THE EFFECT OF RATE AND TIME OF APPLICATION OF *AZOLLA* ON RICE (*Oryza sativa L.*) GRAIN YIELD.

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Thesis
M. Sc.
NYO
1995
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By

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A DISSERTATION SUBMITTED TO THE UNIVERSITY OF ZAMBIA IN PARTIAL FULFILMENT OF THE REQUIREMENTS OF THE DEGREE OF MASTERS OF SCIENCE IN AGRONOMY(CROP SCIENCE)

256030

THE UNIVERSITY OF ZAMBIA

LUSAKA
1995

DECLARATION

I, SHENAL SEBASTIAN NYONI, hereby declare that this dissertation represents my own work and that it has not been previously submitted for a degree at this or another University.

Signature..... Date 14/03/98

APPROVAL

This dissertation of **MR. SHENAL SEBASTIAN NYONI** is approved as fulfilling part of the requirements for the award of the degree of Master of Science in Agronomy by the University of Zambia.

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DEDICATION

To my fiancee Tunu Shaban Mattope and my son Edward for the tolerance, love and patience during the two years I was away from home.

To my parents for all they did for me.

ACKNOWLEDGEMENTS

I would like to extend my sincere gratitude to my supervisors, The late Dr N. C. Mwiindilila, Dr O. A. Yerokun and Dr D. N. Mbewe for their guidance, tolerance and useful criticism during the whole period of carrying out this work. I would also like to thank all members of staff in the Crop Science and Soil Science Departments, who contributed towards the completion of this work.

I wish to express my thanks to Mr B. Shitambi of National Irrigation Research Station, Mazabuka for providing the *azolla* and rice seeds which were used during this study.

My sincere gratitude goes to my sponsors, SACCAR/GTZ for financial support, and my employer in Tanzania, the Ministry of Agriculture for granting me the study leave.

To the course mates and friends who made life enjoyable away from home.

ABSTRACT.

This study was designed to determine the effectiveness of using *azolla* in rice fields and to investigate the appropiate time and quantities of *azolla* to be incorporated. The trial was conducted at the University of Zambia Field Station as a pot experiment. Three different quantities of *azolla pinnata* at 4, 8 and 12 t/ha were used to check yield performance compared to urea at 60 kgN/ha and a control (no *azolla*, no urea). Four times of application i.e during transplanting (DT), DT and 42 days after transplanting (DAT); DT and 56 DAT; DT and 70 DAT were studied. The experiment was arranged as a randomized complete block design with three replications. There was a slight increase in rice yield with increasing quantities of *azolla*. The highest rice yield with *azolla* was obtained when *azolla* was applied at the rate of 12 t/ha (209 gm/pot), but this did not differ significantly from that obtained with *azolla* applied at the rate of 8 t/ha (203.52 gm/pot). Yield obtained with urea (60 kgN/ha) (217.11 gm/pot) was the highest. The lowest yield was from the control plants (130.44 gm/pot). The best treatment combination was when *azolla* at a rate of 12 t/ha was applied at the time of transplanting and 56 days after transplanting. This gave a yield of 261.27 gm/pot,as compared to a yield of 128.4 gm/pot under the control treatment. Other parameter studied were plant height,paicle length,tiller per plant,1000 grain weight,days to 50% flowering,days to maturity and harvest index. From the study it shows that the use of *azolla* increases rice yield but not beyond that obtained with inorganic fertilizers also *azolla* can substitute inorganic fertilizer in rice cultivation.

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ABSTRACT

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CHAPTER 1

INTRODUCTION.

Rice (*Oryza sativa* L.) is the staple food of millions of people in the world. About 40 percent of the world population depends on rice as a major source of calories (De Datta, 1981). It is the third most important source of food, after maize and wheat, respectively (Cock, 1982). It is estimated that total rice production world wide is about 473 million tonnes per year (Sasson, 1990). The highest average yield in the world is about 6.8 t/ha (De Datta, 1981). In Zambia, rice is the fourth important source of food after maize, wheat and sorghum, respectively. Yields of up to 2.4 t/ha have been reported but for most small scale farmers, yield is less than 1.0 t/ha. The overall production of rice in Zambia for the seasons 1989/90, 1990/91,1991/92 was 10,455, 16,427 and 15,745 tonnes, respectively, while the corresponding cultivated areas were 9628, 13,450 and 13 802 ha, respectively. (MAFF, 1994).

The amount of rice produced in Zambia does not meet the national requirement. There are several reasons which have led to this situation, including the high price of fertilizers. Small scale farmers cannot afford to buy these fertilizers and as a result, they grow rice without using fertilizers. This results in low yields from their fields. Other reasons are government placing more emphasis on the production of maize, and lack of proper marketing arrangements for rice (MAFF,1994).

Rice grows well from latitudes 50^0N to 40^0S and from sea level to 2500 m above sea level. Temperature requirement varies from 20^0C to 37.7^0C, while soil pH for proper growth of the rice plant varies from 3 to 10 depending on climatic conditions (De Datta, 1981).

Nitrogen (N) is the key element in increasing the yield of rice. It is essential for vegetative growth, grain protein, maximizing the number of panicles and seed maturity (Becking, 1979). The most common source of N used in rice production is inorganic nitrogen in the form of urea and ammonium sulphate (De Datta, 1981). However, this inorganic N is easily lost in the field through denitrification, volatilization, leaching and surface run off (De Datta, 1981;Grist,1986). Therefore, there is a need to look for a cheaper source of nitrogen which will help to reduce dependence on commercial fertilizers for rice production.

Biological nitrogen fixation can be the best and most efficient way of providing nitrogen for the rice plant. *Azolla* in association with blue green algae *Anabaena-Azollae* could be the best source of nitrogen in the fields of rice, since the combination constitutes a naturally built-in slow release system for providing nitrogen to feed the rice plant. By so doing nutrient loss and environmental pollution are minimized (Becking, 1979; Grist, 1986).

Therefore this study was designed to evaluate the effectiveness of using *azolla* as a source of nitrogen in rice production and to investigate the appropriate time and amount of *azolla* to be incorporated in the rice field.

CHAPTER 2

LITERATURE REVIEW.

2.1 Rice cultivation.

There are two main systems of rice cultivation; upland system which depends on rainfall and the lowland system in which the land is inundated and the crop is grown in water from transplanting until harvesting (Grist, 1986; Purseglove, 1991). In Zambia, the lowland rainfed system is mainly used. Farmers grow rice in swamp areas where water is easily available (MAFF, 1991).

2.2 Mineral nutrition of rice.

The rice plant, just like any other crop plant, needs to be supplied with adequate amount of nutrients so as to make it produce more from a given area. The most important nutrients needed by the rice plant are nitrogen, phosphorous and potassium (De Datta, 1981).

2.2.1. Nitrogen.

The form of nitrogen used by the rice plant can be inorganic N, such as urea; organic manure or biologically fixed nitrogen. Requirement varies from place to place and also depends on varieties used, e.g., Japonica varieties require 110 kgN/ha; while Indica varieties requirements vary from 30-35 kgN/ha (Grist, 1986). In Zambia, urea at a rate of 55 - 75 KgN/ha is usually applied (MAFF, 1991). Application methods depend on the type of cultivation. It can be broadcast or drilled under. A large amount of nitrogen is required at the early and mid

tillering stages, while excessive nitrogen application may weaken the plant and make it vulnerable to diseases. Deficiency results in stunted growth with narrow and short leaves (De Datta, 1981).

2.2.2 Phosphorous.

Rice requires considerable quantities of phosphorous for vigorous growth and high grain yield. It stimulates root development, promotes early flowering and ripening (De Datta, 1981; Kondo et al, 1989). Phosphorous can be supplied as triple super phosphate (P_2O_5) 46%P and the rates to be applied depend on soil fertility but values of 30 - 80 kgP/ha are normally applied in various parts of the world (Grist, 1986). In Zambia, values of 50 - 75 KgP/ha are normally applied to the soil depending on the soil status of the field (MAFF, 1991).

2.2.3. Potassium.

In rice fields, potassium is usually applied in the form of potash (K_2O) during planting or at final land preparation. Rates of application vary from 20 - 120 kgK/ha depending on the soil status. Its main function is to stimulate the build-up and translocation of carbohydrates to the grain and it strengthens plant cell walls (Grist, 1986).

2.3. *Azolla*.

2.3.1 Ecology of *Azolla*.

Azolla is a small aquatic fern of the family Sulvanaceae, which has a hereditary symbiotic relationship with the cyanobacterium

Anabaena- Azollae, as a result of which appreciable quantities of nitrogen can be fixed (FAO, 1977; Watanabe, 1977; Van Hove, 1989). There are six existing forms of *azolla* which are divided into two genera: genera I consists of *Azolla filiculorides* Lam, *Azolla caroliana* wild, *Azolla mexicans* Pres and *Azolla microphylla.* All these are originally found in North and South America. Genera II covers *Azolla pinnata* R.Br and *Azolla millotica* which are wide spread in the Eastern hemisphere, tropical Africa and South Africa (Becking, 1975; IRRI, 1978;Van Hove,1989).

The fern is tolerant to considerable variation of temperature; from 10 -25 ^{0}C but *Azolla pinnata* can withstand temperatures of over 35^{0}C to 40^{0}C. Soil pH requirement is from 4-10. High soil pH reduces growth of *azolla* (Becking, 1976; IRRI, 1978). *Azolla* grows well in soils which are rich in phosphorous. Without it *Azolla* growth is affected and hence its multiplication and nitrogen release capacity (Van Hove, 1989). *Azolla* can grow very rapidly, doubling its weight in three to four days (Hera, 1995).

2.3.2. Nitrogen production.

Anabaena- Azollae can grow and develop perfectly without any source of nitrogen compound since the symbiont, like a number of other cyanobacteria, has an enzymatic complex called nitrogenase, capable of transforming atmospheric nitrogen into ammonia (Ladha and Watanabe, 1987). The production of ammonia from nitrogen occurs in the heterocyst cells found in *Anabaena - Azollae*. The heterocyst cells then excrete a great deal of ammonia into the

leaf cavity where it is collected by the fern (Van Hove, 1989). The nitrogen will then be freed and made available to the rice plant when the fern dies and decays.

2.3.3 *Azolla* incorporation.

The best time to incorporate *Azolla spp.* into the soil is when the ferns are healthy and have just covered the plot, with no overlapping fronds (Ventura and Watanabe, 1993). Decomposition and mineralization studies conducted by Ventura *et al.*(1987) showed that nitrogen is released rapidly from *Azolla* during the first 2-3 weeks after incorporation. Ventura and Watanabe (1993) showed that incorporation of *Azolla* spp 4 weeks after transplanting rice gave a very low grain straw ratio. Krock *et al.*(1987) reported that 25 days after the first application of *Azolla*, appreciable amounts of *Azolla* N was made available to the rice crop. Ventura *et al.* (1987) reported that 20 days after incorporating *Azolla* into the soil, *Azolla* N would be available to the rice plant.

2.3.4 Amount of N released by *Azolla*.

It is estimated that incorporation of *Azolla* of a good quality has an effect similar to that obtained when applying 30-40 kg N/ha (Lumpkin and Plucknett, 1980;Van Hove,1989). Fiore and Gutbrod (1981) observed that when *Azolla* is incorporated , it can produce an equivalent of 20-60 kg N/ha as a mineral fertilizer before sowing and produces 5-100 kg N/ha when intercropped. Loudhapasitiporn and Kanarengsa (1973) found that, one crop of *Azolla* incorporated into the soil or allowed to dry on the soil

surface before or after transplanting, yields N almost equivalent to 30 kg N/ha as urea or ammonium sulphate. Kannaiyan (1981) reported that incorporation of *Azolla* at 2 t/ha has an effect on crop yield equal to that when 25 kg fertilizer N/ha is applied. Ventura and Watanabe (1993) reported that the amount of nitrogen produced by *Azolla spp.* ranged from 70 - 110 Kg N/ha.

It has been observed that higher production of nitrogen is obtained when the field is supplied with phosphorous fertilizer (Van Hove, 1989). Application of 25Kg/ha is necessary for *Azolla* culture in the rice field. A split application of phosphate at 7-10 day intervals is important in order to promote good growth of *azolla* and hence nitrogen release (Loudhapasitiporn and Kanarengsa, 1973).

2.3.5. Effect of *Azolla* on rice yield .

There are different ways of using *Azolla* in rice field. *Azolla* can be grown as a monocrop and incorporated into the soils as a basal green manure before rice is transplanted. Also, *Azolla* can be incorporated as a top dressing after rice has been transplanted (Lumpkin and Plucknett, 1980).

In a trial conducted by Brotonegoro *et al.* (1981), it was observed that higher grain yields were obtained when plots were incorporated with *Azolla* before and after transplanting. They also observed that incorporation of *Azolla* after transplanting rice seedlings gave higher yields than when *azolla* was incorporated before transplanting. The yields in the incorporated

plots were significantly better than those achieved when using 90 kg N/ha.

Watanabe (1977), reported that when *Azolla* was grown as an intercrop without incorporation, it increased the rice yield, while applying *Azolla* only after transplanting gave lower yield. He also observed that *Azolla* before or after transplanting rice gave yields equivalent to those obtained from 30 kg N/ha as a chemical fertilizer. The yields on plots incorporated with *Azolla* were higher compared to treatments without *Azolla*. Kulasooriya *et al.* (1978) reported that *Azolla* incorporation in the rice cultivation cycle gave rice yields equivalent to applying 55 -85 kgN/ha as urea.

Ventura *et al.* (1987) reported that *Azolla* and *Sesbania sp.* significantly increased rice grain yield from 1.4 to 2.1 t/ha. In a trial conducted by Ventura *et al.* (1992), they reported an increase in grain yield from *Azolla* spp plots by 0.5 tons/ha over those in the control and the urea plots. Krock *et al.*(1987) found that incorporation of 1 t/ha fresh *Azolla* increased grain yield by 28 kg/ha. They also reported that 20 tons of fresh *Azolla* per ha incorporated before or after transplanting increased grain yield by 0.5 t/ha. Ventura and Watanabe (1992) reported an increase in rice grain yield by 1.8 - 3.9 t/ha due to the green manure *Azolla*. The yields were almost similar to the application of 60 Kg N/ha as urea.

2.4. Effect of *Azolla* on rice yield components.

Grain yield of rice is determined by the number of panicles per m^2, number of grains per panicle, number of grains and 1000 grain weight. These yield components depend on the availability of nitrogen in the soil. Matsushima (1980) reported that fertilization has an effect on the number of tillers to be formed, number of panicles, number of grains and encourages kernel development. Grist (1986) found that panicle length was positively correlated with rice grain yield, while panicle weight was a good indicator of higher grain yield in rice. The same author reported that a large number of tillers is not necessarily conducive to high grain yield, but higher grain yield is governed by effective tillers.

Zhang *et al.*(1987) and Wang *et al.*(1983) concluded that when *Azolla* is incorporated, it decomposes and contributes nutrients for growth and development of the rice plant, promoting tillers, enhancing the growth of panicles and it increases the number of grains per panicle.

Van Dat and Peterson (1983) and Van Dat *et al.*(1978) reported that 1000 grain weight differed significantly among N levels, as N levels increases, 1000 grain weight declines. They also observed highly significant increase in number of panicles per m^2 with increasing levels of N, but there was a trend towards reduced panicle weight with increasing N levels for both varieties tested.

In a trial conducted by Jones *et al.* (1979), they concluded that grain filling rate was positively and highly significantly correlated with actual panicle weight and 1000 grain weight, but negatively correlated with panicle weight per m^2. Krock *et al.*(1987) reported that application of *Azolla* slightly increased the number of panicles and it also increased the number of filled grains per panicle.

CHAPTER 3

MATERIALS AND METHODS.

3.1 Site description.

The experiment was conducted at the University of Zambia at the Field Station (15^0 23'S, 28^0 28'E and 1140 m above sea level) as a pot experiment during the 1994/95 cropping season. The pots used were 50cm in diameter and 30 cm deep. They were filled with 20 kg soil per pot.

Soil analysis was carried out to determine the initial fertility level. Soil was collected from a depth of 0-20 cm with the use of an auger, from sampling sites which were randomly selected across the field. The soil samples were composited, mixed and placed in pots. The soil type used during this trial was a Ngwerere sandy clay loam (Typic Paleaustalf) with a texture analysis of: sand 58%, silt 28% and clay 14%; soil organic matter was 0.8% while soil pH was 7.58. Nitrogen content in the soil was 0.17%.

3.2. Variety.

Variety Xiang Zhou 5 (commonly known as Mulunga in Zambia) was used for the experiment. It is a lowland, early maturing and high yielding variety, with medium tillering ability, short straw and resistant to rice blight. The variety performs well in dambo areas.

3.3. Nursery.

A plot of 1.5 x 3.0 m was dug using a hand hole, and the soil was

made fine before sowing. Triple super phosphate and urea at a rate of 8 gm and 5 gm, respectively, were broadcast uniformly in the plot. Rice seeds were sown and seedling raised for 4 weeks. Watering was done every day to avoid wilting of the seedlings. Weeding was done by hand, there was no pest attack observed.

3.4. Transplanting.

Triple super phosphate at the rate of 60 kg P/ha (2.69 grams per pot) was uniformly broadcasted in the pots at the time of transplanting. Transplanting of rice seedlings was done when seedlings attained a height of 28 cm and they were spaced at 15 x 15 cm, with six plants per pot. Pots were then flooded with water from the time of transplanting up to two weeks before harvesting.

3.5 Multiplication of *Azolla*.

Azolla pinnata was used as a source of nitrogen for rice plants. *Azolla* were collected from the National Irrigation Research Station in Mazabuka. Multiplication of *Azolla* was done in 10 basins half filled with water. Triple super phosphate fertilizer at a rate of 15 kg P/ha (0.67 gm per pot) was applied to *azolla* to promote good growth and fast multiplication.

3.6. *Azolla* incorporation.

Incorporation of *azolla* was done by hand after draining water from the pots. Different quantities of *azolla* were then incorporated into the pots as per treatments. The pots were then

flooded with water to a level of six cm from the top soil surface in the pots.

3.7 Experimental Design and treatments.

The treatments were arranged in a Randomized Complete Block Design (RCBD) with three replications, time of application and the quantities of *azolla* and urea were the factors assigned in the pots. Different quantities of *Azolla* were used to supply nitrogen to the rice plants. The quantities were 1). 4 t/ha (15 KgN/ha), 2). 8 t/ha (30KgN/ha) and 3). 12 t/ha (45KgN/ha). Treatments with control (no *azolla*, no urea) and Urea at 60 kgN/ha (2 gm/pot) were included to compare the performance with *azolla* treatments. *Azolla* and urea was incorporated in the pots at different growth periods. The periods were 1). during transplanting (DT) 2). DT and 42 days after transplanting (DAT) 3) DT and 56 DAT. 4) DT and 70 DAT. Incorporation of *azolla* was done in split application at a ratio of 50:50 application DT and DAT. There was a total of 20 treatments per replication.

3.8. Data collection.

The following data were collected during the growing season:-

1. Number of plants per pot which survived after transplanting.
2. Number of tillers per plant. This was obtained by counting the number of tillers in a pot, then divided by the number of plants per pot.
3. Days to 50% flowering. Number of days counted from transplanting to when 50% of the plants in a pot flowered.
4. Days to maturity. Number of days counted from transplanting

to when rice in a pot is read for harvesting.

5.Plant height at maturity. This was obtained by measuring plant height from ground level to the leaf tip of a flag leaf.

6.1000 grain weight. One thousand grains from each treatment were counted and weighed.

7.Panicle length. Ten different panicles were selected at random from each pot and their lengths measured. The average represented the length of a panicle per pot.

8.Panicle weight. Ten panicles were selected at random from each pot, weighed and the average represented the weight of panicle per pot.

9.Nitrogen content in whole plant sample at harvesting was determined by the Micro Kjeldal technique (Yoshinda, 1972).

11.Grain yield. Grain harvesting was done on six plants from each pot. Total grain yield was obtained by adding the corresponding treatments from the replication, the average of which represented the yield per treatment.

12.Nitrogen content in the grains. Grains from each treatment were ground and the nitrogen content in the grains was determined by the Micro Kjeldal technique (Yoshinda, 1972).

13.Harvest index was obtained from the ratio of grain yield to that of straw plus the grains harvested.

3.9. Data analysis.

Data analysis was done using the MSTAT computer programme. Mean separation for various parameters was done using Duncan's Multiple Range Test (DMRT). Correlation analysis was done to determine the interrelationships among the parameters studied.

CHAPTER 4

RESULTS.

4.1 General observations.

The trend of growth of the control treatments (no nitrogen, no *Azolla*) was different when compared to other treatments which were supplied with nitrogen fertilizer or *azolla*. Plants in the control pots appeared yellowish for most of the growing period, while it was difficult to notice any visual differences in plants which were supplied with different levels of *azolla* and nitrogen. There was no severe disease attack on the rice plants during the period of this experiment. However, they attracted birds which were easy to control by scaring.

4.2. Effect of time of application on rice grain yield and yield components.

Rice grain yields were significantly affected ($P \leq 0.05$) by time of application of *azolla* and urea as sources of nitrogen. There was a general increase in rice grain yield as number of days to second incorporation of *azolla* increased. Table 1 shows that best rice yield was obtained when *azolla* was incorporated during transplanting and 56 days after transplanting (209.73 gm/pot) while the lowest yield of 159.81 gm/pot was recorded when no N application was made. However, the highest yield was not different from the treatment where *azolla* was applied during transplanting and 70 days after transplanting (200.23 gm/pot)(Table 1 and Fig 1). Application of *azolla* during transplanting and 70 days after transplanting recorded highest panicle length (22.65 cm), but it was not different from other

Table 1. Effect of time of application of *azolla* and urea on the yield and yield components of rice variety Xiang Zhou 5.

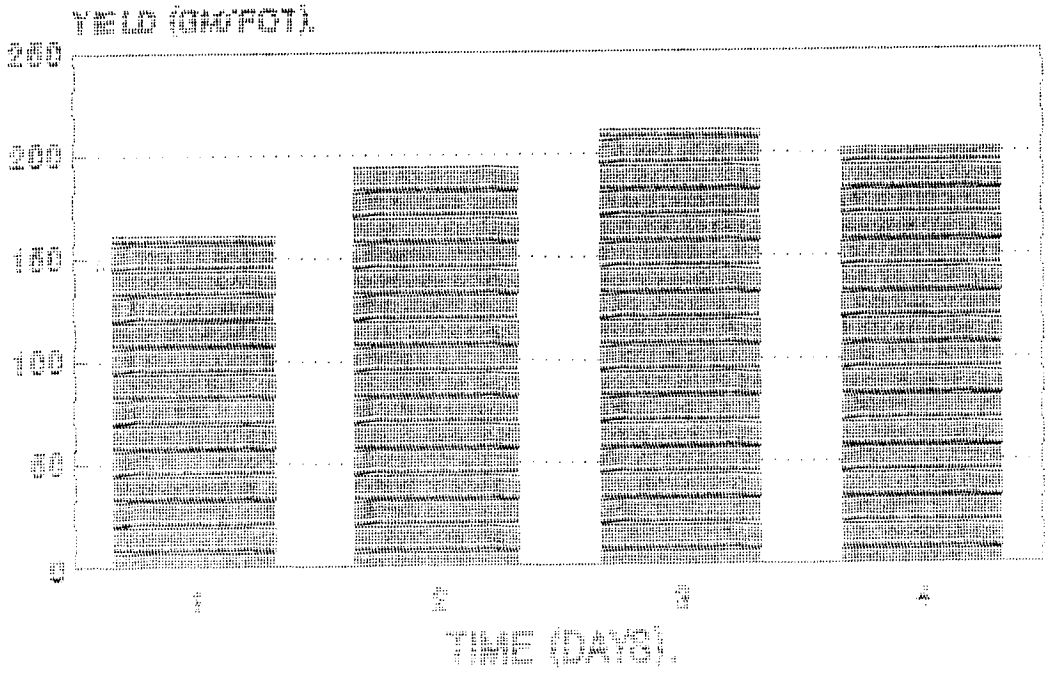
	Yield gm/pot	Panicle length (cm)	Panicle weight (gm)	Days to 50% flo	Days to maturity	Plant height (cm)	1000 gr wt (gm)	Tiller/ plant
DT	159.81c	21.38 b	4.22 a	66 c	127 b	70.51 a	30.44 a	8 b
DT42AT	192.64b	22.44 a	4.29 a	67 b	126 c	69.82 a	30.35 a	9 ab
DT56AT	209.73a	21.85 ab	4.20 a	67 b	127 b	69.35 a	30.34 a	9 b
DT70AT	200.23ab	22.65 a	4.29 a	69 a	129 a	69.99 a	29.29 a	11 a
LSD	11.081	0.954	0.162	0.625	1.049	0.699	2.774	1.940
CV%	7.87	5.85	5.17	3.33	11.10	15.32	12.47	27.58

Means in the same column followed by the same letter are not significantly different from each other, according to Duncans Multiple Range Test ($P \leq 0.05$).

Legend: DT during transplanting.
DT 42 AT during transplanting and 42 days after transplanting.
DT 56 AT during transplanting and 56 days after transplanting.
DT 70 AT during transplanting and 70 days after transplanting.

Figure 1

Effect of time on rice yield.



1-DT 2-DT42AT 3-DT59AT 4-DT70AT

levels, the lowest panicle length being the control (21.38 cm) (Table 1). A higher number of tillers was observed when *azolla* was incorporated during transplanting and 70 days after transplanting, while the lowest was from the control (8.27). Days to 50% flowering was highest when *azolla* was incorporated during transplanting and 70 days after transplanting (69 days). It took 66 days to 50% flowering in the control treatment.

Rice in the control pots matured in 127 days while rice in pots incorporated with *azolla* during transplanting and 70 days after transplanting matured in 129 days. Significant differences were observed in nitrogen content of the plant after harvesting. Highest level of nitrogen were observed in plants supplied with *azolla* at 70 days after transplanting (3.52 mg) (Table 2). The lowest level of nitrogen in the plant was recorded in the control pots (2.41 mg). The nitrogen content in grain showed no significant differences between grains harvested from pots which were supplied with different levels of azolla and urea. Higher dry matter was observed from plants supplied with *azolla* at transplanting and 70 days after transplanting (124.88 gm) while the lowest dry matter was from plants which were supplied with N during transplanting and 42 days after transplanting (110.34 gm) but did not differ from that when no *azolla* was applied (111.34 gm). There was no significant effect ($P \leq 0.05$) observed on panicle weight, plant height, 1000 grain weight and harvest index.

Table 2. Effect of time of application of *azolla* and urea on plant N, grain N, dry matter production and Harvest index of a rice variety Xiang Zhou 5.

	(mg)	N in Plant (gm)	N-Grain (gm)	Dry Matter	HI
DT	2.41 c	1.75 b	111.34 c	0.58 a	
DT 42 AT	2.74 b	2.14 a	110.34 c	0.60 a	
DT 56 AT	3.22 a	1.96 a	118.48 b	0.55 a	
DT 70 AT	3.52 a	1.97 a	124.88 a	0.60 a	
LSD	0.317	0.192	2.402	0.062	
CV%	14.46	13.19	12.79	14.81	

Means in the same column followed by the same letter are not significantly different from each other according to Duncan's Multiple Range Test ($P \leq 0.05$).

4.3 Effect of level of azolla and nitrogen on grain yield and yield components.

There was a general increase in rice grain yield with the increase in levels of *azolla* (Table 3). Of the Azolla treatment the highest grain yield was obtained when *azolla* was applied at the rate of 12 t/ha (209.76 gm/pot), but the yield did not significantly differ from that obtained when *azolla* was applied at the rate of 8 t/ha (203.52 gm/pot). The yield obtained with urea at 60 kgN/ha was the highest (217.11 gm/pot), but it was not different from those obtained from 8 and 12 t/ha of *azolla*, respectively,(See also figure 2). The lowest yield, of 130.44 gm/pot, was recorded from the control plants.

Yield components showed significant differences ($P<0.05$) except for 1000 grain weight (Table 3). The highest panicle length was observed when 12 t/ha *azolla* was used (23.31 cm) but there was no statistical difference with other levels of *azolla* and nitrogen. The lowest panicle length was (19.22 cm) recorded from the control. Highest panicle weight was found when *azolla* was applied at a rate of 8 t/ha (4.45 gm), but the weight did not differ significantly with the weight observed when urea (60 kgN/ha) was used. The lowest panicle weight was recorded in the control treatment (3.95 gm). Days to 50% flowering (Table 3) shows that when *azolla* was applied at the rate of 8 t/ha and 12 t/ha, respectively, rice flowered late and there was no significant difference between the two levels. Early flowering was observed in the control plants (66 days). Days to maturity did not differ much, and early maturity was observed in control

Table 3. Effect of *Azolla* level and urea on the yield and yield components of rice variety Xiang Zhou 5.

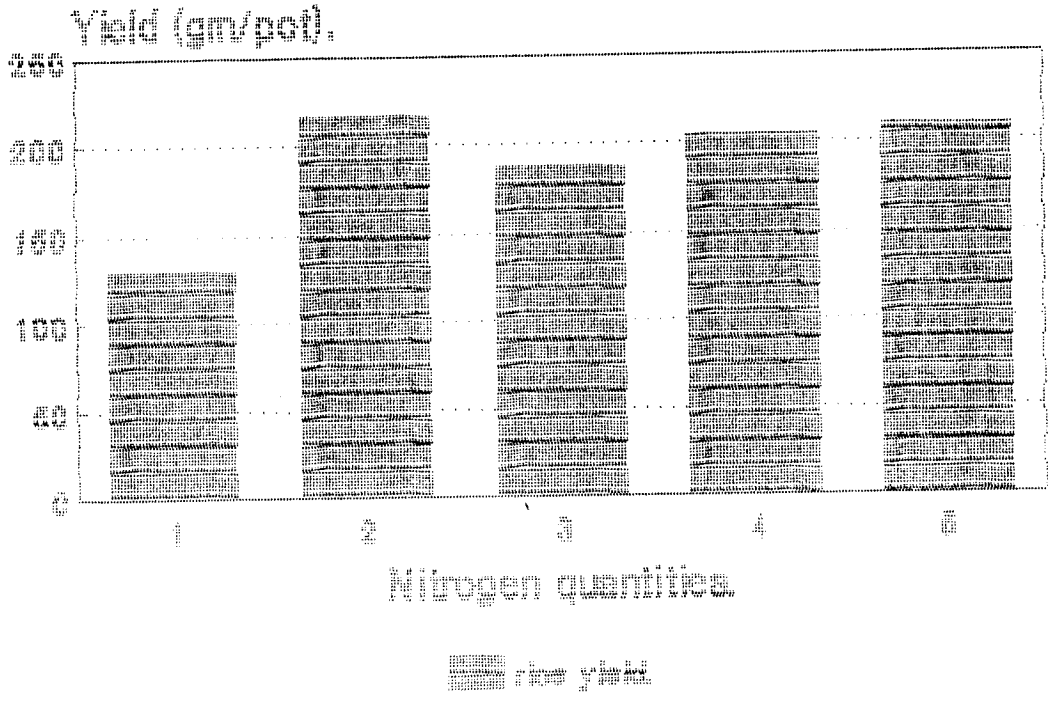
	Yield gm/pot	Panicle length	Panicle weight (cm)	Days to 50%flo (gm)	Days to maturity	Plant height	1000 gr.wt	Tiller/ plant (gm)
Control	130.44c	19.12b	3.95c	66 c	124 c	57.71c	29.70a	6 c
Urea	217.11a	22.54a	4.32ab	67 b	127 b	73.39a	31.33a	10 ab
Az.4t/ha	187.18b	22.78a	4.25b	68 a	128 a	72.94a	30.61a	9 ab
Az.8t/ha	203.52a	22.62a	4.45a	68 a	128 a	70.46b	30.71a	10 ab
Az.12t/ha	209.76a	23.31a	4.17b	68 a	129 a	73.10a	28.51a	12 a
LSD	12.389	1.066	0.181	0.699	1.172	1.402	3.012	2.169
CV%	7.87	5.85	5.17	3.33	11.10	15.32	12.47	27.58

Means in the same column followed by the same letter are not significantly different from each other, according to Duncans Multiple Range Test ($P \leq 0.05$)

Key: Az. - *Azolla*.

Figure 2.

Effect of N on rice yield.



1 = no N, 2 = urea 60KgN/ha, 3= Azolla 4t/ha, 4 = Azolla 8t/ha, 5=Azolla 12t/ha

plants (124 days) while late maturity was observed when 12 t/ha *azolla* was used (129 days). The latter did not differ from the 4 and 8 *azolla* t/ha treatments (Table 3).

The tallest plants (73.10 cm) were observed when *azolla* at a rate of 12 t/ha was used, but there was no significant difference with that obtained when urea at (60 kgN/ha) and 4 t/ha *azolla* was used. The shortest plants at maturity were observed from the control plants (57.71 cm). The highest tiller number was observed when 12 t/ha of *azolla* was applied but there was no statistical difference with that obtained when urea at (60 kgN/ha) and *azolla* at 8 t/ha were used. The lowest number of tillers was recorded from the control plants at 6 tillers per plant while urea and the *azolla* treatment had similar number of tillers per plant.

Table 4 shows that higher dry matter was observed when *azolla* at 8 t/ha was applied (121.92 gm), but did not differ from that obtained when *azolla* at 12 t/ha was applied (120.90 gm). The lowest dry matter was from the control plants (101.38 gm). Harvest index observed from fertilized plants did not differ statistically. However a higher harvest index was observed when urea (60 kg/ha) was applied (0.61) while the lowest was from the treatment supplied *with azolla* at a rate of 12 t/ha (0.54). A difference was observed in plants which were supplied with nitrogen. Table 4 shows that the highest amount of nitrogen in the plants (3.52 mg) was recorded when 12 t/ha *azolla* was used. The lowest amount of nitrogen (1.95 mg) was recorded from the control plants but did not differ from the 4 and 8t/ha

Table 4. Effect of *azolla* level and urea on plant N, grain N and dry matter production of rice variety Xiang Zhou 5.

	Plant N (mg)	Grains N (mg)	Dry matter (gm)	HI
Control.	1.95 c	0.62 b	101.38 d	0.56 a
Urea 60 KgN/ha.	2.87 b	2.38 a	119.03 bc	0.61 a
Azolla 4 t/ha.	3.22 ab	2.33 a	118.05 c	0.61 a
Azolla 8 t/ha.	3.30 a	2.17 a	121.92 a	0.60 a
*Azolla*12 t/ha.	3.52 a	2.28 a	120.90 ab	0.54 a
LSD	0.124	0.214	2.685	0.062
CV%	14.46	13.19	12.79	14.81

Means in the same column followed by the same letter are not significantly differently from each other, according to Duncans Multiple Range Test ($P \leq 0.05$).

treatments. Nitrogen content in the grains harvested from fertilized plants did not differ statistically. The highest amount of nitrogen (2.38 mg) was recorded from plants which were supplied with urea (60 kgN/ha). The lowest nitrogen content was recorded from the grains in the control plants (0.62 mg).

4.4. Interaction of time of application of *azolla* and nitrogen on rice yield and yield components.

There was no clear pattern observed in yield when levels of *azolla* and time of *azolla* application was increased. Table 5 shows that higher yield was found when *azolla* at a rate of 12 t/ha was incorporated during transplanting and 56 days after transplanting (261.27 gm). The lowest yield was from the control plants at 128.40 gm.

Highest dry matter was found when *azolla* was applied at the rate of 8 t/ha at transplanting and 56 days after transplanting (135.20 gm). Lowest dry matter was observed from the control (96.94 gm). Days to 50% flowering of rice in the control plants took 66 days where as the longest time was 71 days in plants supplied with 8 t/ha and 12 t/ha *azolla* at transplanting and 70 days after transplanting. Rice in the control pots took 124 days to maturity while plants supplied with *azolla* at rates of 8 t/ha and 12 t/ha azolla incorporated during transplanting and 70 days after transplanting took about 131 days to reach maturity.

Nitrogen in the grain was highest in plants supplied with *azolla* at 8 t/ha at transplanting and 56 days after transplanting (2.63

mg). Lowest nitrogen content in the grains was from the control plants (0.49 mg). Highest dry matter was obtained when *azolla* at 8 t/ha was applied at transplanting and 70 days after transplanting (135.20 gm) but did not differ with that obtained when *azolla* at 12 t/ha was incorporated at transplanting and 70 days after transplanting (132.18 gm).

Table 6 shows that highest panicle length was found when *azolla* was supplied at a rate of 4 t/ha during transplanting and 42 days after transplanting (23.82 cm) and the lowest panicle length was observed from the control plants (18.32 cm). Across the treatments, panicle length did not differ very much except for the control. Lowest number of tillers per plant were found from the control plants (5.00), while the highest number of tillers was recorded from plants supplied with 8 t/ha *azolla* applied during transplanting and 56 days after transplanting. Highest plant height was observed when *azolla* at a rate of 4 t/ha was used at transplanting and 42 days after transplanting (74.12 cm). Lowest plant height was from the control plants (58.70cm), but the trend of plant height was not clear. Higher harvest index was observed when urea at a rate of 60 KgN/ha was applied (0.63) and the lowest (0.39) was from treatments supplied with *azolla* at a rate of 12 t/ha at transplanting and 56 days after transplanting.

Table 5. Effect of rate and time of application of *azolla* and urea on the yield and some yield components of rice, variety Xiang Zhou 5.

	Days to 50% flo	days to maturity	Dry matter (gm)	Nitrogen in grain (mg)	Yield gm/pot
N1DT	66.00 g	124.00 f	104.37 g	0.49 f	128.40 g
N2DT	67.33 defg	127.33 cde	115.16 ef	2.09 bcde	180.70 ef
N3DT	66.67 efg	127.00 cde	110.14 f	2.17 abcde	160.03 f
N4DT	67.33 defg	128.33 bcde	115.25 ef	2.07 cde	160.20 f
N5DT	67.00 efg	128.67 abcd	111.74 f	1.95 e	169.73 ef
N1W6	66.00 g	124.00 f	96.94 h	0.68 f	150.90 g
N2W6	67.67 def	126.33 def	120.07 de	2.33 abcde	232.23 b
N3W6	68.00 cde	128.00 cde	112.14 f	2.63 a	187.63 de
N4W6	70.00 ab	126.00 def	110.87 f	2.47 abcd	218.70 bc
N5W6	67.67 def	127.00 cde	111.66 f	2.59 ab	193.73 cde
N1W8	67.33 defg	125.67 ef	100.25 gh	0.59 f	131.93 g
N2W8	66.33 defg	126.67 def	115.30 ef	2.53 abc	231.13 b
N3W8	67.67 efg	128.00 cde	122.47 cd	1.97 de	189.73 de
N4W8	67.00 efg	127.33 cde	126.36 bc	2.16 abcde	234.57 b
N5W8	69.33 bc	129.67 abc	128.02 bc	2.56 abc	261.27 a
N1W10	66.33 fg	126.00 def	103.97 g	0.72 f	130.53 g
N2W10	68.00 cde	127.67 cde	125.59 cd	2.57 ab	224.37 b
N3W10	71.00 a	130.67 ab	127.43 bc	2.55 abc	211.33 bcd
N4W10	71.00 a	131.00 a	135.20 a	1.99 de	220.60 bc
N5W10	68.67 bcd	131.00 a	132.18 ab	2.01 de	214.30 bcd
LSD	1.398	2.345	5.370	0.429	24.779
CV %	3.33	11.10	12.79	13.19	7.87

Means in the same column followed by the same letter are not significantly different from each other, according to Duncans Multiple Range Test ($P \leq 0.05$).

DT- during transplanting.
W6- DT plus 42 days after transplanting.
W8- DT plus 56 days after transplanting.
W10-DT plus 70 days after transplanting.

N1- No azolla no urea.
N2- Urea 60 KgN/ha.
N3- *Azolla* 4 t/ha.
N4- *Azolla* 8 t/ha.
N5- *Azolla* 12t/ha.

Table 6. Effect of time and rate of application of *azolla* on the components of rice, variety Xiang Zhou 5.

	Panicle length (cm)	Panicle weight (gm)	1000 grain (gm)	Tillers plant	Plant height (cm)	Harvest index
N1DT	18.32 f	3.79 fg	24.79 ab	5.00 e	60.47 d	0.55 a
N2DT	22.69 abcd	4.46 abc	32.30 a	10.33 abcd	71.23 ab	0.60 a
N3DT	21.98 bcd	4.26 bcde	31.60 a	7.67 bcde	71.64 abc	0.59 a
N4DT	21.58 bcd	3.95 efg	28.93 ab	8.67 abcde	72.84 ab	0.58 a
N5DT	22.33 abcd	4.19 bcdef	29.90 ab	9.00 abcde	72.39 ab	0.60 a
N1W6	18.58 f	4.01 defg	29.93 ab	6.00 cde	58.70 d	0.57 a
N2W6	22.88 abcd	4.29 abcde	31.47 a	10.33 abcd	73.67 ab	0.63 a
N3W6	23.24 abcd	4.49 ab	30.80 a	11.00 abc	74.12 ab	0.62 a
N4W6	22.89 abcd	4.69 a	29.90 ab	8.33 abcde	69.69 bc	0.60 a
N5W6	24.79 a	3.95 defg	29.67 ab	11.33 ab	72.91 ab	0.58 a
N1W8	20.92 de	4.04 cdefg	29.67 ab	5.67 de	60.04 d	0.56 a
N2W8	21.02 cd	4.36 abcde	31.03 a	9.67 abcde	72.19 abc	0.59 a
N3W8	22.07 bcd	3.74 g	29.43 ab	9.67 abcde	73.18 ab	0.61 a
N4W8	22.55 abcd	4.49 ab	31.20 a	10.00 abcde	67.94 c	0.62 a
N5W8	22.44 abcd	4.37 abcd	30.07 ab	11.33 ab	73.40 ab	0.39 b
N1W10	18.85 ef	3.96 defg	29.43 ab	6.00 cde	59.62 d	0.55 a
N2W10	23.42 abcd	4.15 bcdefg	30.53 a	10.00 abcde	72.44 ab	0.62 a
N3W10	23.83 ab	4.51 ab	30.63 a	10.33 abcd	72.80 ab	0.62 a
N4W10	23.48 abc	4.68 a	32.93 a	13.00 a	71.39 abc	0.62 a
N5W10	23.67 abc	4.15 bcdefg	22.97 b	13.00 a	73.71 ab	0.60 a
LSD	2.133	0.362	6.203	1.088	1.398	0.138
CV%	5.85	5.17	12.47	27.58	15.32	14.81

Means in the same column followed by the same letter are not significantly different from each other, according to Duncans Multiple Range Test ($P \leq 0.05$).

DT - During transplanting
W6 - DT plus 42 days after transplanting.
W8 - DT plus 56 days after transplanting.
W10 - DT plus 70 days after transplanting.

N1- No azolla no urea
N2- Urea 60 Kg/ha
N3- *Azolla* 4t/ha
N4- *Azolla* 8t/ha
N5- *Azolla* 12t/ha

4.5 Correlations between various components of rice.

Table 7 shows positive correlations between yield and several yield components. Yield was found to be positively correlated with panicle length, days to 50% flowering, plant height at maturity, days to maturity and tillers per plant, but a weak positive correlation was observed between yield and 1000 grain weight. Other parameters tested which showed positive correlations with rice grain yield were dry matter, grain nitrogen,plant nitrogen content and harvest index.

Table 7. Linear correlation coefficients between and among the yield and yield components of a rice variety Xiang Zhou 5.

	r-1	r-2	r-3	r-4	r-5	r-6	r-7	r-8	r-9	r-10	r-11
r-2	0.62*										
r-3	0.12	0.36									
r-4	0.31	0.16	0.49								
r-5	0.51*	0.04	0.34	0.50*							
r-6	0.55*	0.04	0.31	0.36	0.73*						
r-7	0.51*	-0.09	0.28	0.57*	0.57*	0.54*					
r-8	0.61*	0.01	0.36	0.58*	0.56*	0.61*	0.69*				
r-9	0.64*	-0.12	0.27	0.50*	0.50*	0.52*	0.58*	0.82*			
r-10	0.56*	-0.02	0.42	0.43	0.68*	0.83*	0.47*	0.57*	0.51*		
r-11	0.53*	0.01	0.45*	0.55*	0.52*	0.59*	0.49*	0.72*	0.62*	0.74*	
r-12	0.31	0.09	0.31	0.46*	0.43	0.42	0.32	0.38	0.26	0.61*	0.73*

* significant at $P < 0.05$

Legend

- r-1 Tillers per plant.
- r-2 1000 grain weight (gm)
- r-3 Panicle weight (gm)
- r-4 Days to 50% flowering
- r-5 Panicle length (cm)
- r-6 Plant height (cm)
- r-7 Days to maturity
- r-8 Dry matter (gm)
- r-9 Nitrogen in the plant (mg)
- r-10 Nitrogen in the grains (mg)
- r-11 Yield gm/pot
- r-12 Harvest Index

CHAPTER 5

5.0 DISCUSSION.

Results obtained from this trial show that an increase in *azolla* rates tends to increase rice yield, but in order to attain higher yield, time of incorporation of *azolla* has to be taken into consideration. Among the four times of application of *azolla* used during this trial, it was observed that when higher rates of *azolla* were incorporated during transplanting and 56 days after transplanting, higher rice yield was obtained (261.27 gm/pot). This suggests that the time of incorporating nitrogen in a rice field has a significant influence on the yield of rice. Krock *et al.* (1987) reported that good rice yield can be obtained when *azolla* is incorporated 45 days before heading. The findings also agree with that of Hall (1965) who observed that varieties produce higher yields when they are fertilized 45-60 days before heading. This means that if fertilization is properly timed with the reproductive cycle of the rice plant, nitrogen application can increase the number of florets and eventually produce high grain yield.

When comparing the yield obtained from different levels of *azolla* and that of urea, it shows that higher yield was obtained when urea(60 kgN/ha) was used (217.11gm/pot). This suggests that inorganic fertilizer can give higher yield compared to organic manure when applied to the soil at the same time. The reason could be due to its easy availability to the rice plant compared to that of *azolla* which needs time to decompose in order to release nitrogen to the rice plant. This observation is supported

by Ventura and Watanabe (1993) who reported that *azolla* N is released at later stages in the plant's growth and this could account for the lower grain yield with *azolla* when compared to urea.

On the yield comparison between different quantities of *azolla* and urea, the findings obtained in this study differ with those obtained by Watanabe (1980) who found that incorporating two crops of *azolla* before and after transplanting increases rice yield equivalent to that obtained from 60 kgN/ha as urea. But the finding is supported by Ventura *et al.* (1987) who reported that urea fertilizer can give significantly higher grain yield than *azolla*, while Kulasooriya *et al.* (1980) reported that performance of *azolla* compared to urea is very encouraging, the effect of *azolla* being interpreted as equivalent to yield that would have been obtained from 55-84 kgN/ha as urea. The same observation was made by Hera (1995) who found that when *azolla* was incorporated into the soil, it proved to be as good as urea as a nitrogen source for rice.

Low yields obtained from the control plants where no *azolla* or urea was applied shows that growing rice in the absence of nitrogen normally results in low yield production. This is probably due to the low rate of photosynthesis caused by nitrogen deficiency, since nitrogen is an important constituent of numerous organic compounds which are important in a plant's life cycle. Some of the organic compounds are amino acids, growth hormones, proteins and chlorophyll. Chlorophyll is important for

photosynthesis to take place and hence dry matter accumulation (Hay and Walker,1994). This shows the importance of nitrogen for plant growth and hence rice grain yield. This finding is supported by the observation of Diara *et al.*(1987), who observed that *azolla* always increased rice grain yield compared to treatments without *azolla.* Mengel and Kirkby (1987) also stated that when the level of nitrogen nutrition is insufficient, the plant life cycle is shortened, forcing the plant to mature earlier, resulting in poor economic yield as demonstrated by the present study.

Late fertilization results in excessive vegetative growth, with many tillers. The tillers formed normally produce small panicles with few matured grains, resulting in low yield production. Higher rates of fertilizer applied late in the vegetative stage can lead to excessive vegetative growth and hence formation of higher dry matter.

In this study dry matter production was found to increase with increasing level of *azolla.* This means that increasing the rates of application of nitrogen sources, vegetative growth also increases resulting in more formation of tillers and panicles which are a determining factor for high yield. In this study dry matter was found to be positively correlated with yield. The same observation was reported by Megh (1962) that dry matter synthesis was strongly correlated with rice grain yield.

A positive correlation (r^2=0.53*) was found between rice grain yields and the number of tillers per plant, impliying that the number of tillers have great influence on the yield of rice grains since tillers determine the number of panicles to be formed. Few tillers normally result in low grain yield. Sometimes excessive number of tillers can also result in low grain yield, especially when the nitrogen source is top dressed late. This finding agrees with that of Kawano *et al.*(1968) who reported that tiller number was positively correlated with grain yield. They also observed that nitrogen application markedly increases the tillering capacity of a rice plant. Eastian *et al.*(1969) reported that tillering of rice plant is strongly influenced by genetic factors and by the levels of nitrogen applied to the soil. Russell (1988) also found that nitrogen application increases tillers per plant.

A lack of significant correlation (r^2= 0.01) was observed between yield and 1000 grain weight over different levels of nitrogen application, implies that incorporation of different levels of nitrogen does not affect the weight of the individual grains and hence the grain yield. This suggest that 1000 grain weight does not contribute much towards the yield of a rice plant. Eastian *et al.*(1969) reported similar results, that nitrogen levels had a negligible influence on 1000 grain weight. Darwinkel (1983) also reported that timing of fertilization had a relatively small influence upon individual grain weight, but had a marked influence upon grain nitrogen content. This is probably due to genetic factor of the variety itself, that the amount of nitrogen

taken to the grains is not effected by different quantities of nitrogen applied to the soil.

The findings of Darwinkel (1983) differs slightly from what was observed in this trial. Nitrogen contents in the rice grains, in this case did not differ as the levels of nitrogen increased. There was no significant difference observed between grains harvested from plants incorporated with different levels of *azolla*. A difference was observed when grains from N treated plants were compared with the grains from plants which were not applied with *azolla* or urea.

The time of incorporating *azolla* in the rice field has an influence on days to 50% flowering and days to maturity. In this trial, plants which were applied with *azolla* at transplanting and 70 days after transplanting matured late. This means that late application of fertilizer results in prolonged vegetative growth instead of formation of grains. This delays grain maturity. The findings agree with those of De Datta (1981) and Russell (1988) who reported that late application of nitrogen is not beneficial to the rice plant but delays maturity.

The amount of nitrogen in the plant at harvest was found to increase with the rate of *azolla* application and time of application. This shows that with increasing levels of nitrogen in the soil, plants will extract more nitrogen from the soil resulting in accumulation of nitrogen in the plant. This finding agrees with that made by Ventura *et al.* (1993) who reported that

rice nitrogen uptake increases with the time and rate of application of azolla. In this study, a positive correlation (r^2=0.62*) was observed between nitrogen content in the plant dry matter after harvesting and the rice grain yield.

The significant difference observed in the plant nitrogen content across the treatments, was probably due to the fact that urea N is normally taken up more rapidly by the rice plant but is easily lost from the soils by evaporation or denitrification leaving a smaller amount for plant uptake. Plants applied with *azolla* had more nitrogen content, since *azolla* N is slowly released and not easily lost from the soils, thus most of it is taken up by the plant during growth. This could be the reason why *azolla* treated plants had more nitrogen content at the time of harvesting than the urea and the control treatments. This observation is supported by the findings of (Grist, 1986; De Datta, 1981; Becking, 1979 and Hera, 1995) that inorganic N can easily be lost to the atmosphere through volatization, leaching and surface run off and its efficiency is usually lower compared to that of *azolla* nitrogen.

A positive correlation (r^2=0.73*) was observed between panicle length and plant height, impliying that the size of the panicle to be produced depend on the height of the rice plant, short plants produce small panicles while taller plants produce long panicles. In this case the length of the panicle had an influence on the yield of rice, since the size of the panicle will determine the number of grains to be carried on that panicle; the

more the filled grains the more the potential yield obtained. This finding agrees with that of Grist (1986) who observed a positive correlation between panicle length and rice grain yield.

CHAPTER 6

SUMMARY AND CONCLUSION.

This study shows that growing rice without any source of nitrogen results in low production. The use of *azolla* increases rice yield but not beyond that obtained with inorganic fertilizers. The highest rice yield was obtained when *azolla* at the rate of 12 t/ha was split incorporated at time of transplanting and 56 days after transplanting i.e 261.27 gm/pot (3.62 t/ha).

On the basis of the above findings, it is concluded that where successful *azolla* growth can be achieved, it will have a beneficial effect on rice cultivation. Also *azolla* can substitute inorganic fertilizers in rice cultivation. However, more research is needed to determine the performance of *azolla* in the rice fields under farmers management. Further research is also required to determine the amount of nitrogen contributed by *azolla* in the soil.

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APPENDICES.

Appendix 1. Significance of the F statistic for the yield and yield components of rice variety Xiang Zhou 5 as influenced by time and rate of application of *azolla*.

			1	2	3	4	5	6	7	8	9	10	11	12
Time(T)	*	*	n.s		*	*	n.s	n.s	*		*	*	*	n.s
Nitr(N)	*	*	*		*	*	*	n.s	*		*	*	*	n.s
T x N	*	*	*		*	*	*	n.s	*		*	*	*	n.s

* significant at P≤ 0.05
ns- not significant.

1. Yield
2. Panicle length.
3. Panicle weight.
4. Days to 50% flowering.
5. Days to maturity.
6. Plant height.
7. 1000 grain weight.
8. Tillers per plant.
9. Plant nitrogen.
10. Grain nitrogen
11. Dry matter.
12. Harvest index.

Appendix 2. Climatic data for Lusaka International Airport during 1994/95 cropping season (OCT. 1994- JUNE 1995).

	NOV	DEC	JAN	FEB	MAR	APR	MAY	JUNE
Rain (mm)	43.5	99.5	41.8	105.4	23.9	0	0	0
MMT1	32.3	28.7	28.0	26.9	27.9	28.7	26.6	23.8
MMT2	20.3	19.6	17.4	16.3	15.8	16.7	13.4	10.9

Legend: MMT1 - Mean maximum temperature.
MMT2 - Mean minimum temperature.

Source: Mateorological Department. 1995.