NS	NS
CV (%)	0.8	0.9	4.7	4.6	2.6	6.3

No Azolla inoculation= control; % FG= % filled grains

Azolla cover reduced flood water pH by between 0.7 and 1.7. Reduction in pH can be attributed to the effect of *Azolla* canopy, which reduced flood water temperature thereby minimizing ammonia volatilization from the water body. Reduced ammonia loss increased flood water N content and had the associated effect of lowering flood water pH. The reduction in flood water pH due to *Azolla* biomass cover which limits surface N losses has been reported by Fosu-Mensah et al. (2015).

Azolla growth enhanced the number of adventitious roots in rice plant and reduced the basal internode length. Increase in number, length of adventitious roots and shortening of basal stem internode from *Azolla* treated plots can be attributed to the effect of reduced oxygen balance resulting from *Azolla* biomass growth in flood water. Landsdown (2015) showed that *Azolla* cover on flood water hinders oxygen diffusion. This may have stimulated a physiological complementation strategy of development of shorter basal stem internode for growth of adventitious roots to access oxygen. This adaptive mechanism towards augmentation of depleted oxygen supply has been reported in cereals by Atkinson (2014). Rice plant therefore can physiologically adapt to depleted oxygen supply through the development of adventitious roots.

Azolla growth reduced fibrous root length and enhanced plant biomass. The significant reduction in root length can be attributed to *Azolla* biomass canopy effect, which caused a reduction in water temperatures. This is hypothesized to have reduced enzymatic activities slowing physiological processes of growth and resulting into less root length development. The effect of lower temperature causing a reduction in root length has been reported by Essemine *et al.* (2010). Increase in plant biomass can be attributed to increased N efficiency uptake due to reduced loss of N as a result of low pH as reported by De Macale and Vlek (2004). The increased N levels may have also been supplemented by direct excretion of nitrogen from the *Azolla* anabaena system. Wagner (1997) reported that *Azolla anabaen*a system directly excretes about 5% of its nitrogen fixed into the water body. This enhanced vegetative growth and resulted into increased biomass for the *Azolla* treated plots).

Azolla biomass enhanced growth and yield components of rice crop. During the first season, *Azolla* treatment significantly increased plant height, panicle/m^2 and individual grain weight. In the second season, tiller numbers and spikelets/panicle were increased by *Azolla* treatment. The enhancement of growth and yield components may be attributed to the effect of *Azolla* moderating the flood water temperature and hindering pH increase. The reduced temperature and lowered pH may have had the effect of reducing ammonia volatilization and consequently increasing efficiency of nitrogen utilization from applied fertilizers and N excreted from *Azolla anabaena* symbiosis. De Macale and Vlek (2004) reported increased efficiency of N utilization resulting from reduced pH. Wagner (1997) also reported that up to 3-4% N from the *Azolla anabaena* symbiosis can be directly excreted into the water body to the benefit of the rice crop. *Azolla* growth in flood water is therefore beneficial to farmers through increased fertilizer efficiency uptake, increased grain yield and biomass, which is becoming more useful in straw livestock production.

CONCLUSION

Azolla biomass growth in flooded paddies shields direct sunlight, consequently reducing flood water temperature, with a net effect of reducing N loss from water body. This enhances efficiency of N uptake and increases paddy rice yields

REFERENCES

Campbell, R. 2011. *Azolla* growth in farm dams. Agriculture Victoria. online-http/agriculture.vic.gov.au/agriculture/farm. date accessed 14/3/2016.

de Macale, M.A.R., Vlek, P.L. 2004. The role of *Azolla* cover in improving the nitrogen use efficiency of lowland rice. *Plant and Soil,* 263, 311-321.

Essemine, J., Ammar, S., Bouzid, S. 2010. Effect of temperature on root and shoot development in wheat seedlings during early growth stage. *Asian Journal of Plant Science,* 9, 375-379.

Faichney, G.J., White, G.A. 1983. *Methods for the analysis of feeds eaten by ruminants*. Melbourne, Australia, CSIRO.

Fosu-Mensah, B.Y., Vlek, P. L. G., Manske,G., Mensah, M. 2015. The Influence of *Azolla pinnata* on flood water chemistry, grain yield and nitrogen uptake of rice in Dano, south western Burkina Faso. *Journal of Agricultural Science,* 7, 118-128.

Hussner, A. 2010. NOBANIS–Invasive alien species Fact Sheet–*Azolla filiculoides*, online http/ *www.nobanis.org*. *D*ate of accessed: 5/4/2014

Ivens, G.W. 1989. *East African weeds and their control*. 2nd ed. Nairobi, Oxford University Press.

Jackson, M.L. 1958**.** *Soil chemical analysis*. Englewood Cliffs, N.J., USA, Verlag:Prentice-Hall, Inc.

Kitoh, S., Shiomi, N. 1991. Effect of mineral nutrients and combined nitrogen sources in the medium on growth and nitrogen fixation of the *Azolla anabaena* association. *Soil Science and Plant Nutrition*, 37(3), 419-426. https://doi.org/10.1080/00380768.1991.10415054

Kitoh, S., Shiomi, N., Uheda, E. 1993. The growth and nitrogen fixation of *Azolla filiculoides Lam*. in polluted water. *Aquatic Botany*, 46,129-139. https://doi.org/10.1016/0304-3770(93)90041-T

Landsdown, R.V. 2015.Water fern, *Azolla filiculoides*. Online, http/ *www.nonnativespecies.org/factsheet*. Date accessed 5/10/2016.

Mertz, E.T.; Misra, P. S., Jambunathan, R. 1974. Rapid ninhydrin color test for screening high-lysine mutants of maize, sorghum, barley and other cereal grains. *Cereal Chemistry,* 51, 304-307.

Sage, R.F., Kubien, D.S. 2007. The temperature response of C_3 and C_4 photosynthesis. *Plant Cell Environment*, 30, 1086–1106.

Wagner, M.G. 1997. *Azolla*, a review of its biology and utilization. *The Botanical Review*, 63,1-26.

Wanjogu RK, Mugambi G, Adoli HL, Shisanya SO, Tamura M. 1992. Mwea Rice Production manual. Nairobi. Mwea Irrigation Agricultural Development Project. National Irrigation Board library.