CHAPTER: I
INTRODUCTION

1.1 Background

Nepal is an agricultural country with an area of 147,181 sq. km. and population of 23.1 millions people (MPE 2001 report). It is located in between the latitude 26º 22’N to 30º 27’ North and longitude 80º 04’E to 88º 12’ East. About 65.7% of total population of country is engaged in agriculture. Among various agricultural crops rice (*Oryza sativa* L.) is the major cereal crop and it is the staple food of Nepalese people. Rice is one of the oldest cultivated crops originated as early as 3000 BC in South East Asia. It is cultivated throughout the world and half of world’s population depends on rice. In Nepal, it is grown in 55% of total cultivated land in all agro-ecological conditions (FAO, 2001) which ranges from lower terai to Chhumchaur of Jumla, the highest point of the world where rice is cultivated.

In Nepal the area of rice cultivation has increased from 1.368 million hectares (1994/95) to 1.542 million hectares (2004/05) and the annual production of rice has increased from 2.906 million metric tons (Mt) (1994/95) to 4.29 million metric tons (2004/05) (MOAC, 2006), but even this increase in area and annual production are not able to fulfill the need of increasing population of Nepal. The growth in rice production rate is low (grain yield 2.07% per annum) compared to the rate of population growth (2.25% per annum). This trend has led the country to become a net rice importing country after being an exporting country in the 1960's and 1970's (Sah, 2000).

Nitrogen is widely recognized as the most limiting nutrient for production of wetland rice. Further, the introduction of new high yielding rice cultivars has increased the demand for nitrogen in rice production. In developed countries the required nutrient nitrogen is fulfilled by nitrogenous fertilizers manufactured by very high energy consuming process using fossil fuels, as source of energy. Although, chemical fertilizers have proved to be effective, the marginal farmers of Nepal living under poverty are compelled to use very low dose of fertilizers than recommended due to unaffordable price. A geographical limitation is another causal factor that makes difficulties in transportation of chemical fertilizer. Considering its importance, fertilizer is one of the key input investments prioritized by the Nepal government next to irrigation and road construction. The nutrient application rate increased from 20 kg ha^{-1} in 1992 to 42 kg ha^{-1} in 1999. The Agricultural prospective plan (APP) of Nepal aimed to increase the input of fertilizer nutrients to 68 kg ha^{-1} by 2002 and forecasts 150 kg ha^{-1} by 2014-2015 (Pandey and Joshy, 2000). However, this has led to an unbalanced and haphazard use of fertilizers which may have a negative effect in soil quality, on the economy and on the environment.

Many researchers have demonstrated the low efficiency of N- fertilizer in low land rice (De Datta, 1987; Vlek and Creswell, 1981), which is caused by high losses of N through different mechanisms among which ammonia volatilization is recognized to dominate in flooded rice (Roger *et al.*, 1987). Generally, fertilizer N recovery by rice ranges from 10-60% (Creswell and

Vlek 1979; Fillery *et al.*, 1984). On the other hand, it would not be economical for farmers to apply more fertilizers since marginal productivity tends closer to real price of fertilizer. Further, intensive fertilizer use also causes chemical soil pollution, which has occasionally been observed as a consequence of an increased content of elements such as Zinc, Lead, Nickel, Chromium etc. which are normally present as traces and which causes toxicity to plants at higher levels and causes yield depression (Cottenie, 1872).

The nitrogen constitutes about 78% of the atmosphere; it is not directly available for most of the plants including the cereal crops. It is converted into combined form of organic compounds by some prokaryotic organisms through biological reaction, a phenomenon known as Biological Nitrogen Fixation (BNF) (Prasad, 2003). To achieve food security through sustainable agriculture, the requirement for fixed nitrogen must increasingly be met by Biological nitrogen fixation rather than by using nitrogen fixed industrially (Ladha and Reddy, 2003), which leads introduction of biofertilizer. The term biofertilizer denotes all the nutrient inputs of biological origin for plant growth (Subba Rao, 1982), which are ecofriendly, fuel independent, cost-effective and easily available alternative source of nutrient Nitrogen. The main agents of biological nitrogen fixation in rice fields are free-living blue green algae and symbiotic blue green algae with *Azolla*. Utilization of *Azolla* as biofertilizer will be more effective because it produce relatively more biomass and fixes more nitrogen than free-living BGA. It is also easy to handel and culture as well.

1.2 *Azolla*

Azolla, the smallest pteridophytes, is delicate free floating fern with aquatic and semi aquatic habitat which is commonly found in still water in ponds, ditches and paddy field from temperate to tropical regions. It is commonly known as mosquito fern, water velvet, water fern etc. The most favourable mean air temperature range for growth of all *Azolla* species is between 20-30ºC and P^H between 4.5-7. The genus was established by Lamark in 1983 which is derived from two Latin words: '*azo*'- to dry and '*ollyo*'- to kill which signify its nature of habitat.

Azolla is heterosporous leptosporangiate fern, represent by thirty-one species, out of which seven are extant species. Genus *Azolla* belongs to family Azollaceae of the order Salviniales and divided into two sections *Azolla* and *Rhizosperma* mainly on the basis of number of floats in megaspore and type of massulae trichomes or glochidia. Section *Azolla* includes five species viz. *Azolla caroliniana* Willdenow, *A. filiculoides* Lam., *A. mexicana* Presl, *A. microphylla* Kaulf., *A. rubra* R.Br., these species have three floats and arrow shaped glochidia. Section *Rhizosperma* includes two species namely *A. nilotica* Decaisne, and *A. pinnata* R.Br. These species have nine floats and massula without or with simple glochidia.

Azolla has been of interest to botanist for years because of its remarkable feature that it is the only genus of pteridophytes which exist in a symbiotic association with Nitrogen fixing prokaryotes the *Anabaena azollae*. *Anabaena azollae* occurs in the cavity of dorsal lobe of *Azolla* leaves. The delicate fern provides nutrients and shelter for the *Anabaena* which in turn provides nitrogen for the fern. The symbiotic relationship is most active in flooded soil where it can fix as much as four kg of atmospheric nitrogen per hectare per day. The significance of *Azolla* as biofertilizer in rice field was realized in China and Vietnam and have been described as miniature nitrogen fertilizer factories.

Trichomes of *Anabaena azollae* consists of larger and conspicuous cells called Heterocysts, the actual site of Nitrogen fixation which are rich in enzyme nitrogenase, the enzyme responsible for biological nitrogen fixation. There are two types of heterocysts, intercalary and terminal. Heterocysts can be distinguished from vegetative cells by one (in terminal heterocyst) and two pores (in intercalary heterocyst) through which these remain connected to adjacent vegetative cells. Heterocysts lack enzymes such as ribulose biphosphate (rubisco) and those of Calvin cycle. Thus, it lacks PS II activities and carbondioxide fixation as done by vegetative cells. The heterocyst provides an anaerobic environment for the protection of nitrogenase because nitrogenase becomes inactive in presence of oxygen. Since oxygen is not evolved in protoplast due to absence of PS II, heterocyst, thus provide ideal atmosphere for nitrogen fixation process and for the activities of nitrogenase.

CHAPTER : II
OBJECTIVES

2.1 Objectives

The following are the objectives of the present investigation:

- To determine the heterocyst frequency, doubling time, relative growth rate, amino nitrogen and chlorophyll content in *Azolla caroliniana* and *A. pinnata*.
- To study the effect of *Azolla* and Urea on chlorophyll content in leaf of rice variety at different stages.
- To study the effect of *Azolla* and Urea on yield and yield components in rice variety.
- To study the effect of *Azolla* and Urea on Nitrogen and Organic matter content of soil.

2.2 Justification of the study

The population growth rate is higher than the growth rate of rice production. This forces the farmers to increase production to feed the growing population. The increase in production by increasing the area of land under cultivation is not an effective solution rather than increasing the productivity of the area. Productivity can be increased by increasing the dose of the chemical fertilizer but that will not be good option for sustainable agriculture.

The chemical fertilizers are manufactured by consuming the fossil fuels. Due to increase in the cost of the non renewable fossil fuels, the price of chemical fertilizers is increasing day by day and which becomes unaffordable to the marginal farmers. Further, for those farmers, using limited dose of chemical fertilizers, it would not be economical to apply more fertilizers. Also in places where fertilizer is in adequate supply, farmers are using it in haphazard way due to insufficient knowledge of using chemical fertilizers. This haphazard use of chemical fertilizer is not only spoiling the ecosystem but also degrading the natural fertility of soil.

Biofertilizers which are eco-friendly and cost-effective are the most suitable option to achieve food security through sustainable agriculture. The paddy field forms an ideal environment for luxurious growth and multiplication of *Azolla*. The algal symbiont inside *Azolla* is well known to fix atmospheric nitrogen and makes it available to rice plants. Apart from direct nutritional effect, *Azolla* can also act as a physical barrier to the escaping NH_3 and also checks weed growth in rice field. Thus applying them to rice field will definitely have positive effect on rice productivity which can enhance the economic status of farmers and finally of the country.

CHAPTER : III

LETERATURE REVIEW

3.1 *Azolla* as biofertilizer

Watanabe *et al.* (1977) carried out an experiment on utilization of the *Azolla* –*Anabaena* complex as a nitrogen fertilizer for rice. Pot experiments revealed that nitrogen in dried *Azolla* increased rice growth but its availability to rice was 40% lower than that of ammonium fertilizer nitrogen. Five crops of *Azolla* produced a total of 117 kg N ha^{-1} in 106 days. Inoculating *Azolla* with phosphorous at the time of transplanting rice and incorporating it after 40 days of growth increased the yield of dry-season rice over that in plots with only mid-season rice puddling.

Maskey and Bhattarai (1982) conducted an experiment to see the effect of *Azolla pinnata* on rice crop and found that single incorporation of *Azolla* increased the yield of rice by 25% which was equivalent to the yield due to application of 30 kg of nitrogen in the form of urea. When *Azolla* was incorporated twice at the time of transplanting and during growth period rice yield increased by 40% over control.

Mandal and Bharati (1983) conducted an experiment on the sandy clay soil and used *Azolla pinnata* as organic manure for rice. The result showed 33-47% increase in yield, 35% in straw yield and 31% in N content in soil after harvested.

Islam *et al*. (1984) reported that use of *Azolla* at the rate of 60 kg ha^{-1} either alone or in combination with urea significantly increased the tiller number of rice but did not affect the plant height. Compared with control, the increase in straw and grain yield were up to 33% and 15% respectively with *Azolla*. After 4 and 8 weeks of application a significant amounts of available N was released in *Azolla* treatments because of decay of the added *Azolla*.

Mian (1984) conducted a ^{15}N – tracer pot study with ' IR 8' rice (*Oryza sativa* Limm.) and found that nitrogen of more labile fractions of both *Azolla* and *Anabaena* was rapidly released for uptake by rice plants and the remaining nitrogen in the more refractory fractions of them was slowly released for plant uptake. The study showed that only 10 and 14% of ^{15}N applied as *Azolla* and *Anabaena* were available to the second crop compared with 25 and 32% applied to the first crop.

Ito and Watanabe (1985) conducted an experiment on availability to rice plants of nitrogen fixed by *Azolla*. It was found that rice plants grown in a pot absorbed 50% ^{15}N – labeled *Azolla* nitrogen incorporated at the time of transplanting. When *Azolla* was kept on the surface of water, less than 10% of its nitrogen was available to the rice plant. The field study showed that larger amounts of *Azolla* nitrogen were available to rice when *Azolla* was incorporated than when it was placed on the soil surface. However, the availability following incorporation (12-27%) was much lower than pots. A later application (78 days after transplanting) resulted in a higher contribution of *Azolla* - nitrogen to grain - nitrogen than an earlier application (30 or 53 days after transplanting).

Bhattarai and Maskey (1987) conducted an experiment, with *Azolla pinnata* to be used as green manure on paddy under green house and field conditions at Khumaltar Agriculture Section and found that 17% increase in grain yield paddy. A favourable effect of *Azolla* compost as a manure has been observed in wheat, chilies and potato crops.

Chaudhary and Mahato (1987) reported that *Azolla* alone could help augment rice yields, provided that water is available during the growing season. Incorporation of *Azolla* at the rate of 1 t ha^{-1} produced grain yield of 3.2-3.4 t ha^{-1}.

Singh (1987) conducted an experiment in which few selected isolates from each species of *Azolla* were studied in detail with respect to Phosphorus and nitrogen fertilization. The growth and nitrogen fixation of *Azolla* in rice fields in presence of pesticides, herbicides, chemical fertilizers and their interaction with rice plants were investigated *Azolla* decomposition its N, P availability to rice crop microorganisms involved in decomposition and nitrogen balance were examined. *Azolla caroliniana* was found to fix more nitrogen in rice field and tolerated pests and disease to a greater extent than *A. pinnata*.

Mishra and Singh (1988) studied on the effect of different methods of *Azolla* application on the growth and nitrogen fixation of *Azolla* and grain yield of rice varieties and concluded that the treatment comprising basal incorporation plus dual cropping of *Azolla* was significantly superior to all other treatments.

Singh and Singh (1988) have stated that *Azolla* possesses the ability of self decomposition after mat formation. The decomposition begins from those plants, which are in lower parts of the canopy. Incorporation of the fern into soil increases the rates of decomposition and nitrogen release.

Singh (1988) conducted that thrice *Azolla* cropping once a basal monocarp and twice as intercrop could fix 60-90 kg N ha^{-1} during a rice crop. *Azolla* is also known to control weeds, check water evaporation, increase nutrient availability and improve soil fertility.

Singh *et al.* (1988) conducted an experiment on *Azolla* and Blue green algae intercropping with rice to analyze the effect of different level of chemical nitrogen (urea). It was found that application of higher levels (60 and 90 kg N ha^{-1}) of nitrogen fertilizer (urea) inhibited the growth of *Azolla pinnata* and blue green algae though the reduction was more in BGA than *Azolla*. Inoculation of 500 kg N $ha^{-1}$of fresh *Azolla* 10 days after transplanting (DAT) in rice fields receiving 30, 60 and 90 kg N ha^{-1}as urea produced an average of 16.5, 15.0 and 13.0 t ha^{-1} fresh biomass of *Azolla* at 30 DAT, which contained 31.31 and 27 kg N ha^{-1}, respectively. The intercropping of *Azolla* and rice in combination with 30, 60 and 90 kg N ha^{-1}as urea showed the yields, yield attributes and nitrogen uptake in rice at par with those obtained by applying 60, 90 and 120 kg N ha^{-1} as urea respectively.

Singh and Singh (1990) evaluated that *Azolla* decomposes after 8-10 days of incorporation into the soil and rice plants are benefited noticeably after 20-30 days. One crop of *Azolla* green manuring provides 20-40 kg N ha^{-1}. The increased grain yield due to incorporation of 10-12 t $ha^{-1}$of *Azolla pinnata* and application of 30 and 50 kg N ha^{-1} as ammonium sulphate was comparable to that obtained with 60 and 80 kg N ha^{-1} of ammonium sulphate respectively. The incorporation of *Azolla* gives higher grain yield than non-incorporated treatment and incorporation of 5-15 t ha^{-1} of *Azolla* one month after transplantation was found to increase rice yield by 12-33% over unfertilized control.

Roger and Ladha (1992) conducted an experiment on estimation and contribution to nitrogen balance by *Azolla* in wetland rice fields. It was found that standing crop of *Azolla* averaging 30-40 kg N $ha^{-1}$and the accumulation of 50-90 kg N ha^{-1} for two crops of *Azolla* grown before and after transplanting rice. Estimates of % Nitrogen derived from atmosphere by 15N dilution and delta 15N methods range from 51 to 99%. N balance in long-term fertility experiments range from 19-98 kg N ha^{-1} crop (average 50 kg N) in field with no N fertilizer applied. Balances are usually highest in flooded planted pots exposed to light and receiving no N fertilizer: extrapolated values range from 16 to 70 kg N ha^{-1} $crop^{-1}$ (average 38 kg N). A compilation of balance experiments with rice soil shows an average balance of about 30 kg N ha^{-1} $crop^{-1}$ in soils where no inorganic fertilizer N was applied.

Watanabe and Liu (1992) conducted an experiment and found that hybrids between *Azolla microphylla* and *A. filiculoides* (male) produced higher annual biomass than either parent. When *Anabaena* form high temperature tolerate *A. microphylla* was transferred to *Anabaena*- free *A. filiculoides*. *A. filiculoides* became tolerant of high temperature. A study using *Azolla* labeled with ^{15}N showed the reduction of N losses by fish uptake of N. The

Azolla mat could also reduce losses of urea N by lowering flood water P^H and storing a part of applied N in *Azolla*.

Adhikari (1997) conducted an experiment to evaluate the effect of methods of *Azolla* cultivation on its growth rate, N production rate and rice production. The result showed that *Azolla* growth rate was not affected by cultivation method used. But N production by monocrop 20 days before transplanting was found superior in N production rate compared to that of N rate produced by dual cropped *Azolla*. The efficiency of urea N in grain production was increased when *Azolla* and urea N were applied together to the rice crop. *Azolla* grown as a dual crop with rice produced economically higher yields compared to that of other cultivation practice used.

Rao and Sitaramaya (1997) conducted pot experiment to test the changes in soil nitrogen forms uptake and grain yield due to integrated nutrient management of rice through conjunctive use of fertilizers urea with FYM biogas slurry, poultry manure and the green manures *Gliricidia* and *Azolla*. *Azolla* application significantly enhanced grain yield followed by *Gliricidia*. Nitrogen uptake by rice was highest among all treatment.

Mandal *et al.* (1999) conducted an experiment to study the beneficial effects of blue green algae and *Azolla* on wetland rice field and found that *Azolla* prevented rise in P^H, reduced water temperature, curb NH_3 volatilization and suppressed weeds. On decomposing, they influenced the redox activity and resulted in the formation of different organic acids in soil. All such changes brought about by BGA and *Azolla* in soil may ultimately influence plant available nutrients and also soil characteristics.

Vandan *et al.* (1999) conducted in which fresh fronds of *Azolla microphylla* were inoculated at 1 t ha^{-1} at 10 DAT and incorporated at 30 DAT (day after transplantation). It was found that a layer of *Azolla* covering a hectare of rice field supplies about 25-30kg N ha^{-1}. Also the results clearly revealed that dual crapping of *A. microphylla* with rice enhanced soil available N status and grain yield.

Adhikari *et al.* (2001) found that use of *Azolla* in rice fields increased 37% of the nitrogen in soil. Studies also medicated that approximately 38 kg ha^{-1} of nitrogen (N) is produced by the introduction of single crop of *Azolla* to the paddies. Incorporation of *Azolla* during rice transplantation produced similar grain yields to those obtained from 100 kg $ha^{-1}$of urea N application at the same level of phosphorous and potassium.

Cisse and Vlek (2003) have stated that nitrogen losses are notoriously high in flooded rice fertilized with urea. An *Azolla* intercrop can reduce such losses by immobilizing urea nitrogen during periods of potentially high nitrogen loss. The reduction in nitrogen loss linked with the absorption and remobilization of urea nitrogen by *Azolla*.

Tuladhar (2003) carried out an experiment to study the effect of *Azolla* and nitrogen use efficiency in rice wheat rotations of Nepal. The combined use of *Azolla* and urea intercropping increased the grain yield by 5-15% in field and dry matter accumulation by 20% in the pot study. On average, the yield following application of *Azolla* alone was corresponded to an application of 30 kg urea N ha^{-1}. Agronomic efficiency, physiological efficiency and apparent recovery efficiency were also increased by combined application of *Azolla* and urea. *Azolla* cover decreased the flood water P^H by 1.4-2.2 units and the NH_3 partial pressure by 0.336 pa, thus reducing the partial for NH_3 voltilization from the flooded rice field after urea application. The agronomic N use efficiency increased from 8.5 kg grain kg^{-1} N applied in sole urea to 21.8 kg^{-1} N applied in the presence of *Azolla*.

Aroro and Singh (2004) studied on six different *Azolla* species namely *A. filiculoides*, *A. mexicana*, *A. microphylla*, *A. pinnata*, *A. rubra* and *A. caroliniana* in a polyhouse to assess their growth potential by determining their maximal biomass productivity, doubling time and relative growth rates. Among them *A. microphylla* gave highest biomass production and relative growth rate followed by *A. caroliniana*. Both these had high nitrogenase activity also. Peak nitrogen use activity of these strains was found on 14^{th} day of growth and it declined of further incubation. On the other hand *A. pinnata* exhibited low biomass production, relative growth rate and lower nitrogenase activity compared to other species.

Nayak *et al*. (2004) studied the effect of urea, blue green algae and *Azolla* on nitrogen fixing potential in terms of acetylene reducing activity (ARA) and biomass accumulation (in terms of chlorophyll) using surface and below surface soil cores collected from rice fields 45-90 days after transplanting (DAT). Application of biofertilizers brought about a significant enhancement in chlorophyll accumulation and its nitrogenase activity, when measured 45 DAT.

3.2 Chemical fertilizer

Kolenbrander (1972) stated that nitrogen loss from fertilizer is higher on light soils than those on clay soils. Nitrogen losses increase as the amount of applied fertilizer increases. Among the plant nutrients responsible for eutrophication, phosphorus and nitrogen has been found to be the most important elements.

Dhyani and Mishra (1993) studied the effect of nitrogenous fertilizer applied at different growth stages on utilization of soil and fertilizer N by rice. The treatment in which 60 kg N ha-1was applied as basal (T1) showed the lowest fertilizer N recovery and the highest contribution of soil N to the plant N during both the years, while 120 kg N ha-1 applied in three splits (50%basal + 25%at tillering + 25%at panicle initiation) showed the highest fertilizer N recovery and the lowest contribution of the soil N. The basal application of N without subsequent top dressing the plants have to depend on soil N during later growth stages. A large portion of the nitrogen applied as basal was subjected to loss through different mechanisms; plants derive most of the required nitrogen from the soil. It was also concluded that the fertilizer N applied at later stages is better utilized by the rice plant than basal application of N, particularly for grain production where as N applied up to flowering is utilized for vegetative growth to a greater extent.

Masayoshi (1994) has stated that most nutrient losses from the paddy field occur during the time of fertilizer application-transplanting. Soon after the application and puddling, high concentration of nitrogen and phosphorus are found in the floodwater. Draining the floodwater soon after the application results in higher looses of nutrients from paddy fields.

Motsara (1994) stated that the fraction of plant nutrients escaping from the soil-plant environment in the hydrosphere or atmosphere becomes an environment pollutant.

Bhattarai *et al.* (2002) have recommended dose of mineral fertilizers on the basis of soil type, crop species, yield level etc. The recommended dose of NPK is 100:40:30 kg ha^{-1}. All the Phosphatic and potassic fertilizer is recommended to use as basal dose while for better efficiency, nitrogenous fertilizer is recommended to apply in 2-3 splits.

Basnet (2004) stated that there is 60-70% loss of nitrogen when it is top dressed through urea under submerged condition.

CHAPTER : IV
MATERIALS AND METHODS

4.1 Study area

The effect of *Azolla* and Urea on the biochemical parameters and yield of rice variety was investigated in the field and pot experiments in the year 2005.

4.1.1 Location and topography

Bhainsepati of Sainbu V.D.C., Lalitpur was selected as research site for field experiment. The field lies in between 27º 39.003’ North to 27º 39.012’ North latitude and 85 º 18.171 ’East to 85 º 18.182’ East longitude. The pot experiment was conducted in the Central Department of Botany TU, Kirtipur, located in between 27º 40’ to 27º 41’ North latitude and 85º 16’ to 85º 18’ East longitude in the south west region of Kathmandu valley and cover 2.76 sq. km at an altitude ranging from 1280m to 1400m above sea level.

4.1.2 Climate

The climatic data of Lalitpur and Kathmandu representing the climate of field and pot experimental site were collected from Department of Hydrology and Meterology , Babarmahal, Kathmandu. In the year 2005, the mean maximum and minimum temperature in Lalitpur was 27.86 ºC and 17.7ºC respectively during the experimental period. The annual rainfall was 1182.3 mm and 74.78% of it was received only in five months i.e. June to

October.The mean maximum and minimum temperature in Kathmandu was 28.9ºC and 18.78ºC during the experimental period respectively. The annual rainfall was 1235.9mm and 84.01 %of it was received only in experimental period.

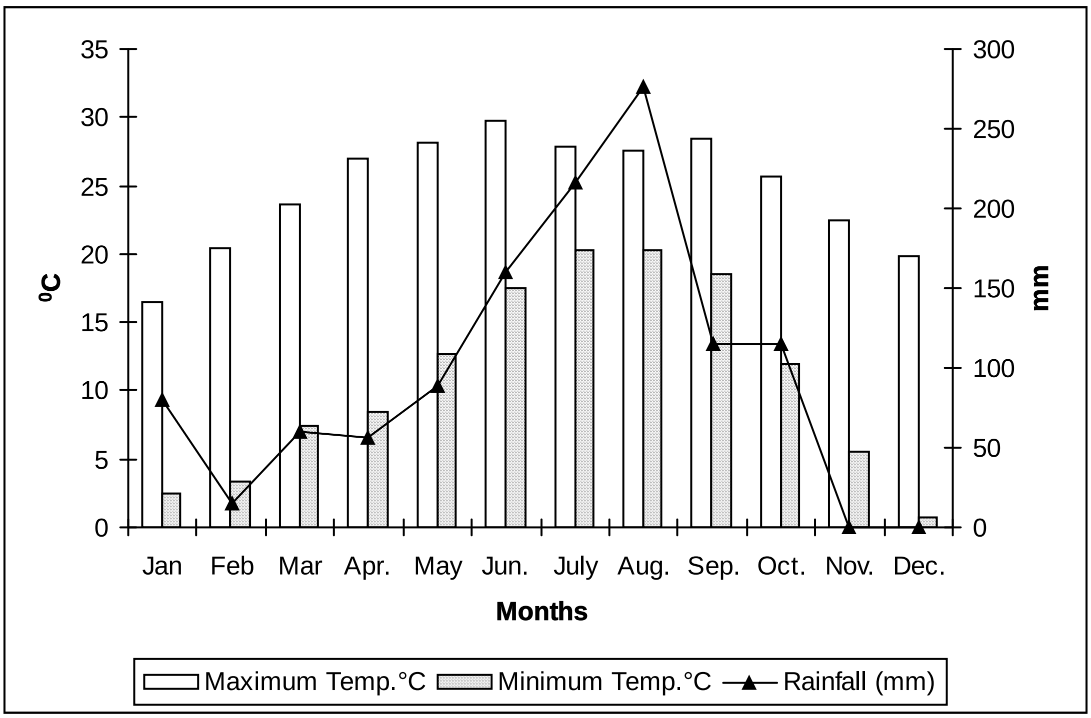

Fig.1: Graphical Representation of Climatic data of Lalitpur (2005).

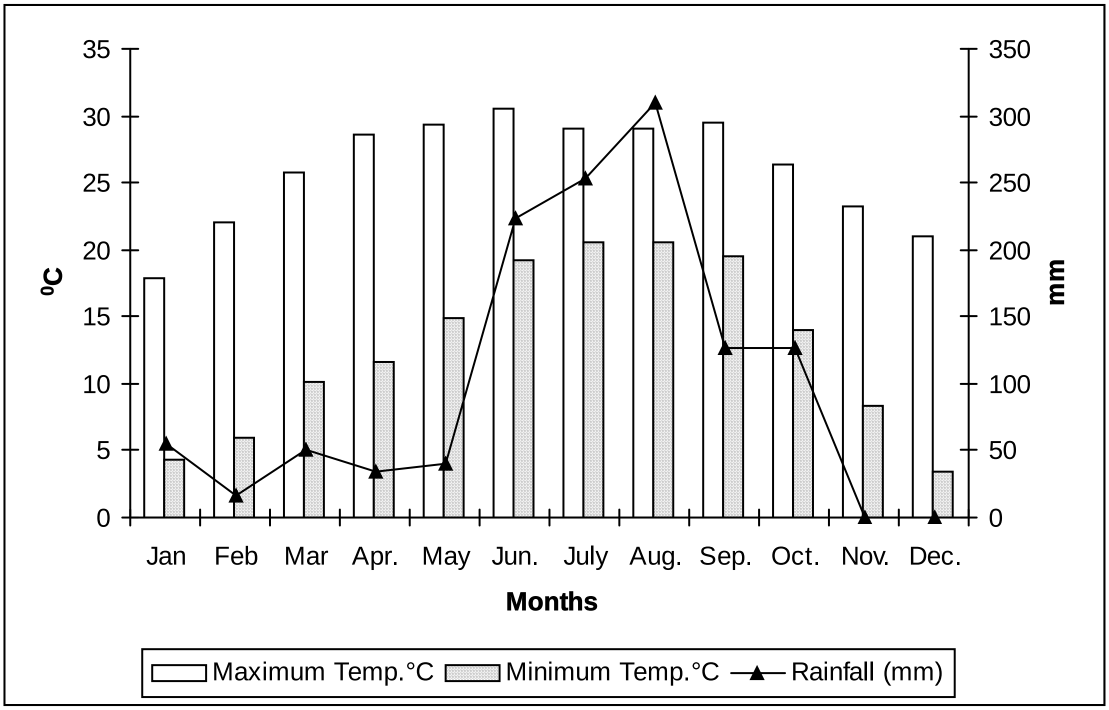

Fig.2: Graphical Representation of Climatic data of Kathmandu (2005).

4.1.3 Soil

The soil of the Bhainsepati was found near neutral (P^H 6.9). Texture of soil was silt loam. The organic matter and nitrogen content was found to be 0.45% and 0.16% respectively. The soil of the Kirtipur was found more acidic (P^H 5.4) than that in Bhainsepati while the soil in Kirtipur was richer in organic matter and nitrogen content and it was found to be 0.646% and 0.231% respectively. Texture of soil of Kirtipur was also silt loam.

4.2 Materials

4.2.1 Plant materials

A breeding genotype of rice, NR-10414 having crop duration of 140 days and production up to 9.8 t ha^{-1} was collected from the Agriculture Botany Division, Nepal Agriculture and Research Council (NARC) , Khumaltar.

Seeds were soaked 24 hours before seeding and seeded in a seed bed measuring 5m × 1.5m. Twenty-five days old rice seedlings were transplanted with 20cm × 20cm spacing in the flooded experimental plots with three to four seedlings per hill while in pot six rice seedlings were transplanted, one in each hill and only four healthy plants per pot were maintained.

4.2.2 *Azolla*

Azolla caroliniana required for the field experiment was collected from nearest pond to the experimental site and it was maintained in the nursery, where the constant level of water was also maintained. Super phosphate (P_2O_5 @ 15kg ha^{-1}) was broadcasted in the pond. *Azolla pinnata* was collected from Bishazari Tal, Bharatpur, Chitawan and both species of *Azolla* required for lab experiments were maintained in large pots separately. The pot for the *A. pinnata* was maintained in the green house . *Azolla* was incorporated in the field and pots where it was grown as monocrop at the rate of 1 t ha^{-1} , 2 t ha^{-1} respectively 7 days before rice plantation. *Azolla* was applied at the rate of 1 t ha^{-1} in the field and at the rate of 2 t ha^{-1} in pots, where it was grown as dual crop, 7 days after rice transplantation.

4.2.3 Chemical fertilizer

The nitrogenous, phosphatic and potassic fertilizers were used in the ratio 80:40:30 kg ha^{-1}. Nitrogenous fertilizer was applied in field and pot experiment in three split doses in the ratio 50:25:25 %. The chemical formula and nutrient content of the chemical fertilizers used in the experiment are shown in table 1.

Table 1: Chemical formula and nutrient content of chemical fertilizers used in the experiment.

Name of fertilizer	Chemical formula	Grade			Form
		N %	P %	K %	
Urea	$CO(NH_2)_2$	46	-	-	White color of prills free floating, soluble in water.
Single super phosphate	$Ca(H_2PO_4)$ $+CaSO_4$	-	16	-	Dirty grey powder/ granular hygroscopic form.
Muriate of potash	KCl	-	-	60	Reddish/ light grey crystalline, non hygroscopic form

4.3 Methods

4.3.1 Field Experiment

Field experiment was carried out in Bhainsepati of Sainbu VDC, Lalitpur. The field was designed as Completely Randomized Design (CRD) with three replications. Each plot measured 3m × 2m and the number of replication was three while the numbers of treatments were seven. Hills were arranged in rows at 20 cm apart and 20 cm between the hills. Three to four seedlings were transplanted per hill in the fields. The methods of chemical fertilizer and biofertilizer (*Azolla*) application were described as shown in table 2.

Table 2: Treatments in the field experiments

Treatments	Chemical fertilizer			*Azolla*	Method of application
	N (kg ha^{-1})	P (kg ha^{-1})	K (kg ha^{-1})		
T_1	0	0	0	-	-
T_2	80	40	30	-	Urea in three split dose.
T_3	40	0	0	+	Urea in three split dose + *Azolla* inoculated 7 DAT,incorporated during tillering stage and 2nd *Azolla* inoculation (a).
T_4	30	0	0	+	Urea in three split dose + *Azolla* used as in (a) + 2nd incorporation during panicle formation (b).
T_5	20	0	0	+	Urea in three split dose + *Azolla* used as in (b) + 3rd inoculation (c).
T_6	10	0	0	+	Urea in three split dose + *Azolla* grown before transplantation, 1st incorporation 7 days before transplantation+ 2nd *Azolla* inoculation 7 DAT+2nd incorporation during tillering stage, 3rd inoculation+3rd incorporation during panicle formation (d).
T_7	0	0	0	+	*Azolla* application as in (d)+ 4th *Azolla* inoculation.

4.3.2 Pot experiment

Pot experiments were carried out in green house in CDB, T.U. Kirtipur. Each pots lined with polyethylene were filled with 6 kg soil, sieved to 2mm. The number of replication was three and the total number of treatments was seven. The methods of chemical and biofertilizer application were same as in the field experiment. Only difference that only four healthy plants were maintained in each pot. The water level was maintained in each pot at 5cm during the whole experiment period.

4.3.3 Measurement of heterocyst frequency in *Anabaena azollae* inhabiting in *Azolla caroliniana* and *A. pinnata*

The frequency of heterocyst in the cyanosymbiont *Anabaena azollae* present in *Azolla* frond was calculated according to the method describe by Kannaiyan and Kumar (1993). Properly washed *Azolla* frond was crushed and the total number of vegetative cells and heterocyst in each microscopic fields was recorded. Further, the observed heterocysts were identified whether individual or multiple heterocyst. The heterocyst frequencies were calculated by the expressions below

$$\text{Frequency of single heterocyst (\%)} = \frac{\text{Total no. of single heterocyst}}{\text{Total no. of vegetative cells}} \times 100$$

$$\text{Frequency of multiple heterocyst(\%)} = \frac{\text{Total no. of multiple heterocyst}}{\text{Total no. of vegetative cells}} \times 100$$

$$\text{Total heterocyst frequency (\%)} = \frac{\text{Total number of heterocyst}}{\text{Total number of vegetative cells}} \times 100$$

4.3.4 Measurement of Doubling time (Dt) and Relative Growth Rate (RGR) of *Azolla caroliniana* and *A. pinnata*

The doubling time of *Azolla* can be defined as time taken by *Azolla* to double its biomass under optimum growth conditions. The method described by Kannaiyan and Kumar (1993) was followed for the doubling time and the relative growth rate measurement. For this, first soil extract medium was prepared and in this medium fix weight of *Azolla* was inoculated in bottles and bucket. After few weeks the final weight was taken. The doubling time and relative growth rate was calculated by the following expression:-

Doubling time = t/r

Where, t = experimental period

$$r = \frac{\log (W1/W0)}{0.301}$$

Where,

W1 = weight after days

W0 = weight of initial inoculum

0.301 = constant

$$\text{Relative Growth Rate (RGR)} = \frac{0.693}{\text{Doubling time}}$$

Where,

0.693 = constant

4.3.5 Estimation of chlorophyll in *Azolla caroliniana* and *A. pinnata*

Chlorophyll was estimated by following the method described by Arnon, 1949. For this, 0.3 gm of *Azolla* frond was taken and chlorophyll was extracted in 8 ml acetone and absorbance readings were taken at 645, 652 and 663 nm wave length in the spectrophotometer. The chlorophyll a chlorophyll b and total chlorophyll was calculated by the following expression:-

Chlorophyll a $=\{12.7(OD^{663})-2.69(OD^{645})\}$ V/1000W mg g^{-1} fresh weight of leaf tissue(fwl)

Chlorophyll b = $\{22.9(OD^{645})-4.68(OD^{663})\}$ V/1000W mg g^{-1} fwl

$$\text{Total chlorophyll} = \frac{OD^{652} \times 1000 \times V}{34.5 \times 1000 \times W} \text{ mg g}^{-1} \text{ fwl}$$

Where,

OD^{645} =optical density at 645nm

OD^{663} =optical density at 663nm

OD^{652} = optical density at 652nm

V = final volume of chlorophyll extract

W = the weight of *Azolla* frond taken

4.3.6 Measurement of amino N_2 in *Azolla caroliniana* and *A. pinnata*

First, different concentration of Glycine solution of final volume 4 ml was prepared and 4 ml of 1% Ninhydrin reagent prepared in 90% acetone was added, then incubated for some times in water bath and absorbance readings were taken at 570nm. Same procedure were done for 4 ml of pure extract of *Azolla* and total amino acids as glycine was estimated by the calibration curve method. And finally, total amino nitrogen was calculated by multiplying the total amino acids by fraction 14/75.

Total amino nitrogen = Total amino acids as Glycine × 14/75

4.3.7 Chemical analysis of soil sample.

Soil samples were collected before rice cultivation and after harvest. Air dry soil samples were kept in airtight polyethylene bags and brought to the laboratory for further analysis.

4.3.7.1 Determination of P^H.

The P^H of the soil samples from both the research sites before the cultivation of rice seedling was taken. For the P^H determination, 1:1 soil and water was mixed well. The soil was allowed to settle down and P^H was measured directly by a P^H meter.

4.3.7.2 Estimation of total nitrogen

The total nitrogen content of the soil was determined by Modified Kjeldahl method (Jackson, 1973). The organic matter was oxidized by treating soil with conc. H_2SO_4 to convert organic compound into $(NH_4)_2SO_4$ and also to drop ammonium ions present in soil. The liberated ammonia was estimated by collecting it in a conical flask containing mixed indicator. A part of indicator neutralize by ammonia was determined by titrating against an acid of known strength (0.01 N HCl).The method included three steps:

1. Digestion
2. Distillation
3. Titration

1.Digestion

1gm of dry soil sample was mixed with 3.5gm K_2SO_4 and 0.4gm $CuSO_4$ in a digestion flask. Then, 10ml of Nitrogen free conc.H_2SO_4 was added. The mixture was digested over a heating mantle. The duration of digestion was 2-3 hrs. After a complete digestion, the flask was allowed to cool and about 50ml of distilled water was added to the digested mixture.

2.Distillation

The digested solution was transferred to distillation apparatus. Then about 40ml of 40% NaOH was added. Then, the mouth of distillation apparatus was closed. 10ml of mixed indicator solution (0.3gm in 500ml 95% ethanol) was placed in a conical flask. The distillation apparatus was connected in such a way that the end of condenser was dipped below the surface by boiling the solution in the round bottom flask. Up on steam distillation, NaOH reacted with $(NH_4)_2SO_4$ to liberate ammonia which was collected in mixed indicator solution. The distillation was continued for 7 to 10 minutes.

3.Titration

After about 40ml of distillate was collected in the conical flask, it was disconnected from the condenser and titration was carried out with 0.01 N HCl.

The volumes of acid consumed by both blank and samples were noted and the total nitrogen content (N %) was calculated by using following formula:

$$N\ \% = \frac{(T\ Z B)\ x\ 14x\ N\ \ x\ 100}{Weight\ of\ \ sample\ (gm)}$$

Where,

T = Volume of acid used for the titration of sample

B = Volume of acid used for the titration of blank

N = Normality of the acid used

4.3.7.3 Determination of Organic matter

The organic matter was determined by Walkey-Black (1934) method. In this method, 0.5gm of air dry soil sample was taken in a conical flask of 500ml. To this, added 5ml of 1N $K_2Cr_2O_7$. After that, 10ml of Conc.H_2SO_4 was added and swirled. It was allowed to rest for 30minutes. There after 100ml of distilled water was added to dilute the reaction mixture. Then 5ml of H_2SO_4 solution and 0.5ml of diphenylamine indicator were added one by one. Then the solution was titrated against 0.5N ferrous ammonium sulphate

$[Fe(NH_4)_2SO_4]$ till the blue violet color of the solution mixture was changed to green. A blank (without soil) was also run simultaneously.

The organic matter present in the soil sample was calculated by following formula:

$$\%\text{Carbon in soil} = N\frac{(B-C)}{\text{Wt. of soil (gm)}}\times 0.003\times 100$$

Where,

N = Normality of ferrous ammonium sulphate $[FeSO_4\ (NH_4)$ $2SO_4\ \ 6H_2O]$.

B = Volume of ferrous ammonium sulphate used for blank.

C = Volume of ferrous ammonium sulphate used for sample.

There is incomplete oxidation of the organic matter in this procedure. Therefore, the organic carbon obtained by above method is multiplied by a factor 1.3 based on the assumption that there is 77% recovery.

Organic carbon = organic carbon estimated×1.3

Since, organic matter contains 58% organic carbon, the percentage of organic matter content can be calculated by multiplying above organic carbon by Van Bemmelen factor of 1.724.

Therefore,

% of organic matter content = organic carbon estimated×1.3×1.724

4.3.8 Estimation of chlorophyll in rice leaf.

The Chlorophyll estimation of rice leaves was done for four times during 60, 70, 80 and 90 days after transplanting the rice, following the method described by Arnon, 1949. 0.2gm of fresh leaves of *Oryza sativa* L. was taken and ground in morter adding 80% acetone. The mixture was filtered and the final volume of filtrate was made 8ml. The absorbance was measured at 652nm using as a reference by spectrophotometer.

Expression used for chlorophyll estimation:

$$\text{Total chlorophyll} = \frac{OD^{652} \times 1000 \times V}{34.5 \times 1000 \times W} \text{ mg g}^{-1} \text{ fwl}$$

Where,

OD^{652} = optical density at 652nm

V = final volume of chlorophyll extract

W = the weight of *Azolla* frond taken

4.3.9 Measurement of yield and yield components

In the pot and field experiments, plant height was recorded from ground to the tip of longest leaf at the time of plant maturity-Grain and straw yield were measured after harvest. The yield components, such as 1,000 grain weight, percentage of filled grains, number primary branches per panicle were measured from panicles of 10 hills randomly sampled from each plot in field experiment. The number of tillers per hill was counted from 10 hills from each plot at harvest. The same components in the pot experiments were measured by using each and every plant.

4.3.10 Statistical analysis

Statistical analysis was done by using Analysis of Variance (ANOVA) one way classification system. The data obtained were analyzed using application software-SPSS 11.5.

CHAPTER : V

RESULTS

5.1 Results on Heterocyst frequency in *Anabaena azollae* inhabiting in *Azolla caroliniana* and *A. pinnata*

In *Azolla caroliniana*, single heterocyst frequency, multiple heterocyst frequency and total heterocyst frequency were found to be 16.89%, 3.22% and 20.11% respectively. Similarly, in *A. pinnata*, single heterocyst frequency, multiple heterocyst frequency and total heterocyst frequency were found to be 11.90%, 1.09%, 12.99% respectively.

Table 3: Heterocyst frequency in *Anabaena azollae* of *A. caroliniana*

No of obs.	**Total no of vege-tative cell**	**No. of single heter-ocyst**	**Frequeny of single hetero-cyst %**	**No. of multiple heteroc-yst**	**Frequeny of multiple hetero-cyst %**	**Total heteroc-yst**	**Total hetero-cyst frequency %**
1.	80	7	8.75	2	2.5	9	11.25
2.	150	30	20	4	2.67	34	22.67
3.	296	61	20.61	10	3.38	71	23.99
4.	132	23	17.42	6	4.55	29	21.97
5.	66	47	17.67	8	3.01	55	20.68
Mean			**16.89**		**3.22**		**20.11**

Table 4: Heterocyst frequency in *Anabaena azollae* of *A. pinnata*

No of obs.	**Total no of vegeta-tive cell**	**No. of single heter-ocyst**	**Frequeny of single hetero-cyst %**	**No. of multiple hetero-cyst**	**Frequeny of multiple hetero-cyst %**	**Total hetero-cyst**	**Total hetero-cyst frequency %**
1.	210	19	9.05	2	0.95	21	10
2.	118	16	13.56	0	0	16	13.56
3.	194	15	7.73	0	0	15	7.73
4.	160	18	11.25	2	1.25	20	12.5
5.	246	44	17.89	8	3.25	52	21.14
Mean			**11.90**		**1.09**		**12.99**

5.2 Results on Doubling time (Dt) and Relative Growth Rate (RGR) of *Azolla caroliniana* and *A. pinnata*.

Doubling time and Relative Growth Rate of *A. carolinilna* were found to be 9.64 days and 0.074 per day respectively. These parameters of *A. pinnata* were found to be 10.22 days and 0.07 per days respectively.

Table 5: Dt and RGR in *Azolla caroliniana* and *A. pinnata*.

Date of inocul-ation	*Azola* spp.	Initial Wt.(g)	Date of observa-tion	Final wt.(g)	Days of interval	Dt (days)	RGR (per days)
05-07-05	*A. carol-iniana*	0.5	05-07-27	2.01	22	10.95	0.063
		0.5		2.13	22	10.52	0.066
		5		38.69	22	7.45	0.093
	Mean					**9.64**	**0.074**
05-07-05	*A. pinn-ata*	0.5	05-07-27	1.7	22	12.46	0.056
		0.5		2.38	22	9.77	0.071
		5		30.46	22	8.43	0.082
	Mean					**10.22**	**0.07**

5.3 Results on chlorophyll content in *Azolla caroliniana* and *A. pinnata*.

In *A. caroliniana* chlorophyll a, chlorophyll b and total chlorophyll were found to be 0.089, 0.017 and 0.124 mg g^{-1} fresh weight of leaf tissue respectively. Similarly, in *A. pinnata* chlorophyll a, chlorophyll b and total chlorophyll were found to be 0.079, 0.014 and 0.109 mg g^{-1} fresh weight of leaf tissue respectively.

Table 6: Absorbance reading and chlorophyll content in *Azolla caroliniana* and *A. pinnata*

Species of *Azolla*	Absorbance reading (nm)			Chlorophyll a (mg g-1 fwl)	Chlorophyll b (mg g-1 fwl)	Total chlorophyll (mg g-1 fwl)
	645	652	663			
A. caroli-niana	0.085	0.161	0.281	**0.089**	**0.017**	**0.124**
A. pinnata	0.074	0.141	0.248	**0.079**	**0.014**	**0.109**

5.4 Results on amino nitrogen content in *Azolla caroliniana* and *A. pinnata*

From calibration curve method, total amino acids as Glycine in *A. caroliniana* and *A. pinnata* were found to be 4.3 µg/ml and 3.25 µg/ml of pure extract respectively. Finally amino nitrogen content in *A. caroliniana* and *A. pinnata* were found to be equals to 0.80 and 0.61 µg/ml of pure extract respectively.

Table 7: Absorbance reading of different Glycine concentration and extract of *A. caroliniana* and *A. pinnata*.

Test tubes	Vol. of Glycine(ml)	Vol. of H_2O	Total vol.(ml)	Conc. of Glycine µg/ml	Absorbance
1	0.4	3.6	4	1	0.190
2	0.8	3.2	4	2	0.415
3	1.2	2.8	4	3	0.628
4	1.6	2.4	4	4	0.810
5	-	4	4	-	0
6	*A. caroliniana*		4	-	0.891
7	*A. pinnata*		4	-	0.670

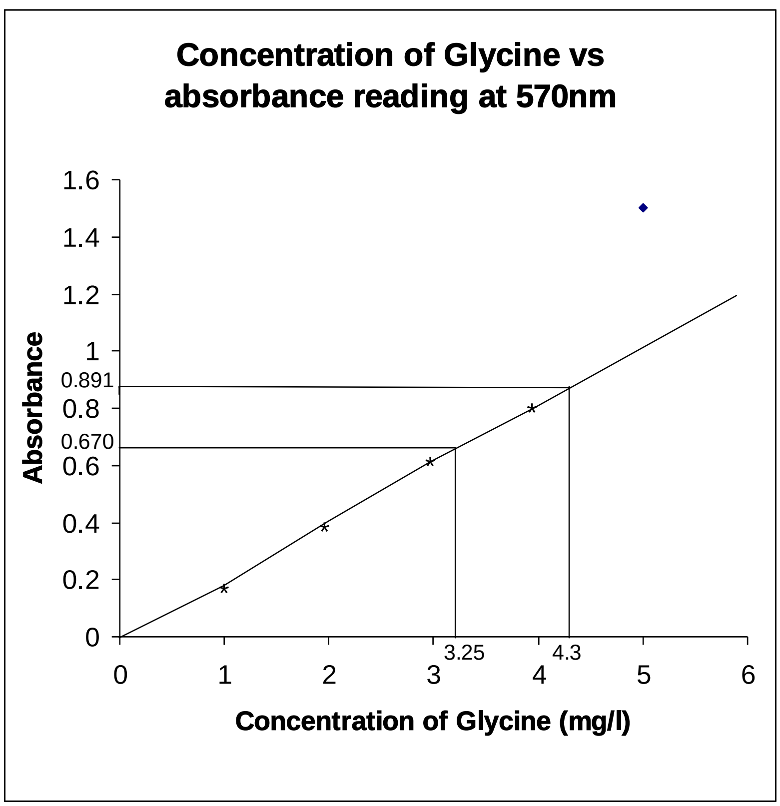

Fig.3: Determination of amino acid from standard calibration curve.

From calibration curve,

Total amino acids as Glycine in *A. caroliniana* = 4.3 µg/ml of pure extract

Amino N_2 in *A. caroliniana* = 4.3 × 14/75

=0.80 µg/ml

Total amino acids as Glycine in *A. pinnata* = 3.25 µg/ml(mg/l) of pure extract

Amino N_2 in *A. pinnata* = 3.25 × 14/75

=0.61µg/ml

5.5 Result on the effect of *Azolla* and urea in chlorophyll content of rice leaf.

Regarding the chlorophyll content of rice leaves in the field experiment, the highest amounts of total chlorophylls were found in the treatment seven in each estimation while the least values were found in the control treatment. The increase in total chlorophyll was found to be 43.18%, 42.55%, 44.23% and 42.38% in rice plant of 60 DAT, 70DAT, 80DAT and 90DAT respectively. Similar results were found in the pot experiment. The increase in total chlorophyll was found to be 46.34%, 43.78%, 43.14% and 50.00% in rice plant of pot in 60DAT, 70DAT, 80DAT and 90DAT respectively. Total chlorophyll was increasing up to 80DAT then it started to decrease in all treatments.

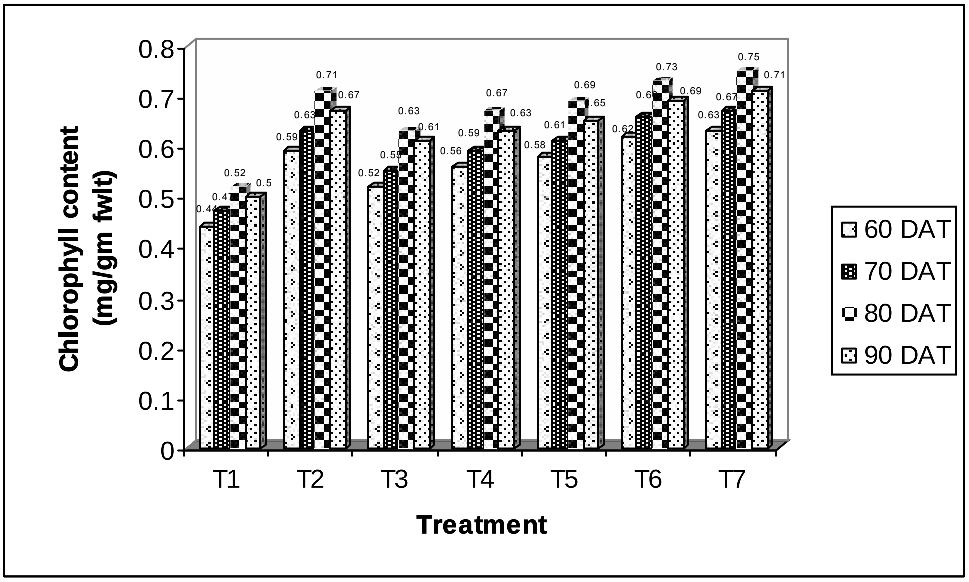

Fig.4: Graphical representation of chlorophyll content in rice leaf of field at different DAT.

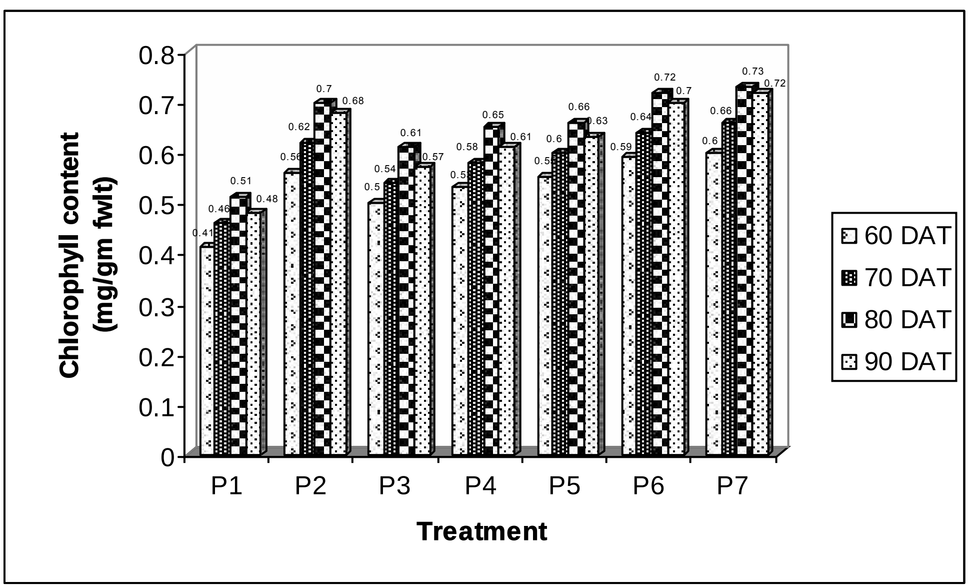

Fig.5: Graphical representation of chlorophyll content in rice leaf of pot at different DAT.

5.6 Results on the effect of *Azolla* and Urea on the rice yield and yield components.

The results clearly revealed that in both experimental sites, all the treatments have better performance than the control. *Azolla* utilization has positive effect on all the yield components. In the field experiment the highest grain yield 8.76 t ha^{-1} and straw yield 6.92 t ha^{-1} were obtained in the treatment seven (T_7) where four crops of *Azolla* was grown and incorporated three times, once before transplantation and twice after transplantation, that was 46.49% and 35.95% higher over control and 8.89% and 4.83% higher than the treatment where 80:40:30 kg ha^{-1} of NPK were used (T_2). Among the *Azolla* and urea treated sets, highest grain yield 8.62 t ha^{-1} and straw yield 6.86 t ha^{-1} were found in the treatment six where three crops of *Azolla* was grown and incorporated thrice plus 10 kg N ha^{-1} as Urea, which was also higher than the treatment two (T_2). Other *Azolla* and urea treated sets had grain yield and straw yield comparable to that of treatment two.

In pot experiments similar results were obtained. The highest grain yield 26.13 gm pot^{-1} and straw yield 21.47 gm pot^{-1}were obtained in the treatment seven (P_7) where *Azolla* was applied in the same way as in the treatment seven of the field experiment. That grain yield and straw yield values were respectively 47.13% and 40.97% higher over control set and 10.7% and 8.1% higher over treatment two (P_2) where recommended dose of fertilizers were used. Among the *Azolla* and Urea treated sets highest grain yield 25.40 gm pot^{-1} and straw yield 21.02 gm pot^{-1} were found in treatment six (P_6) where *Azolla* and urea applied in the same way as in treatment six of field experiment (T_6). The grain yield and straw yield values were respectively 43.02% and 38.02% higher over control set.

The yield components such as plant height, panicle per hill, number of primary branches per panicle, number of filled grain per panicle and percentage of filled grains were also found highest in the treatment seven in both field and pot experiment.

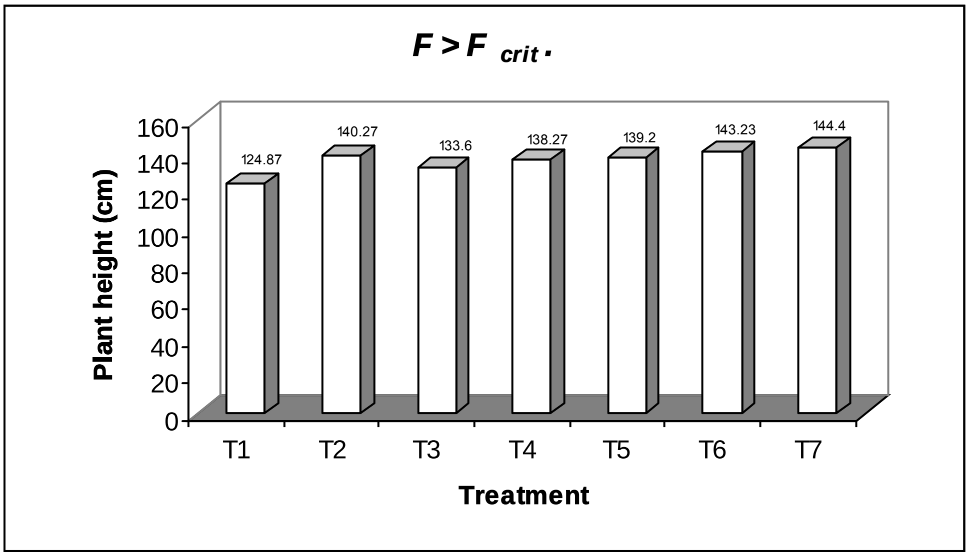

Fig.6: Graphical representation of plant height (cm) in the field.

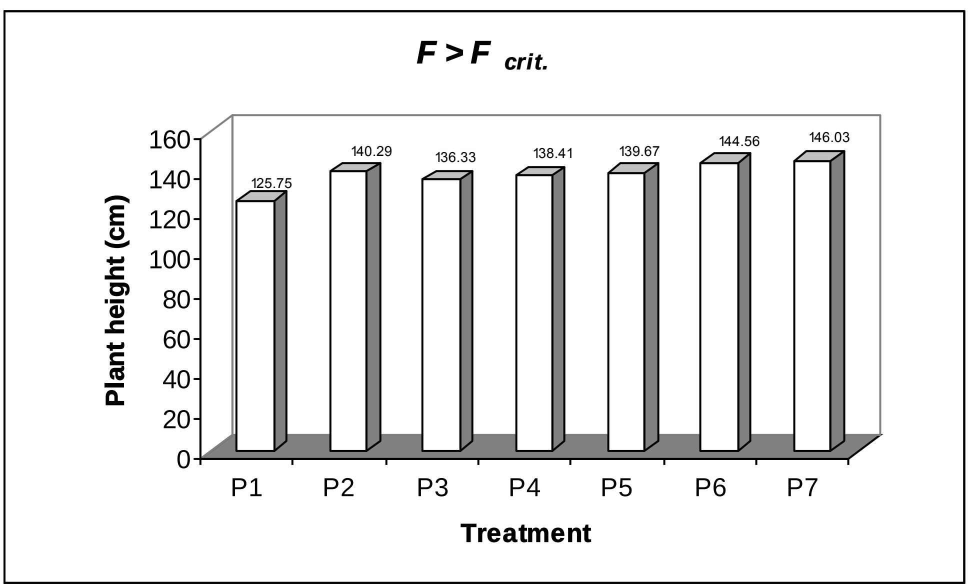

Fig.7: Graphical representation of plant height (cm) in the pot.

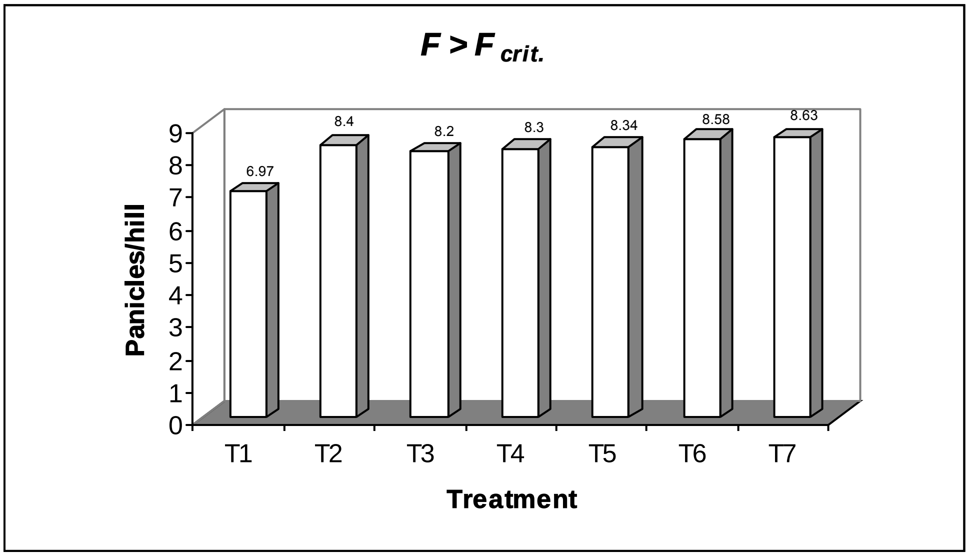

Fig.8: Graphical representation of no. of panicles/hill in the field.

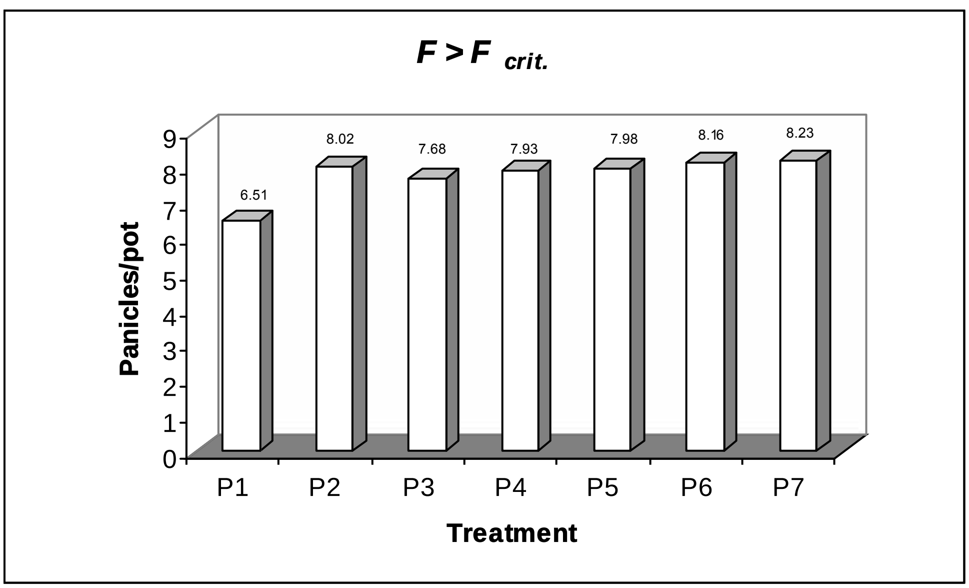

Fig.9: Graphical representation of no. of panicles/hill in the pot.

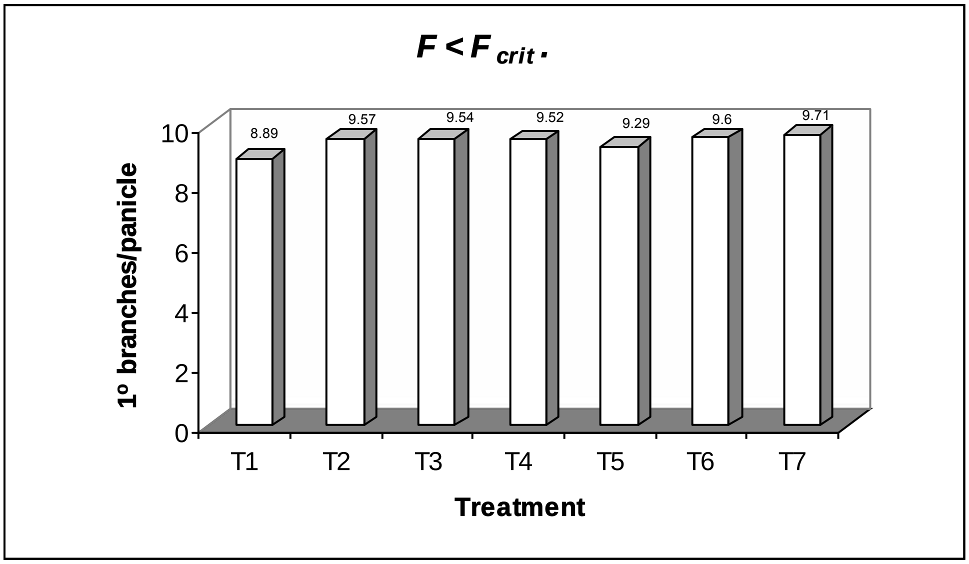

Fig.10: Graphical representation of no. of 1º branches/panicle in the field.

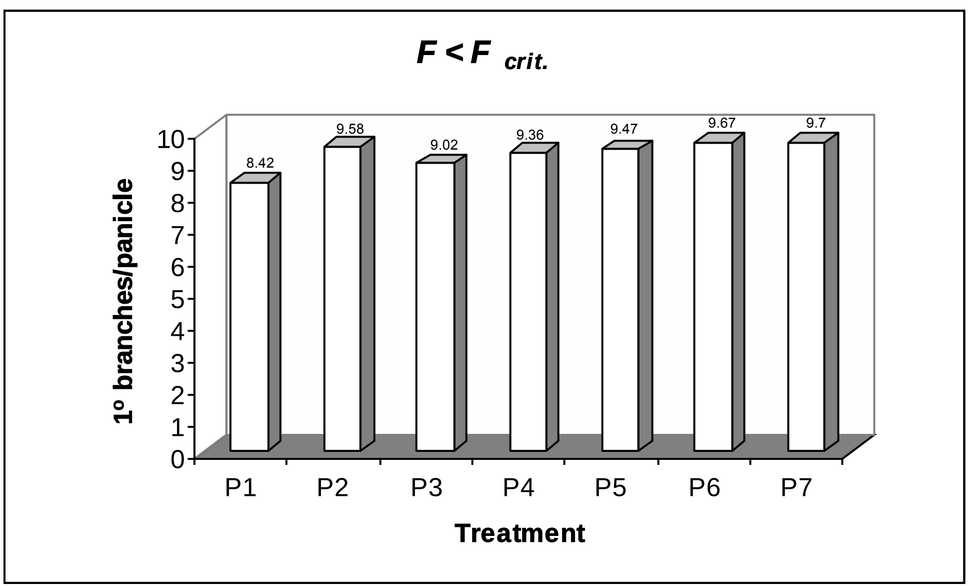

Fig.11: Graphical representation of no. of 1º branches/panicle in the pot.

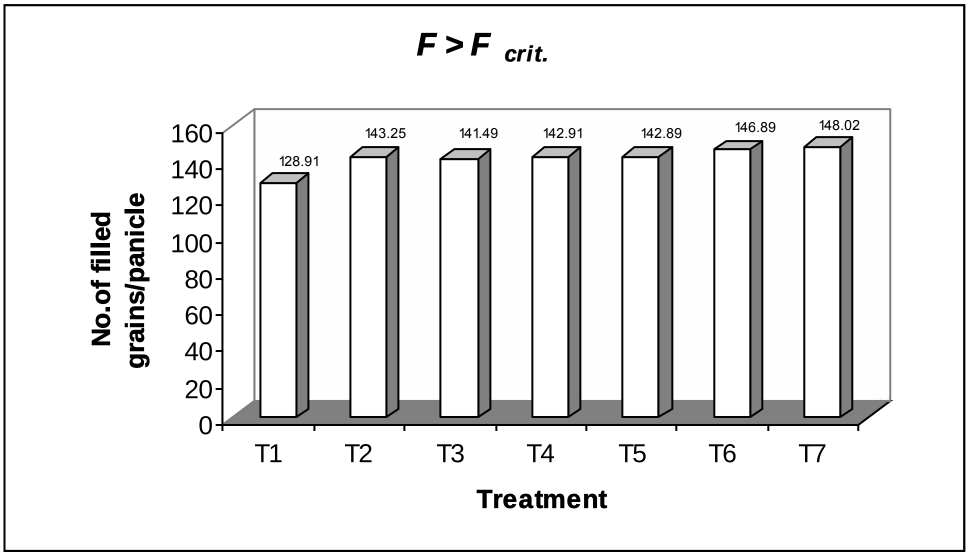

Fig.12: Graphical representation of no. of filled grains/panicle in the field.

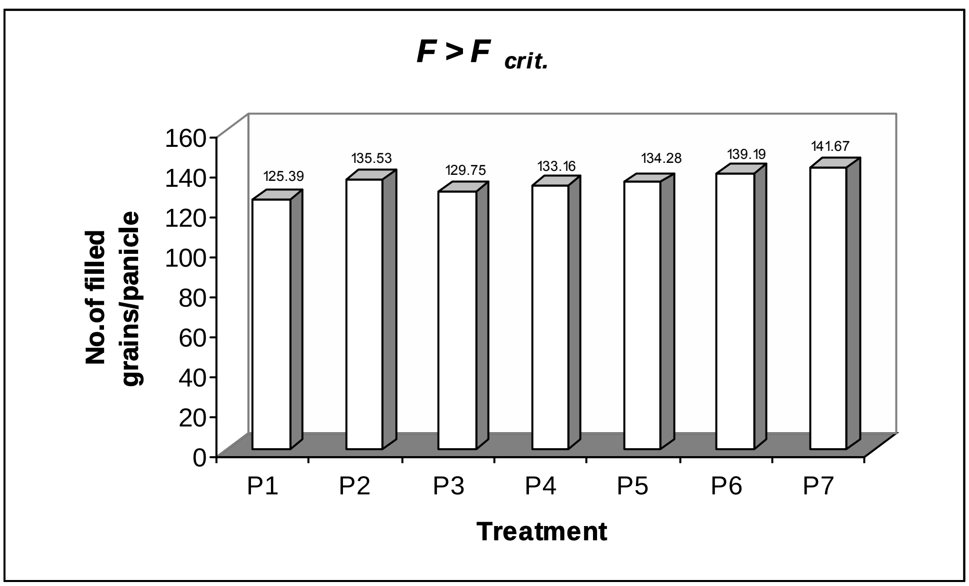

Fig.13: Graphical representation of no. of filled grains/panicle in the pot.

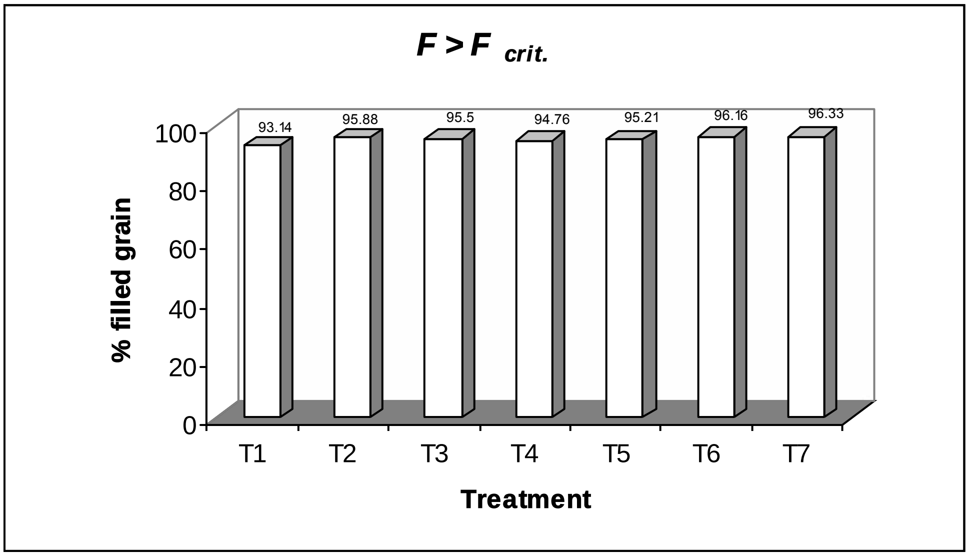

Fig.14: Graphical representation of % filled grain in the field.

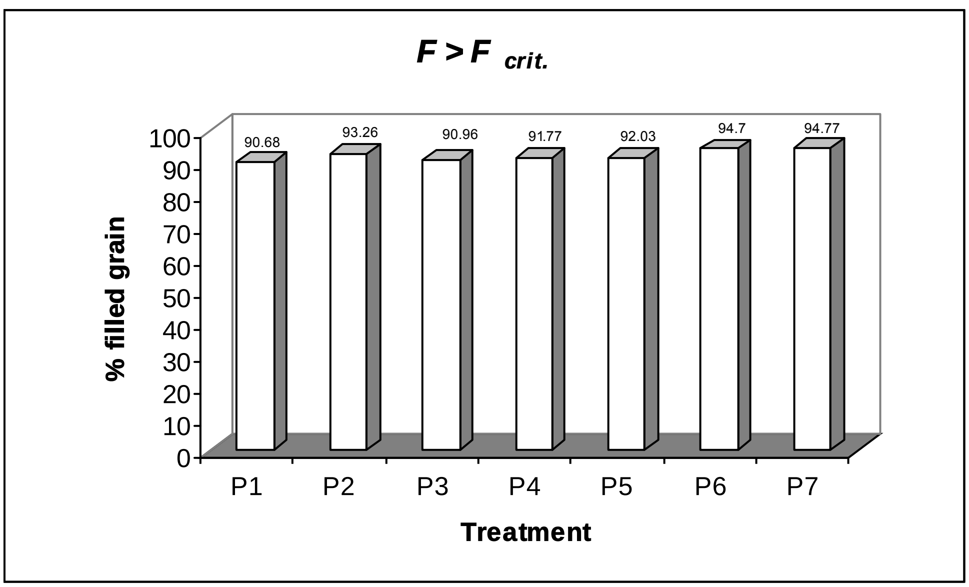

Fig.15: Graphical representation of % filled grain in the pot.

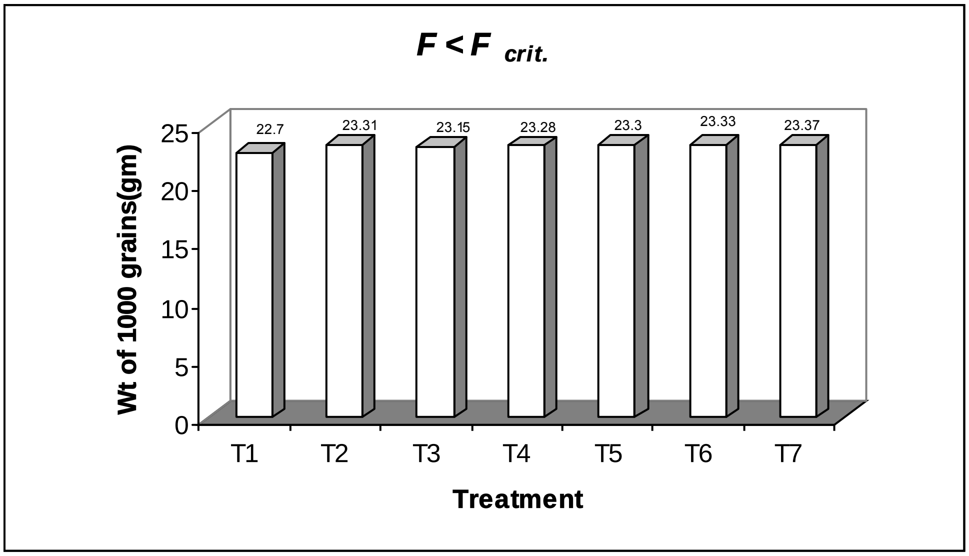

Fig.16: Graphical representation of wt. of 1000 grains (gm) in the field.

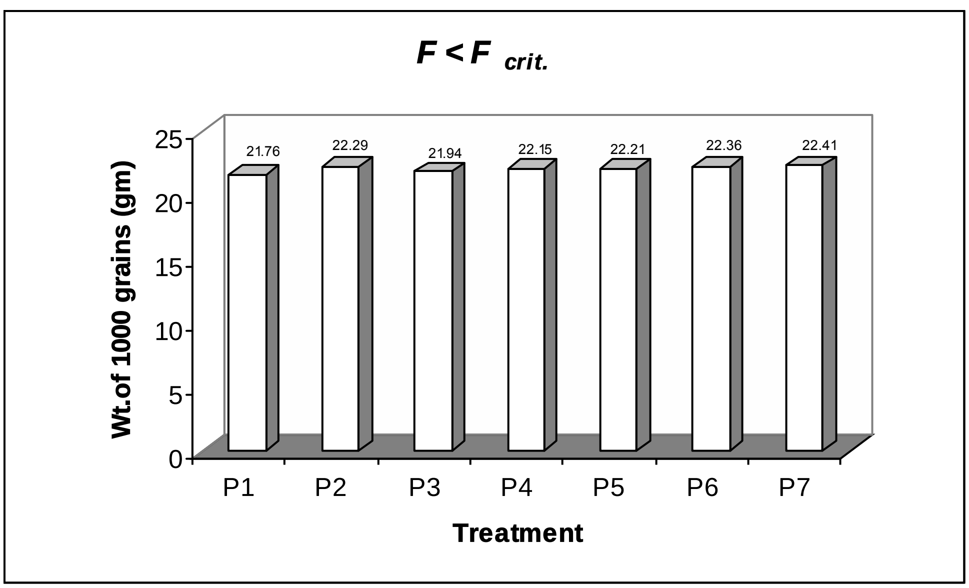

Fig.17: Graphical representation of wt. of 1000 grains (gm) in the pot.

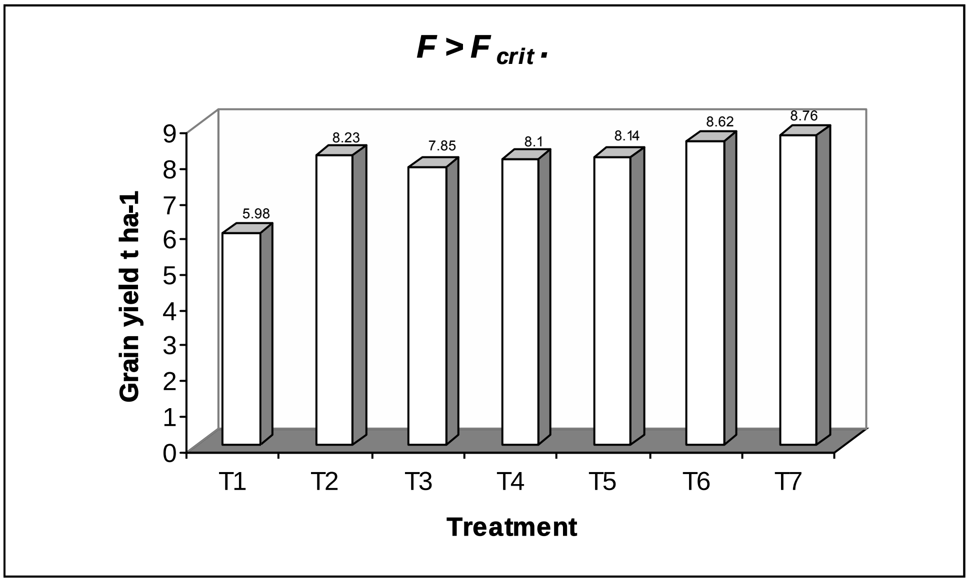

Fig.18: Graphical representation of grain yield t ha^{-1} in the field.

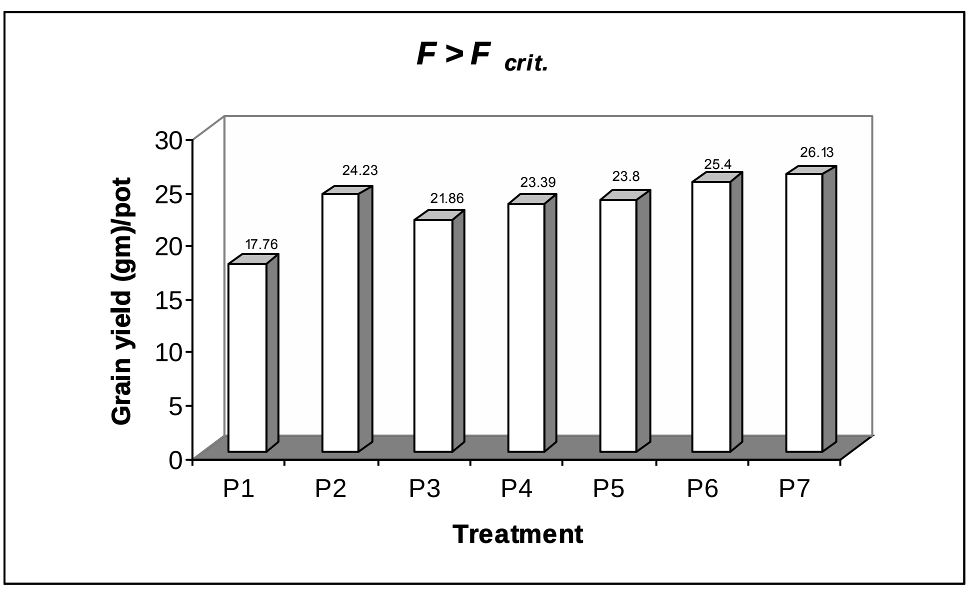

Fig19: Graphical representation of grain yield (gm)/pot in the pot.

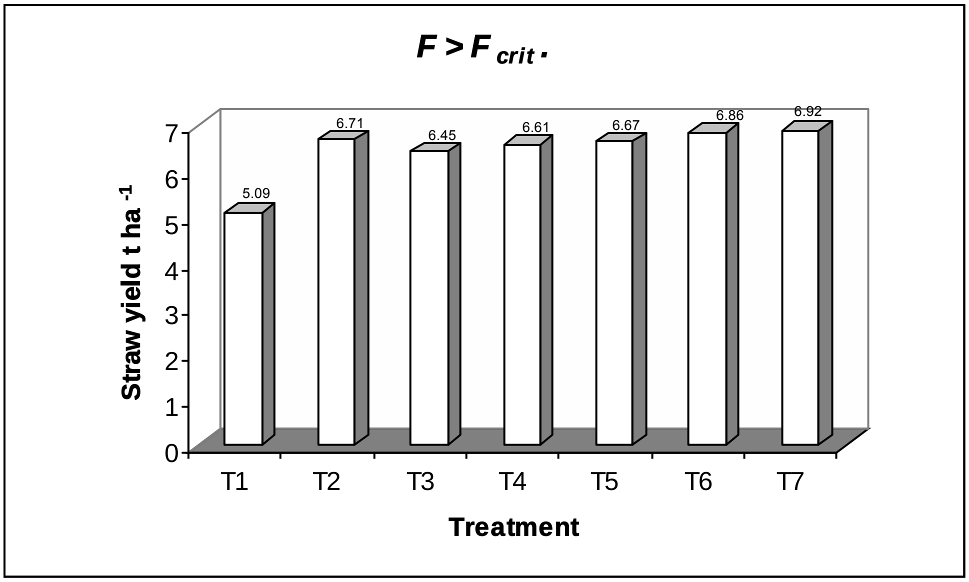

Fig.20: Graphical representation of straw yield t ha^{-1} in the field.

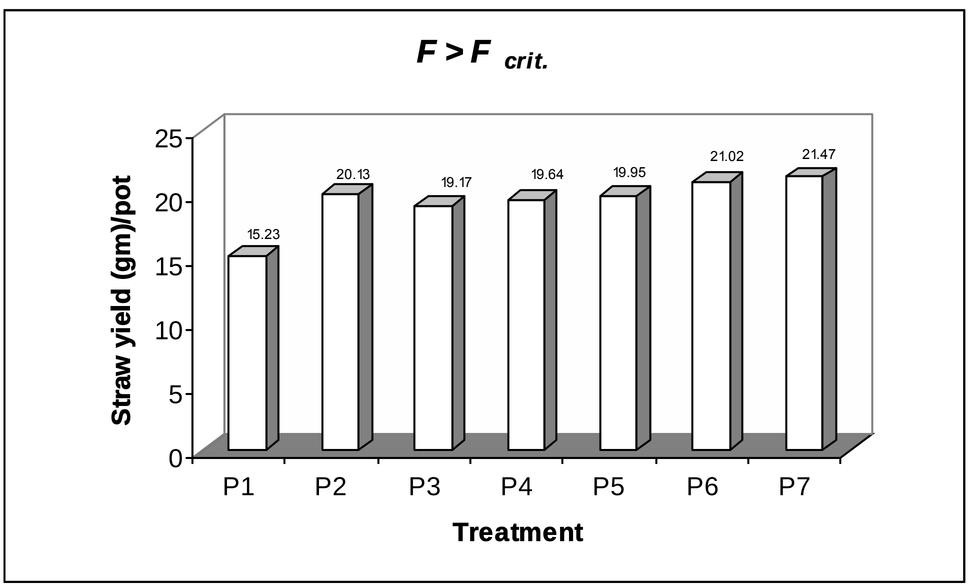

Fig.21: Graphical representation of straw yield (gm)/pot in the pot.

5.7 Results on the effect of *Azolla* and Urea on N content of soil

Nitrogen content of soil was found highest in the treatment where four crops of *Azolla* was grown and incorporated three times, and no chemical fertilizers were used, followed by treatment where three crops of *Azolla* was grown and incorporated three times plus 10kg N ha^{-1} Urea used. Least value of N content in soil was observed in control sets. Among *Azolla* treated sets, least value in soil was observed in sets where two crops of *Azolla* was grown and incorporated once plus 40 kg N ha^{-1} as urea.

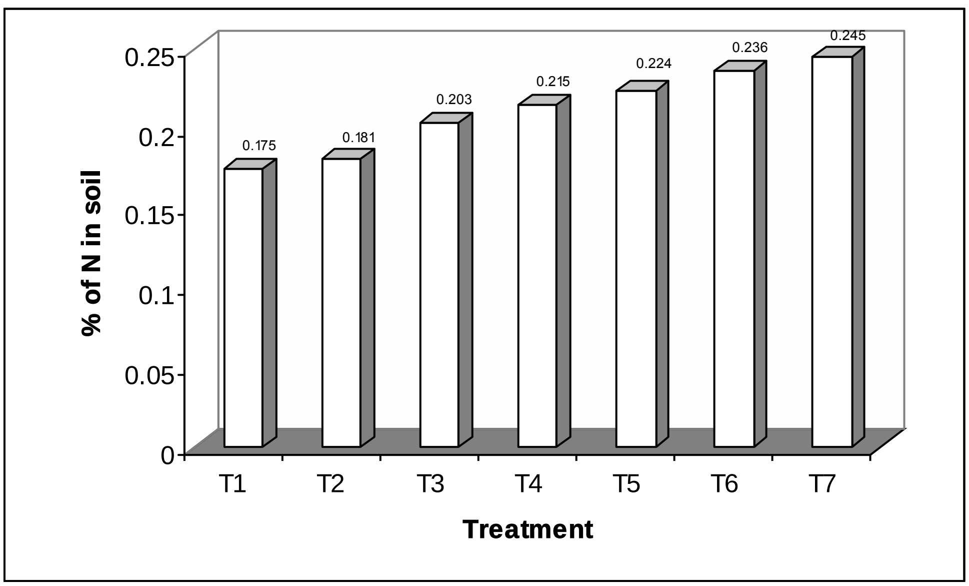

Fig.22: Graphical representation of Nitrogen content in soil in the field.

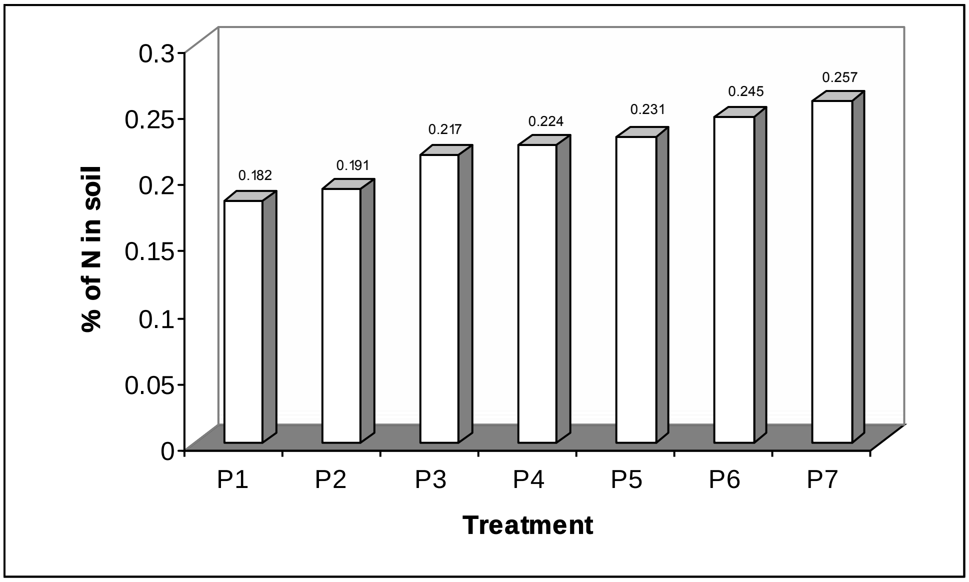

Fig.23: Graphical representation of Nitrogen content in soil in the pot.

5.8 Result on the effect of *Azolla* and Urea on organic matter content of soil

Organic matter content of the soil was found highest in the treatment where four crops of *Azolla* was grown and incorporated thrice and no chemical fertilizers were used; followed by treatment where three crops of *Azolla* was grown and incorporated thrice, plus 10 kg N ha^{-1} as Urea. Least value of organic matter content in soil was observed in controlled sets. Among *Azolla* treated sets, least value in soil organic matter was observed in sets where two crops of *Azolla* was grown and incorporated once, plus 40 kg N ha^{-1} as urea.

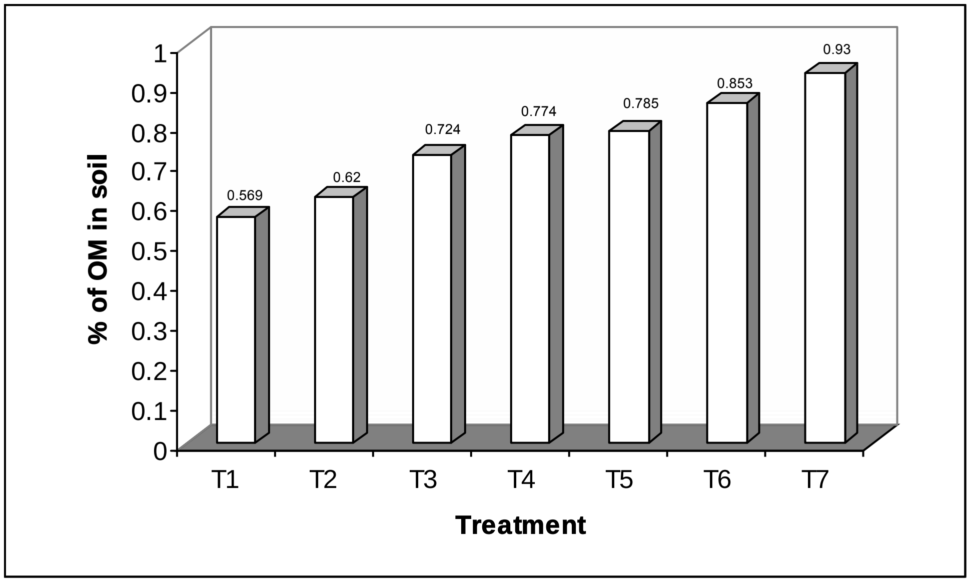

Fig.24: Graphical representation of Organic matter content in soil in the field.

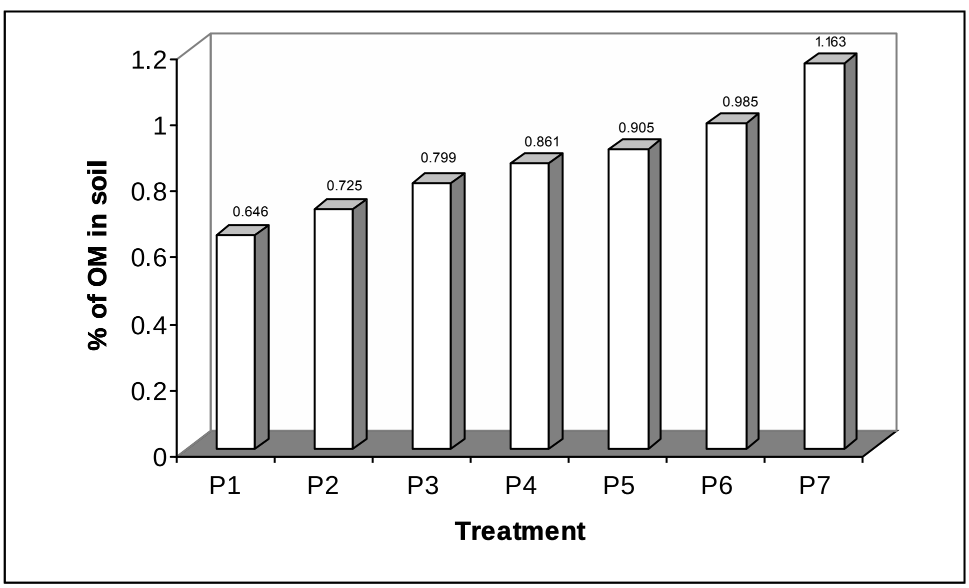

Fig.25: Graphical representation of Organic matter content in soil in the pot.

CHAPTER : VI
DISCUSSIONS

6.1 Discussion on the heterocyst frequency in *Anabaena azollae* of *Azolla caroliniana* and *A. pinnata*

The heterocyst frequency 12.99% and 20.11% of *A. pinnata* and *A. caroliniana* respectively, determined in the present study is supported by the findings of various scientists. Peters *et al.* (1979) reported heterocyst frequency up to 30%. Lumpkin and Pluknett (1982) also reported 20-30% heterocyst frequency of *Anabaena azollae*. The lower heterocyst frequency in the *A. pinnata* could be due to unfavourable climatic conditions because it was collected from tropical area.

6.2 Discussion on Doubling time (Dt) and Relative Growth Rate (RGR) of *A. caroliniana* and *A. pinnata*

The lower doubling time (9.64 days) and higher Relative growth rate (0.074 per day) of *Azolla caroliniana* than that of *A. pinnata*. Aroro and Singh (2004) reported *Azolla caroliniana* exhibited higher relative growth rate and *A. pinnata* exhibited low biomass production and relative growth rate compared to other species.

6.3 Discussion on chlorophyll and amino nitrogen content in *A. caroliniana* and *A. pinnata*

The high chlorophyll (0.124 mg g-1 fwl) and amino nitrogen (0.80 µg/ml) content of *Azolla caroliniana* than *A. pinnata* could be due to higher heterocyst frequency in *Anabaena azollae* inhabiting in *A. caroliniana* which fixed more atmospheric nitrogen and made available to the *Azolla*. *Azolla* use that nitrogen for various metabolic activities, some of these are chlorophyll and amino-acid bio-synthesis. Another cause of having lower chlorophyll and amino nitrogen content in *A. pinnata* might be due to its inability to grow and develop properly on unavailability of proper environmental conditions required for its growth and development.

6.4 Discussion on effect of *Azolla* and Urea on chlorophyll content in rice leaf

The highest value of chlorophyll content in the rice leaves of treatment seven might be due to higher supply of nitrogen and other requiring nutrients by *Azolla* to rice. Nayak *et al.* (2004) reported the application of biofertilizers brought about a significant enhancement in chlorophyll accumulation. Nitrogen is one of the constituent elements of chlorophyll and chlorophyll in its turn, major pigment responsible for photosynthesis which finally affects the productivity directly. Among the Urea and *Azolla* treated sets, the chlorophyll content in rice leaf was increasing from treatment three to six though the Urea was decreasing. The result could be due to increasing amount of nitrogen supplied by *Azolla* which was treated in such way that rate of *Azolla* decomposition was increasing from treatment three to six.

Among four chlorophyll estimation, chlorophyll was increasing up to third estimation (80DAT) then it was decreasing. That might be due to different growth stages of rice plant. During panicle formation and its development stage the rice plant becomes more dark green due to accumulation of greater amount of chlorophyll which synthesize more reserve food material required for rice grain development, and during the late stage of panicle development the rice plant starts to become light green due to decreasing amount of chlorophyll.

6.5 Discussion on the effect of *Azolla* and Urea on rice yield and yield components

Best performance of the set where four crops of *Azolla* was grown and incorporated thrice, first before transplantation and second and third after transplantation could be attributed to the continue *Azolla* growth and synchronized release of nitrogen from incorporation of *Azolla* with the plant need . Misra and Singh (1988) also reported that basal incorporation of *Azolla* plus dual cropping was more effective for rice yield. Ito and Watanabe(1985) also reported about 50% of *Azolla* nitrogen was absorbed by rice plants when *Azolla* was incorporated at the time of transplanting, but when *Azolla* was kept on surface of water less than 10% of nitrogen was available to the rice plant. In sets where four crops of *Azolla* was grown and incorporated thrice, grain yield increased up to 47.13% and straw yield increased by 40.97%. This finding was supported by Mandal and Bharati (1983) who reported 33-47% increase in grain yield and 35% increase in straw yield. Maskey and Bhattarai (1982) reported rice yield increased by 40% over control when *Azolla* was incorporated twice at the time of transplanting and during growth period.

In the sets where three crops of *Azolla* was grown and incorporated twice after transplanting rice with 20 kg N ha^{-1} as urea, the increase in grain yield 36.12% and straw yield 31.04% which is comparable to that yield by using recommended dose of NPK fertilizers (80:40:30 kg ha^{-1}). On the other hand, sets where three crops of *Azolla* grown and incorporated thrice, first before transplantation and second and third after transplantation with 10 kg N ha^{-1} as urea, the increase in grain and straw yield was 44.15% and 38.02% respectively. The yield was higher not only than the above set but also than recommended dose of fertilizers. The increase in yield might be attributed by one more incorporation of *Azolla* that was grown and incorporated before transplantation which decomposed and released more nitrogen and other nutrients available to rice plant. The result in this study is supported by the reports of different scientists. Singh and Singh (1990) reported one crop of *Azolla* provides 20-40 kg N ha^{-1} and the incorporation of *Azolla* gives higher grain yield than non incorporated treatment, also the incorporation of 5-15 t ha^{-1} of *Azolla* one month after transplantation was found to increase rice yield by 12-33% over unfertilized control. Roger and Ladha (1992) found that standing crop of *Azolla* averaging 30-40 kg N ha^{-1} and accumulation of 50-90 kg N ha^{-1} for two crops of *Azolla* grown before and after transplanting rice.

Finally the increase in grain yield and straw yield might be attributed to the high number of grains, effective tillers, higher numbers of primary branches per panicle, higher number of filled grain per panicle, greater percentage of filled grain, grain weight and the plant height.

6.6 Discussion on effect of *Azolla* and Urea on the N content of soil

The least soil nitrogen content of control sets of experiment might be due lack of addition of any kind of nitrogen sources eg. chemical fertilizer, biological nitrogen fixers etc. Soil nitrogen content was increased up to 41.21% when four crops of *Azolla* were grown and incorporated thrice, once before transplantation and twice after transplantation in the pot experiment (P_7). Adhikari *et al.* (2002) have reported 37.7% increased in soil nitrogen when *Azolla* was incorporated into the soil. Bhattarai and Maskey (1987) also reported that *Azolla* incorporation increased the nitrogen content in the soil by considerable amount. The nitrogen content was increasing from treatment two to treatment six in both field and pot experiments though the amount of Urea was applied in decreasing manner. The above result could be influenced by the amount of *Azolla* inhabiting *Anabaena azollae* (symbioticN_2 fixer) undergoing the decomposition because *Azolla* was applied in the field and pot experiment in such manner that amount of *Azolla* undergoing decomposition was increasing from treatment three to treatment seven. *Anabaena azollae* fixes the atmospheric nitrogen, which increases the nitrogen content of *Azolla*, the death and decay of nitrogen rich *Azolla* tissue during growing period and decomposition of these tissues after incorporation into the soil, increases the soil nitrogen content. Ladha *et al.* (2000) reported no significant change in soil N content by the control and Urea treatment while *Azolla* incorporation increased 344-351 kg N after twenty-seven crops.

6.7 Discussion on the effect of *Azolla* and Urea on organic matter content of the soil

The least soil organic matter content of control set of experiment might be absence of additional source of organic matter supplier. Soil organic matter content was increased up to 80.03% in treatment seven of pot experiment (P_7) where four crops of *Azolla* were grown and incorporated thrice. The order of organic matter content of soil was increasing from treatment two to seven in both field and pot experiments. The result could be due to the amount of *Azolla* biomass undergoing the decomposition because *Azolla* was applied in such manner that amount of *Azolla* biomass undergoing decomposition was increasing in the same scenario. Singh and Singh (1988) have reported that *Azolla* possesses the ability of self decomposition after mat formation and incorporation of the fern into the soil increases the rate of decomposition and nitrogen release.

CHAPTER : VII

CONCLUSION

- *Azolla caroliniana* was found more effective to be used as biofertilizer than *A. pinnata* because *A. caroliniana* exhibited higher heterocyst frequency, lower doubling time (Dt), higher Relative Growth Rate (RGR), higher chlorophyll content and higher amino nitrogen content than *A. pinnata*.
- Different methods of *Azolla* application considerably enhance the soil biochemical parameters eg. soil nitrogen and soil organic matter and found to be increased up to 41.21% and 80.03% respectively.
- Continued growth of *Azolla* with repeated incorporation considerably increased the chlorophyll content of rice leaf.
- Among the different methods of *Azolla* application, growing four crops of *Azolla* with incorporation thrice, first before transplantation, second and third after transplantation significantly increased the grain and straw yield, it was found up to 47.13% and 40.97% respectively.
- Application of *Azolla* can reduce the chemical fertilizer, the yield produced by double incorporation of *Azolla* followed by inoculation with 20 kg N ha^{-1} was almost equal and triple incorporation of *Azolla* with 10 kg N ha^{-1} was greater than recommended dose of fertilizer. *Azolla* can also displace the chemical fertilizer from rice field.
- Incorporation *Azolla* into the soil was found to be more effective than its inoculation, in contributing soil nitrogen, grain and straw yield of rice .

CHAPTER : VIII
RECOMMENDATIONS

Following recommendations have been outlined in order to obtain better rice productivity and sustainable agriculture

- Application of *Azolla* as biofertilizer which reduces or even substitute the use of urea is highly recommended for its cost-effectiveness and eco-friendly nature.
- *Azolla* enhance the rice yield and soil fertility. Its use is highly recommended to achieve food security through sustainable agriculture.
- Although it is labour intensive, *Azolla* should be grown and incorporated before transplanting rice. It is also recommended repeated inoculation and incorporation of *Azolla* at proper time after rice transplanting as far as possible to gain better yield.
- Application of *Azolla* as biofertilizer is recommended only in those areas where stagnant water condition is available or can be maintained. In absence of stagnant water condition (eg. running water, water deficient condition etc.), the relative growth rate of *Azolla* will decrease and it would not be more effective biofertilizer.
- Proper selection of the *Azolla* species on location basis should be done prior using it as biofertilizer, *A. pinnata* for tropical rice fields and *A. caroliniana* for sub-tropical rice fields.
- Recent developed technologies and agricultural instruments for inoculating and incorporating the *Azolla*, should be introduced which minimize the cost of labour expenses.

CHAPTER : IX

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PHOTOPLATE - 1

Azolla caroliniana Azolla pinnata

Azolla nursery for field experiment Expt. sets for Dt. and RGR

Preparing plots for field experiment Rice transplanting in pots

Dense mat of Azolla covering Rice growing in pot experiment

rice plant

PHOTOPLATE - 2

Taking absorbance reading Extract of rice leaf in acetone

for chl. estimation

Counting no. of grains Sample preparation for OM

analysis

Titration of soil Digestion of soil sample for N

for OM estimation

PHOTOPLATE – 3

Dorsal lobe of Azolla pinnata leaf Ventral lobe of Azolla pinnata leaf

Dorsal lobe of Azolla caroliniana Ventral lobe of Azolla caroliniana

leaf leaf

Heterocysts of Anabaena azollae Heterocysts of Anabaena azollae

in Azolla Pinnata in Azolla caroliniana

a. The total monthly rainfall (mm), maximum temperature (°C) and minimum temperature (°C) of the representative meteorological station(Khokana) of Lalitpur, Nepal in the year 2005.

Months	Maximum Temp.°C	Minimum Temp.°C	Rainfall (mm)
January	16.5	2.5	79.4
February	20.4	3.4	15.0
March	23.6	7.5	59.4
April	27.0	8.5	55.9
May	28.2	12.7	88.5
June	29.7	17.5	160.3
July	27.9	20.3	216.8
August	27.5	20.2	276.4
September	28.5	18.5	115.3
October	25.7	12.0	115.3
November	22.5	5.5	0
December	19.9	0.7	0

b. The total monthly rainfall (mm), maximum temperature (°C) and minimum temperature (°C) of the representative meteorological station(Airport) of Kathmandu, Nepal in the year 2005.

Months	Maximum Temp.°C	Minimum Temp.°C	Rainfall (mm)
January	17.9	4.3	55.1
February	22.0	5.9	17.0
March	25.8	10.1	50.1
April	28.6	11.6	34.8
May	29.4	14.9	40.6
June	30.5	19.2	222.9
July	29.1	20.6	253.5
August	29.0	20.6	309.3
September	29.5	19.5	126.5
October	26.4	14.0	126.1
November	23.3	8.4	0
December	21.0	3.5	0

a. Chlorophyll content in leaf of rice plant(mg/gm fwl) at different DAT in the field

	60 DAT	70 DAT	80 DAT	90 DAT
T_1	0.44	0.47	0.52	0.50
T_2	0.59	0.63	0.71	0.67
T_3	0.52	0.55	0.63	0.61
T_4	0.56	0.59	0.67	0.63
T_5	0.58	0.61	0.69	0.65
T_6	0.62	0.66	0.73	0.69
T_7	0.63	0.67	0.75	0.71

b. Chlorophyll content in leaf of rice plant (mg/gm fwl) at different DAT in the pot

	60 DAT	70 DAT	80 DAT	90 DAT
P_1	0.41	0.46	0.51	0.48
P_2	0.56	0.62	0.70	0.68
P_3	0.50	0.54	0.61	0.57
P_4	0.53	0.58	0.65	0.61
P_5	0.55	0.60	0.66	0.63
P_6	0.59	0.64	0.72	0.70
P_7	0.60	0.66	0.73	0.72

a. Effect of *Azolla* and Urea in the yield and yield components of rice in the field experiments.

Treatments	Plant Height (cm)	Panicle / hill	No. of primary branches/ panicle	No. of Filled grain/ panicle	% field grain	Wt.of 1000 grain (gm)	Grain Yield t ha-1	% increase	Straw Yield t ha-1	% increase
T1	124.87	6.97	8.89	128.91	93.14	22.70	5.98	100	5.09	100
T2	140.27	8.4	9.57	143.25	95.88	23.31	8.23	137.6	6.71	131.82
T3	133.6	8.2	9.54	141.49	95.50	23.15	7.85	131.3	6.45	126.72
T4	138.27	8.3	9.52	142.91	94.76	23.28	8.10	135.45	6.61	129.86
T5	139.2	8.34	9.29	142.89	95.21	23.30	8.14	136.12	6.67	131.04
T6	142.23	8.58	9.60	146.89	96.16	23.33	8.62	144.15	6.86	134.77
T7	144.40	8.63	9.71	148.02	96.33	23.37	8.76	146.49	6.92	135.95
GM	137.69	8.20	9.45	142.05	95.28	23.21	7.95		6.47	
CV(%)	1.02	4.31	5.86	4.23	0.55	1.31	3.08		7.65	
F-test	**	**	ns	**	**	ns	**		**	

b. Effect of *Azolla* and Urea in the yield and yield components of rice in the pot experiments

Treatments	Plant Height (cm)	Panicle/ pot	No. of primary branches/ panicle	No.of Filled grain/ panicle	% field grain	Wt.of 1000 grain (gm)	Grain Yield Gm/ pot	% increase	Straw Yield Gm/ pot	% increase
P1	125.75	6.51	8.42	125.39	90.68	21.76	17.76	100	15.23	100
P2	140.29	8.02	9.58	135.53	93.26	22.29	24.23	136.43	20.13	132.17
P3	136.33	7.68	9.02	129.75	90.96	21.94	21.86	123.01	19.17	125.87
P4	138.41	7.93	9.36	133.16	91.77	22.15	23.39	131.70	19.64	128.96
P5	139.67	7.98	9.47	134.28	92.03	22.21	23.80	134.00	19.95	130.99
P6	144.56	8.16	9.67	139.19	94.70	22.36	25.40	143.02	21.02	138.02
P7	146.03	8.23	9.70	141.67	94.77	22.41	26.13	147.13	21.47	140.97
GM	138.72	7.79	9.32	134.14	92.60	22.16	23.14		19.52	
CV(%)	0.47	7.64	5.56	0.39	0.76	2.24	2.52		3.65	
F-test	**	*	ns	**	**	ns	**		**	

** significant at 1%　　* significant at 5%　　ns not significan

a. Effect of *Azolla* and Urea on N content of soil in field and pot experiments.

Field experiment	% of N in soil	Pot experiment	% of N in soil
T_1	0.175	P_1	0.182
T_2	0.181	P_2	0.191
T_3	0.203	P_3	0.217
T_4	0.215	P_4	0.224
T_5	0.224	P_5	0.231
T_6	0.2361	P_6	0.245
T_7	0.245	P_7	0.257

b. Effect of *Azolla* and Urea on organic matter content in soil of field and pot experiments.

Field experiments	%of OM in soil	Pot experiments	% of OM in soil
T_1	0.569	P_1	0.646
T_2	0.620	P_2	0.725
T_3	0.724	P_3	0.799
T_4	0.774	P_4	0.861
T_5	0.785	P_5	0.905
T_6	0.853	P_6	0.985
T_7	0.930	P_7	1.163

ANOVA for Plant height (cm) in the field

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	798.207	6	133.035	66.928	.000
Within Groups	27.828	14	1.988		
Total	826.035	20			

Multiple LSD comparisons of means on dependent variable Plant height (cm) in the field.

(I) Treatment	(J)Treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1.00	2.00	-15.4000(*)	1.15115	.000	-17.8690	-12.9310
	3.00	-8.7300(*)	1.15115	.000	-11.1990	-6.2610
	4.00	-13.4000(*)	1.15115	.000	-15.8690	-10.9310
	5.00	-14.3300(*)	1.15115	.000	-16.7990	-11.8610
	6.00	-18.3600(*)	1.15115	.000	-20.8290	-15.8910
	7.00	-19.5300(*)	1.15115	.000	-21.9990	-17.0610
2.00	1.00	15.4000(*)	1.15115	.000	12.9310	17.8690
	3.00	6.6700(*)	1.15115	.000	4.2010	9.1390
	4.00	2.0000	1.15115	.104	-.4690	4.4690
	5.00	1.0700	1.15115	.368	-1.3990	3.5390
	6.00	-2.9600(*)	1.15115	.022	-5.4290	-.4910
	7.00	-4.1300(*)	1.15115	.003	-6.5990	-1.6610
3.00	1.00	8.7300(*)	1.15115	.000	6.2610	11.1990
	2.00	-6.6700(*)	1.15115	.000	-9.1390	-4.2010
	4.00	-4.6700(*)	1.15115	.001	-7.1390	-2.2010
	5.00	-5.6000(*)	1.15115	.000	-8.0690	-3.1310
	6.00	-9.6300(*)	1.15115	.000	-12.0990	-7.1610
	7.00	-10.8000(*)	1.15115	.000	-13.2690	-8.3310
4.00	1.00	13.4000(*)	1.15115	.000	10.9310	15.8690
	2.00	-2.0000	1.15115	.104	-4.4690	.4690
	3.00	4.6700(*)	1.15115	.001	2.2010	7.1390
	5.00	-.9300	1.15115	.433	-3.3990	1.5390
	6.00	-4.9600(*)	1.15115	.001	-7.4290	-2.4910
	7.00	-6.1300(*)	1.15115	.000	-8.5990	-3.6610
5.00	1.00	14.3300(*)	1.15115	.000	11.8610	16.7990
	2.00	-1.0700	1.15115	.368	-3.5390	1.3990
	3.00	5.6000(*)	1.15115	.000	3.1310	8.0690
	4.00	.9300	1.15115	.433	-1.5390	3.3990
	6.00	-4.0300(*)	1.15115	.004	-6.4990	-1.5610
	7.00	-5.2000(*)	1.15115	.000	-7.6690	-2.7310
6.00	1.00	18.3600(*)	1.15115	.000	15.8910	20.8290
	2.00	2.9600(*)	1.15115	.022	.4910	5.4290
	3.00	9.6300(*)	1.15115	.000	7.1610	12.0990
	4.00	4.9600(*)	1.15115	.001	2.4910	7.4290
	5.00	4.0300(*)	1.15115	.004	1.5610	6.4990
	7.00	-1.1700	1.15115	.327	-3.6390	1.2990
7.00	1.00	19.5300(*)	1.15115	.000	17.0610	21.9990
	2.00	4.1300(*)	1.15115	.003	1.6610	6.5990
	3.00	10.8000(*)	1.15115	.000	8.3310	13.2690
	4.00	6.1300(*)	1.15115	.000	3.6610	8.5990
	5.00	5.2000(*)	1.15115	.000	2.7310	7.6690
	6.00	1.1700	1.15115	.327	-1.2990	3.6390

* The mean difference is significant at the .05 level.

ANOVA for panicles/hill in the field

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	5.735	6	.956	7.638	.001
Within Groups	1.752	14	.125		
Total	7.487	20			

Multiple LSD comparisons of means on dependent variable panicles/hill in the field.

(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	Upper Bound
1.00	2.00	-1.4300(*)	.28884	.000	-2.0495	-.8105
	3.00	-1.2300(*)	.28884	.001	-1.8495	-.6105
	4.00	-1.3300(*)	.28884	.000	-1.9495	-.7105
	5.00	-1.3700(*)	.28884	.000	-1.9895	-.7505
	6.00	-1.6100(*)	.28884	.000	-2.2295	-.9905
	7.00	-1.6600(*)	.28884	.000	-2.2795	-1.0405
2.00	1.00	1.4300(*)	.28884	.000	.8105	2.0495
	3.00	.2000	.28884	.500	-.4195	.8195
	4.00	.1000	.28884	.734	-.5195	.7195
	5.00	.0600	.28884	.838	-.5595	.6795
	6.00	-.1800	.28884	.543	-.7995	.4395
	7.00	-.2300	.28884	.439	-.8495	.3895
3.00	1.00	1.2300(*)	.28884	.001	.6105	1.8495
	2.00	-.2000	.28884	.500	-.8195	.4195
	4.00	-.1000	.28884	.734	-.7195	.5195
	5.00	-.1400	.28884	.635	-.7595	.4795
	6.00	-.3800	.28884	.209	-.9995	.2395
	7.00	-.4300	.28884	.159	-1.0495	.1895
4.00	1.00	1.3300(*)	.28884	.000	.7105	1.9495
	2.00	-.1000	.28884	.734	-.7195	.5195
	3.00	.1000	.28884	.734	-.5195	.7195
	5.00	-.0400	.28884	.892	-.6595	.5795
	6.00	-.2800	.28884	.349	-.8995	.3395
	7.00	-.3300	.28884	.272	-.9495	.2895
5.00	1.00	1.3700(*)	.28884	.000	.7505	1.9895
	2.00	-.0600	.28884	.838	-.6795	.5595
	3.00	.1400	.28884	.635	-.4795	.7595
	4.00	.0400	.28884	.892	-.5795	.6595
	6.00	-.2400	.28884	.420	-.8595	.3795
	7.00	-.2900	.28884	.332	-.9095	.3295
6.00	1.00	1.6100(*)	.28884	.000	.9905	2.2295
	2.00	.1800	.28884	.543	-.4395	.7995
	3.00	.3800	.28884	.209	-.2395	.9995
	4.00	.2800	.28884	.349	-.3395	.8995
	5.00	.2400	.28884	.420	-.3795	.8595
	7.00	-.0500	.28884	.865	-.6695	.5695
7.00	1.00	1.6600(*)	.28884	.000	1.0405	2.2795
	2.00	.2300	.28884	.439	-.3895	.8495
	3.00	.4300	.28884	.159	-.1895	1.0495
	4.00	.3300	.28884	.272	-.2895	.9495
	5.00	.2900	.28884	.332	-.3295	.9095
	6.00	.0500	.28884	.865	-.5695	.6695

* The mean difference is significant at the .05 level.

ANOVA for no. of primary branches/panicle in the field

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1.370	6	.228	.742	.625
Within Groups	4.305	14	.307		
Total	5.675	20			

Multiple LSD comparisons of means on dependent variable no. of primary branches/panicle in the field.

(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
1.00	2.00	-.6800	.45276	.155	-1.6511	.2911
	3.00	-.6500	.45276	.173	-1.6211	.3211
	4.00	-.6300	.45276	.186	-1.6011	.3411
	5.00	-.4000	.45276	.392	-1.3711	.5711
	6.00	-.7100	.45276	.139	-1.6811	.2611
	7.00	-.8200	.45276	.092	-1.7911	.1511
2.00	1.00	.6800	.45276	.155	-.2911	1.6511
	3.00	.0300	.45276	.948	-.9411	1.0011
	4.00	.0500	.45276	.914	-.9211	1.0211
	5.00	.2800	.45276	.546	-.6911	1.2511
	6.00	-.0300	.45276	.948	-1.0011	.9411
	7.00	-.1400	.45276	.762	-1.1111	.8311
3.00	1.00	.6500	.45276	.173	-.3211	1.6211
	2.00	-.0300	.45276	.948	-1.0011	.9411
	4.00	.0200	.45276	.965	-.9511	.9911
	5.00	.2500	.45276	.590	-.7211	1.2211
	6.00	-.0600	.45276	.896	-1.0311	.9111
	7.00	-.1700	.45276	.713	-1.1411	.8011
4.00	1.00	.6300	.45276	.186	-.3411	1.6011
	2.00	-.0500	.45276	.914	-1.0211	.9211
	3.00	-.0200	.45276	.965	-.9911	.9511
	5.00	.2300	.45276	.619	-.7411	1.2011
	6.00	-.0800	.45276	.862	-1.0511	.8911
	7.00	-.1900	.45276	.681	-1.1611	.7811
5.00	1.00	.4000	.45276	.392	-.5711	1.3711
	2.00	-.2800	.45276	.546	-1.2511	.6911
	3.00	-.2500	.45276	.590	-1.2211	.7211
	4.00	-.2300	.45276	.619	-1.2011	.7411
	6.00	-.3100	.45276	.505	-1.2811	.6611
	7.00	-.4200	.45276	.369	-1.3911	.5511
6.00	1.00	.7100	.45276	.139	-.2611	1.6811
	2.00	.0300	.45276	.948	-.9411	1.0011
	3.00	.0600	.45276	.896	-.9111	1.0311
	4.00	.0800	.45276	.862	-.8911	1.0511
	5.00	.3100	.45276	.505	-.6611	1.2811
	7.00	-.1100	.45276	.812	-1.0811	.8611
7.00	1.00	.8200	.45276	.092	-.1511	1.7911
	2.00	.1400	.45276	.762	-.8311	1.1111
	3.00	.1700	.45276	.713	-.8011	1.1411
	4.00	.1900	.45276	.681	-.7811	1.1611
	5.00	.4200	.45276	.369	-.5511	1.3911
	6.00	.1100	.45276	.812	-.8611	1.0811

ANOVA for no. of filled grains in the field

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	704.775	6	117.462	325.497	.000
Within Groups	5.052	14	.361		
Total	709.827	20			

Multiple LSD comparisons of means on dependent variable no. of filled grains in the field.

(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
1.00	2.00	-14.3400(*)	.49049	.000	-15.3920	-13.2880
	3.00	-12.5800(*)	.49049	.000	-13.6320	-11.5280
	4.00	-14.0000(*)	.49049	.000	-15.0520	-12.9480
	5.00	-13.9800(*)	.49049	.000	-15.0320	-12.9280
	6.00	-17.9800(*)	.49049	.000	-19.0320	-16.9280
	7.00	-19.1100(*)	.49049	.000	-20.1620	-18.0580
2.00	1.00	14.3400(*)	.49049	.000	13.2880	15.3920
	3.00	1.7600(*)	.49049	.003	.7080	2.8120
	4.00	.3400	.49049	.500	-.7120	1.3920
	5.00	.3600	.49049	.475	-.6920	1.4120
	6.00	-3.6400(*)	.49049	.000	-4.6920	-2.5880
	7.00	-4.7700(*)	.49049	.000	-5.8220	-3.7180
3.00	1.00	12.5800(*)	.49049	.000	11.5280	13.6320
	2.00	-1.7600(*)	.49049	.003	-2.8120	-.7080
	4.00	-1.4200(*)	.49049	.012	-2.4720	-.3680
	5.00	-1.4000(*)	.49049	.013	-2.4520	-.3480
	6.00	-5.4000(*)	.49049	.000	-6.4520	-4.3480
	7.00	-6.5300(*)	.49049	.000	-7.5820	-5.4780
4.00	1.00	14.0000(*)	.49049	.000	12.9480	15.0520
	2.00	-.3400	.49049	.500	-1.3920	.7120
	3.00	1.4200(*)	.49049	.012	.3680	2.4720
	5.00	.0200	.49049	.968	-1.0320	1.0720
	6.00	-3.9800(*)	.49049	.000	-5.0320	-2.9280
	7.00	-5.1100(*)	.49049	.000	-6.1620	-4.0580
5.00	1.00	13.9800(*)	.49049	.000	12.9280	15.0320
	2.00	-.3600	.49049	.475	-1.4120	.6920
	3.00	1.4000(*)	.49049	.013	.3480	2.4520
	4.00	-.0200	.49049	.968	-1.0720	1.0320
	6.00	-4.0000(*)	.49049	.000	-5.0520	-2.9480
	7.00	-5.1300(*)	.49049	.000	-6.1820	-4.0780
6.00	1.00	17.9800(*)	.49049	.000	16.9280	19.0320
	2.00	3.6400(*)	.49049	.000	2.5880	4.6920
	3.00	5.4000(*)	.49049	.000	4.3480	6.4520
	4.00	3.9800(*)	.49049	.000	2.9280	5.0320
	5.00	4.0000(*)	.49049	.000	2.9480	5.0520
	7.00	-1.1300(*)	.49049	.037	-2.1820	-.0780
7.00	1.00	19.1100(*)	.49049	.000	18.0580	20.1620
	2.00	4.7700(*)	.49049	.000	3.7180	5.8220
	3.00	6.5300(*)	.49049	.000	5.4780	7.5820
	4.00	5.1100(*)	.49049	.000	4.0580	6.1620
	5.00	5.1300(*)	.49049	.000	4.0780	6.1820
	6.00	1.1300(*)	.49049	.037	.0780	2.1820

* The mean difference is significant at the .05 level.

ANOVA for %filled grains in the field

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	21.420	6	3.570	12.822	.000
Within Groups	3.898	14	.278		
Total	25.318	20			

Multiple LSD comparisons of means on dependent variable %filled grains in the field.

(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1.00	2.00	-2.7400(*)	.43084	.000	-3.6640	-1.8160
	3.00	-2.3600(*)	.43084	.000	-3.2840	-1.4360
	4.00	-1.6200(*)	.43084	.002	-2.5440	-.6960
	5.00	-2.0700(*)	.43084	.000	-2.9940	-1.1460
	6.00	-3.0200(*)	.43084	.000	-3.9440	-2.0960
	7.00	-3.1900(*)	.43084	.000	-4.1140	-2.2660
2.00	1.00	2.7400(*)	.43084	.000	1.8160	3.6640
	3.00	.3800	.43084	.393	-.5440	1.3040
	4.00	1.1200(*)	.43084	.021	.1960	2.0440
	5.00	.6700	.43084	.142	-.2540	1.5940
	6.00	-.2800	.43084	.526	-1.2040	.6440
	7.00	-.4500	.43084	.314	-1.3740	.4740
3.00	1.00	2.3600(*)	.43084	.000	1.4360	3.2840
	2.00	-.3800	.43084	.393	-1.3040	.5440
	4.00	.7400	.43084	.108	-.1840	1.6640
	5.00	.2900	.43084	.512	-.6340	1.2140
	6.00	-.6600	.43084	.148	-1.5840	.2640
	7.00	-.8300	.43084	.075	-1.7540	.0940
4.00	1.00	1.6200(*)	.43084	.002	.6960	2.5440
	2.00	-1.1200(*)	.43084	.021	-2.0440	-.1960
	3.00	-.7400	.43084	.108	-1.6640	.1840
	5.00	-.4500	.43084	.314	-1.3740	.4740
	6.00	-1.4000(*)	.43084	.006	-2.3240	-.4760
	7.00	-1.5700(*)	.43084	.003	-2.4940	-.6460
5.00	1.00	2.0700(*)	.43084	.000	1.1460	2.9940
	2.00	-.6700	.43084	.142	-1.5940	.2540
	3.00	-.2900	.43084	.512	-1.2140	.6340
	4.00	.4500	.43084	.314	-.4740	1.3740
	6.00	-.9500(*)	.43084	.045	-1.8740	-.0260
	7.00	-1.1200(*)	.43084	.021	-2.0440	-.1960
6.00	1.00	3.0200(*)	.43084	.000	2.0960	3.9440
	2.00	.2800	.43084	.526	-.6440	1.2040
	3.00	.6600	.43084	.148	-.2640	1.5840
	4.00	1.4000(*)	.43084	.006	.4760	2.3240
	5.00	.9500(*)	.43084	.045	.0260	1.8740
	7.00	-.1700	.43084	.699	-1.0940	.7540
7.00	1.00	3.1900(*)	.43084	.000	2.2660	4.1140
	2.00	.4500	.43084	.314	-.4740	1.3740
	3.00	.8300	.43084	.075	-.0940	1.7540
	4.00	1.5700(*)	.43084	.003	.6460	2.4940
	5.00	1.1200(*)	.43084	.021	.1960	2.0440
	6.00	.1700	.43084	.699	-.7540	1.0940

*The mean difference is significant at the .05 level.

ANOVA for wt. of 1000 grains (gm) in the field

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.980	6	.163	1.759	.180
Within Groups	1.300	14	.093		
Total	2.279	20			

Multiple LSD comparisons of means on dependent variable wt. of 1000 grains (gm) in the field.

					95% Confidence Interval	
(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	Sig.	Lower Bound	Upper Bound
1.00	2.00	-.6100(*)	.24877	.028	-1.1436	-.0764
	3.00	-.4500	.24877	.092	-.9836	.0836
	4.00	-.5800(*)	.24877	.035	-1.1136	-.0464
	5.00	-.6000(*)	.24877	.030	-1.1336	-.0664
	6.00	-.6300(*)	.24877	.024	-1.1636	-.0964
	7.00	-.6700(*)	.24877	.017	-1.2036	-.1364
2.00	1.00	.6100(*)	.24877	.028	.0764	1.1436
	3.00	.1600	.24877	.531	-.3736	.6936
	4.00	.0300	.24877	.906	-.5036	.5636
	5.00	.0100	.24877	.969	-.5236	.5436
	6.00	-.0200	.24877	.937	-.5536	.5136
	7.00	-.0600	.24877	.813	-.5936	.4736
3.00	1.00	.4500	.24877	.092	-.0836	.9836
	2.00	-.1600	.24877	.531	-.6936	.3736
	4.00	-.1300	.24877	.609	-.6636	.4036
	5.00	-.1500	.24877	.556	-.6836	.3836
	6.00	-.1800	.24877	.481	-.7136	.3536
	7.00	-.2200	.24877	.391	-.7536	.3136
4.00	1.00	.5800(*)	.24877	.035	.0464	1.1136
	2.00	-.0300	.24877	.906	-.5636	.5036
	3.00	.1300	.24877	.609	-.4036	.6636
	5.00	-.0200	.24877	.937	-.5536	.5136
	6.00	-.0500	.24877	.844	-.5836	.4836
	7.00	-.0900	.24877	.723	-.6236	.4436
5.00	1.00	.6000(*)	.24877	.030	.0664	1.1336
	2.00	-.0100	.24877	.969	-.5436	.5236
	3.00	.1500	.24877	.556	-.3836	.6836
	4.00	.0200	.24877	.937	-.5136	.5536
	6.00	-.0300	.24877	.906	-.5636	.5036
	7.00	-.0700	.24877	.783	-.6036	.4636
6.00	1.00	.6300(*)	.24877	.024	.0964	1.1636
	2.00	.0200	.24877	.937	-.5136	.5536
	3.00	.1800	.24877	.481	-.3536	.7136
	4.00	.0500	.24877	.844	-.4836	.5836
	5.00	.0300	.24877	.906	-.5036	.5636
	7.00	-.0400	.24877	.875	-.5736	.4936
7.00	1.00	.6700(*)	.24877	.017	.1364	1.2036
	2.00	.0600	.24877	.813	-.4736	.5936
	3.00	.2200	.24877	.391	-.3136	.7536
	4.00	.0900	.24877	.723	-.4436	.6236
	5.00	.0700	.24877	.783	-.4636	.6036
	6.00	.0400	.24877	.875	-.4936	.5736

* The mean difference is significant at the .05 level.

ANOVA for grain yield (t/ha) in the field

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	15.398	6	2.566	42.631	.000
Within Groups	.843	14	.060		
Total	16.241	20			

Multiple LSD comparisons of means on dependent variable grain yield (t/ha) in the field.

(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1.00	2.00	-2.2500(*)	.20033	.000	-2.6797	-1.8203
	3.00	-1.8700(*)	.20033	.000	-2.2997	-1.4403
	4.00	-2.1200(*)	.20033	.000	-2.5497	-1.6903
	5.00	-2.1600(*)	.20033	.000	-2.5897	-1.7303
	6.00	-2.6400(*)	.20033	.000	-3.0697	-2.2103
	7.00	-2.7800(*)	.20033	.000	-3.2097	-2.3503
2.00	1.00	2.2500(*)	.20033	.000	1.8203	2.6797
	3.00	.3800	.20033	.079	-.0497	.8097
	4.00	.1300	.20033	.527	-.2997	.5597
	5.00	.0900	.20033	.660	-.3397	.5197
	6.00	-.3900	.20033	.072	-.8197	.0397
	7.00	-.5300(*)	.20033	.019	-.9597	-.1003
3.00	1.00	1.8700(*)	.20033	.000	1.4403	2.2997
	2.00	-.3800	.20033	.079	-.8097	.0497
	4.00	-.2500	.20033	.233	-.6797	.1797
	5.00	-.2900	.20033	.170	-.7197	.1397
	6.00	-.7700(*)	.20033	.002	-1.1997	-.3403
	7.00	-.9100(*)	.20033	.000	-1.3397	-.4803
4.00	1.00	2.1200(*)	.20033	.000	1.6903	2.5497
	2.00	-.1300	.20033	.527	-.5597	.2997
	3.00	.2500	.20033	.233	-.1797	.6797
	5.00	-.0400	.20033	.845	-.4697	.3897
	6.00	-.5200(*)	.20033	.021	-.9497	-.0903
	7.00	-.6600(*)	.20033	.005	-1.0897	-.2303
5.00	1.00	2.1600(*)	.20033	.000	1.7303	2.5897
	2.00	-.0900	.20033	.660	-.5197	.3397
	3.00	.2900	.20033	.170	-.1397	.7197
	4.00	.0400	.20033	.845	-.3897	.4697
	6.00	-.4800(*)	.20033	.031	-.9097	-.0503
	7.00	-.6200(*)	.20033	.008	-1.0497	-.1903
6.00	1.00	2.6400(*)	.20033	.000	2.2103	3.0697
	2.00	.3900	.20033	.072	-.0397	.8197
	3.00	.7700(*)	.20033	.002	.3403	1.1997
	4.00	.5200(*)	.20033	.021	.0903	.9497
	5.00	.4800(*)	.20033	.031	.0503	.9097
	7.00	-.1400	.20033	.496	-.5697	.2897
7.00	1.00	2.7800(*)	.20033	.000	2.3503	3.2097
	2.00	.5300(*)	.20033	.019	.1003	.9597
	3.00	.9100(*)	.20033	.000	.4803	1.3397
	4.00	.6600(*)	.20033	.005	.2303	1.0897
	5.00	.6200(*)	.20033	.008	.1903	1.0497
	6.00	.1400	.20033	.496	-.2897	.5697

* The mean difference is significant at the .05 level

ANOVA for straw yield (t/ha) in the field

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	7.130	6	1.188	4.842	.007
Within Groups	3.436	14	.245		
Total	10.566	20			

Multiple Comparison Multiple LSD comparisons of means on dependent variable straw yield (t/ha) in the field.

(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1.00	2.00	-1.6200(*)	.40450	.001	-2.4876	-.7524
	3.00	-1.3600(*)	.40450	.005	-2.2276	-.4924
	4.00	-1.5200(*)	.40450	.002	-2.3876	-.6524
	5.00	-1.5800(*)	.40450	.002	-2.4476	-.7124
	6.00	-1.7700(*)	.40450	.001	-2.6376	-.9024
	7.00	-1.8300(*)	.40450	.000	-2.6976	-.9624
2.00	1.00	1.6200(*)	.40450	.001	.7524	2.4876
	3.00	.2600	.40450	.531	-.6076	1.1276
	4.00	.1000	.40450	.808	-.7676	.9676
	5.00	.0400	.40450	.923	-.8276	.9076
	6.00	-.1500	.40450	.716	-1.0176	.7176
	7.00	-.2100	.40450	.612	-1.0776	.6576
3.00	1.00	1.3600(*)	.40450	.005	.4924	2.2276
	2.00	-.2600	.40450	.531	-1.1276	.6076
	4.00	-.1600	.40450	.698	-1.0276	.7076
	5.00	-.2200	.40450	.595	-1.0876	.6476
	6.00	-.4100	.40450	.328	-1.2776	.4576
	7.00	-.4700	.40450	.265	-1.3376	.3976
4.00	1.00	1.5200(*)	.40450	.002	.6524	2.3876
	2.00	-.1000	.40450	.808	-.9676	.7676
	3.00	.1600	.40450	.698	-.7076	1.0276
	5.00	-.0600	.40450	.884	-.9276	.8076
	6.00	-.2500	.40450	.546	-1.1176	.6176
	7.00	-.3100	.40450	.456	-1.1776	.5576
5.00	1.00	1.5800(*)	.40450	.002	.7124	2.4476
	2.00	-.0400	.40450	.923	-.9076	.8276
	3.00	.2200	.40450	.595	-.6476	1.0876
	4.00	.0600	.40450	.884	-.8076	.9276
	6.00	-.1900	.40450	.646	-1.0576	.6776
	7.00	-.2500	.40450	.546	-1.1176	.6176
6.00	1.00	1.7700(*)	.40450	.001	.9024	2.6376
	2.00	.1500	.40450	.716	-.7176	1.0176
	3.00	.4100	.40450	.328	-.4576	1.2776
	4.00	.2500	.40450	.546	-.6176	1.1176
	5.00	.1900	.40450	.646	-.6776	1.0576
	7.00	-.0600	.40450	.884	-.9276	.8076
7.00	1.00	1.8300(*)	.40450	.000	.9624	2.6976
	2.00	.2100	.40450	.612	-.6576	1.0776
	3.00	.4700	.40450	.265	-.3976	1.3376
	4.00	.3100	.40450	.456	-.5576	1.1776
	5.00	.2500	.40450	.546	-.6176	1.1176
	6.00	.0600	.40450	.884	-.8076	.9276

*The mean difference is significant at the .05 level.

ANOVA for plant height (cm) in the pot

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	794.815	6	132.469	310.232	.000
Within Groups	5.978	14	.427		
Total	800.793	20			

Multiple LSD comparisons of means on dependent variable plant height (cm) in the pot.

(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
1.00	2.00	-14.5400(*)	.53354	.000	-15.6843	-13.3957
	3.00	-10.5800(*)	.53354	.000	-11.7243	-9.4357
	4.00	-12.6600(*)	.53354	.000	-13.8043	-11.5157
	5.00	-13.9200(*)	.53354	.000	-15.0643	-12.7757
	6.00	-18.8100(*)	.53354	.000	-19.9543	-17.6657
	7.00	-20.2800(*)	.53354	.000	-21.4243	-19.1357
2.00	1.00	14.5400(*)	.53354	.000	13.3957	15.6843
	3.00	3.9600(*)	.53354	.000	2.8157	5.1043
	4.00	1.8800(*)	.53354	.003	.7357	3.0243
	5.00	.6200	.53354	.265	-.5243	1.7643
	6.00	-4.2700(*)	.53354	.000	-5.4143	-3.1257
	7.00	-5.7400(*)	.53354	.000	-6.8843	-4.5957
3.00	1.00	10.5800(*)	.53354	.000	9.4357	11.7243
	2.00	-3.9600(*)	.53354	.000	-5.1043	-2.8157
	4.00	-2.0800(*)	.53354	.002	-3.2243	-.9357
	5.00	-3.3400(*)	.53354	.000	-4.4843	-2.1957
	6.00	-8.2300(*)	.53354	.000	-9.3743	-7.0857
	7.00	-9.7000(*)	.53354	.000	-10.8443	-8.5557
4.00	1.00	12.6600(*)	.53354	.000	11.5157	13.8043
	2.00	-1.8800(*)	.53354	.003	-3.0243	-.7357
	3.00	2.0800(*)	.53354	.002	.9357	3.2243
	5.00	-1.2600(*)	.53354	.033	-2.4043	-.1157
	6.00	-6.1500(*)	.53354	.000	-7.2943	-5.0057
	7.00	-7.6200(*)	.53354	.000	-8.7643	-6.4757
5.00	1.00	13.9200(*)	.53354	.000	12.7757	15.0643
	2.00	-.6200	.53354	.265	-1.7643	.5243
	3.00	3.3400(*)	.53354	.000	2.1957	4.4843
	4.00	1.2600(*)	.53354	.033	.1157	2.4043
	6.00	-4.8900(*)	.53354	.000	-6.0343	-3.7457
	7.00	-6.3600(*)	.53354	.000	-7.5043	-5.2157
6.00	1.00	18.8100(*)	.53354	.000	17.6657	19.9543
	2.00	4.2700(*)	.53354	.000	3.1257	5.4143
	3.00	8.2300(*)	.53354	.000	7.0857	9.3743
	4.00	6.1500(*)	.53354	.000	5.0057	7.2943
	5.00	4.8900(*)	.53354	.000	3.7457	6.0343
	7.00	-1.4700(*)	.53354	.015	-2.6143	-.3257
7.00	1.00	20.2800(*)	.53354	.000	19.1357	21.4243
	2.00	5.7400(*)	.53354	.000	4.5957	6.8843
	3.00	9.7000(*)	.53354	.000	8.5557	10.8443
	4.00	7.6200(*)	.53354	.000	6.4757	8.7643
	5.00	6.3600(*)	.53354	.000	5.2157	7.5043
	6.00	1.4700(*)	.53354	.015	.3257	2.6143

*The mean difference is significant at the .05 level.

ANOVA for no. of panicles/pot in the pot

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	6.269	6	1.045	2.953	.045
Within Groups	4.953	14	.354		
Total	11.221	20			

Multiple LSD comparisons of means on dependent variable no. of panicles/pot in the pot.

(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1.00	2.00	-1.5100(*)	.48563	.008	-2.5516	-.4684
	3.00	-1.1700(*)	.48563	.030	-2.2116	-.1284
	4.00	-1.4200(*)	.48563	.011	-2.4616	-.3784
	5.00	-1.4700(*)	.48563	.009	-2.5116	-.4284
	6.00	-1.6500(*)	.48563	.004	-2.6916	-.6084
	7.00	-1.7200(*)	.48563	.003	-2.7616	-.6784
2.00	1.00	1.5100(*)	.48563	.008	.4684	2.5516
	3.00	.3400	.48563	.495	-.7016	1.3816
	4.00	.0900	.48563	.856	-.9516	1.1316
	5.00	.0400	.48563	.936	-1.0016	1.0816
	6.00	-.1400	.48563	.777	-1.1816	.9016
	7.00	-.2100	.48563	.672	-1.2516	.8316
3.00	1.00	1.1700(*)	.48563	.030	.1284	2.2116
	2.00	-.3400	.48563	.495	-1.3816	.7016
	4.00	-.2500	.48563	.615	-1.2916	.7916
	5.00	-.3000	.48563	.547	-1.3416	.7416
	6.00	-.4800	.48563	.340	-1.5216	.5616
	7.00	-.5500	.48563	.276	-1.5916	.4916
4.00	1.00	1.4200(*)	.48563	.011	.3784	2.4616
	2.00	-.0900	.48563	.856	-1.1316	.9516
	3.00	.2500	.48563	.615	-.7916	1.2916
	5.00	-.0500	.48563	.919	-1.0916	.9916
	6.00	-.2300	.48563	.643	-1.2716	.8116
	7.00	-.3000	.48563	.547	-1.3416	.7416
5.00	1.00	1.4700(*)	.48563	.009	.4284	2.5116
	2.00	-.0400	.48563	.936	-1.0816	1.0016
	3.00	.3000	.48563	.547	-.7416	1.3416
	4.00	.0500	.48563	.919	-.9916	1.0916
	6.00	-.1800	.48563	.716	-1.2216	.8616
	7.00	-.2500	.48563	.615	-1.2916	.7916
6.00	1.00	1.6500(*)	.48563	.004	.6084	2.6916
	2.00	.1400	.48563	.777	-.9016	1.1816
	3.00	.4800	.48563	.340	-.5616	1.5216
	4.00	.2300	.48563	.643	-.8116	1.2716
	5.00	.1800	.48563	.716	-.8616	1.2216
	7.00	-.0700	.48563	.887	-1.1116	.9716
7.00	1.00	1.7200(*)	.48563	.003	.6784	2.7616
	2.00	.2100	.48563	.672	-.8316	1.2516
	3.00	.5500	.48563	.276	-.4916	1.5916
	4.00	.3000	.48563	.547	-.7416	1.3416
	5.00	.2500	.48563	.615	-.7916	1.2916
	6.00	.0700	.48563	.887	-.9716	1.1116

* The mean difference is significant at the .05 level.

ANOVA for no. of primary branches/panicle in the pot

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	3.776	6	.629	2.337	.089
Within Groups	3.769	14	.269		
Total	7.545	20			

Multiple LSD comparisons of means on dependent variable no. of primary branches/panicle in the pot.

(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1.00	2.00	-1.1600(*)	.42365	.016	-2.0686	-.2514
	3.00	-.6000	.42365	.179	-1.5086	.3086
	4.00	-.9400(*)	.42365	.044	-1.8486	-.0314
	5.00	-1.0500(*)	.42365	.027	-1.9586	-.1414
	6.00	-1.2500(*)	.42365	.011	-2.1586	-.3414
	7.00	-1.2800(*)	.42365	.009	-2.1886	-.3714
2.00	1.00	1.1600(*)	.42365	.016	.2514	2.0686
	3.00	.5600	.42365	.207	-.3486	1.4686
	4.00	.2200	.42365	.612	-.6886	1.1286
	5.00	.1100	.42365	.799	-.7986	1.0186
	6.00	-.0900	.42365	.835	-.9986	.8186
	7.00	-.1200	.42365	.781	-1.0286	.7886
3.00	1.00	.6000	.42365	.179	-.3086	1.5086
	2.00	-.5600	.42365	.207	-1.4686	.3486
	4.00	-.3400	.42365	.436	-1.2486	.5686
	5.00	-.4500	.42365	.306	-1.3586	.4586
	6.00	-.6500	.42365	.147	-1.5586	.2586
	7.00	-.6800	.42365	.131	-1.5886	.2286
4.00	1.00	.9400(*)	.42365	.044	.0314	1.8486
	2.00	-.2200	.42365	.612	-1.1286	.6886
	3.00	.3400	.42365	.436	-.5686	1.2486
	5.00	-.1100	.42365	.799	-1.0186	.7986
	6.00	-.3100	.42365	.476	-1.2186	.5986
	7.00	-.3400	.42365	.436	-1.2486	.5686
5.00	1.00	1.0500(*)	.42365	.027	.1414	1.9586
	2.00	-.1100	.42365	.799	-1.0186	.7986
	3.00	.4500	.42365	.306	-.4586	1.3586
	4.00	.1100	.42365	.799	-.7986	1.0186
	6.00	-.2000	.42365	.644	-1.1086	.7086
	7.00	-.2300	.42365	.596	-1.1386	.6786
6.00	1.00	1.2500(*)	.42365	.011	.3414	2.1586
	2.00	.0900	.42365	.835	-.8186	.9986
	3.00	.6500	.42365	.147	-.2586	1.5586
	4.00	.3100	.42365	.476	-.5986	1.2186
	5.00	.2000	.42365	.644	-.7086	1.1086
	7.00	-.0300	.42365	.945	-.9386	.8786
7.00	1.00	1.2800(*)	.42365	.009	.3714	2.1886
	2.00	.1200	.42365	.781	-.7886	1.0286
	3.00	.6800	.42365	.131	-.2286	1.5886
	4.00	.3400	.42365	.436	-.5686	1.2486
	5.00	.2300	.42365	.596	-.6786	1.1386
	6.00	.0300	.42365	.945	-.8786	.9386

* The mean difference is significant at the .05 level.

ANOVA for no. of filled grains/ panicle in the pot

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	542.850	6	90.475	333.751	.000
Within Groups	3.795	14	.271		
Total	546.645	20			

Multiple LSD comparisons of means on dependent variable no. of filled grains/ panicle in the pot

(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	Upper Bound
1.00	2.00	-10.1400(*)	.42512	.000	-11.0518	-9.2282
	3.00	-4.3600(*)	.42512	.000	-5.2718	-3.4482
	4.00	-7.7700(*)	.42512	.000	-8.6818	-6.8582
	5.00	-8.8900(*)	.42512	.000	-9.8018	-7.9782
	6.00	-13.8000(*)	.42512	.000	-14.7118	-12.8882
	7.00	-16.2800(*)	.42512	.000	-17.1918	-15.3682
2.00	1.00	10.1400(*)	.42512	.000	9.2282	11.0518
	3.00	5.7800(*)	.42512	.000	4.8682	6.6918
	4.00	2.3700(*)	.42512	.000	1.4582	3.2818
	5.00	1.2500(*)	.42512	.011	.3382	2.1618
	6.00	-3.6600(*)	.42512	.000	-4.5718	-2.7482
	7.00	-6.1400(*)	.42512	.000	-7.0518	-5.2282
3.00	1.00	4.3600(*)	.42512	.000	3.4482	5.2718
	2.00	-5.7800(*)	.42512	.000	-6.6918	-4.8682
	4.00	-3.4100(*)	.42512	.000	-4.3218	-2.4982
	5.00	-4.5300(*)	.42512	.000	-5.4418	-3.6182
	6.00	-9.4400(*)	.42512	.000	-10.3518	-8.5282
	7.00	-11.9200(*)	.42512	.000	-12.8318	-11.0082
4.00	1.00	7.7700(*)	.42512	.000	6.8582	8.6818
	2.00	-2.3700(*)	.42512	.000	-3.2818	-1.4582
	3.00	3.4100(*)	.42512	.000	2.4982	4.3218
	5.00	-1.1200(*)	.42512	.020	-2.0318	-.2082
	6.00	-6.0300(*)	.42512	.000	-6.9418	-5.1182
	7.00	-8.5100(*)	.42512	.000	-9.4218	-7.5982
5.00	1.00	8.8900(*)	.42512	.000	7.9782	9.8018
	2.00	-1.2500(*)	.42512	.011	-2.1618	-.3382
	3.00	4.5300(*)	.42512	.000	3.6182	5.4418
	4.00	1.1200(*)	.42512	.020	.2082	2.0318
	6.00	-4.9100(*)	.42512	.000	-5.8218	-3.9982
	7.00	-7.3900(*)	.42512	.000	-8.3018	-6.4782
6.00	1.00	13.8000(*)	.42512	.000	12.8882	14.7118
	2.00	3.6600(*)	.42512	.000	2.7482	4.5718
	3.00	9.4400(*)	.42512	.000	8.5282	10.3518
	4.00	6.0300(*)	.42512	.000	5.1182	6.9418
	5.00	4.9100(*)	.42512	.000	3.9982	5.8218
	7.00	-2.4800(*)	.42512	.000	-3.3918	-1.5682
7.00	1.00	16.2800(*)	.42512	.000	15.3682	17.1918
	2.00	6.1400(*)	.42512	.000	5.2282	7.0518
	3.00	11.9200(*)	.42512	.000	11.0082	12.8318
	4.00	8.5100(*)	.42512	.000	7.5982	9.4218
	5.00	7.3900(*)	.42512	.000	6.4782	8.3018
	6.00	2.4800(*)	.42512	.000	1.5682	3.3918

*The mean difference is significant at the .05 level.

ANOVA for % of filled grains in the pot

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	50.833	6	8.472	17.214	.000
Within Groups	6.890	14	.492		
Total	57.723	20			

Multiple LSD comparisons of means on dependent variable % of filled grains in the pot.

(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
1.00	2.00	-2.5800(*)	.57280	.000	-3.8085	-1.3515
	3.00	-.2800	.57280	.633	-1.5085	.9485
	4.00	-1.0900	.57280	.078	-2.3185	.1385
	5.00	-1.3500(*)	.57280	.034	-2.5785	-.1215
	6.00	-4.0200(*)	.57280	.000	-5.2485	-2.7915
	7.00	-4.0900(*)	.57280	.000	-5.3185	-2.8615
2.00	1.00	2.5800(*)	.57280	.000	1.3515	3.8085
	3.00	2.3000(*)	.57280	.001	1.0715	3.5285
	4.00	1.4900(*)	.57280	.021	.2615	2.7185
	5.00	1.2300(*)	.57280	.050	.0015	2.4585
	6.00	-1.4400(*)	.57280	.025	-2.6685	-.2115
	7.00	-1.5100(*)	.57280	.020	-2.7385	-.2815
3.00	1.00	.2800	.57280	.633	-.9485	1.5085
	2.00	-2.3000(*)	.57280	.001	-3.5285	-1.0715
	4.00	-.8100	.57280	.179	-2.0385	.4185
	5.00	-1.0700	.57280	.083	-2.2985	.1585
	6.00	-3.7400(*)	.57280	.000	-4.9685	-2.5115
	7.00	-3.8100(*)	.57280	.000	-5.0385	-2.5815
4.00	1.00	1.0900	.57280	.078	-.1385	2.3185
	2.00	-1.4900(*)	.57280	.021	-2.7185	-.2615
	3.00	.8100	.57280	.179	-.4185	2.0385
	5.00	-.2600	.57280	.657	-1.4885	.9685
	6.00	-2.9300(*)	.57280	.000	-4.1585	-1.7015
	7.00	-3.0000(*)	.57280	.000	-4.2285	-1.7715
5.00	1.00	1.3500(*)	.57280	.034	.1215	2.5785
	2.00	-1.2300(*)	.57280	.050	-2.4585	-.0015
	3.00	1.0700	.57280	.083	-.1585	2.2985
	4.00	.2600	.57280	.657	-.9685	1.4885
	6.00	-2.6700(*)	.57280	.000	-3.8985	-1.4415
	7.00	-2.7400(*)	.57280	.000	-3.9685	-1.5115
6.00	1.00	4.0200(*)	.57280	.000	2.7915	5.2485
	2.00	1.4400(*)	.57280	.025	.2115	2.6685
	3.00	3.7400(*)	.57280	.000	2.5115	4.9685
	4.00	2.9300(*)	.57280	.000	1.7015	4.1585
	5.00	2.6700(*)	.57280	.000	1.4415	3.8985
	7.00	-.0700	.57280	.904	-1.2985	1.1585
7.00	1.00	4.0900(*)	.57280	.000	2.8615	5.3185
	2.00	1.5100(*)	.57280	.020	.2815	2.7385
	3.00	3.8100(*)	.57280	.000	2.5815	5.0385
	4.00	3.0000(*)	.57280	.000	1.7715	4.2285
	5.00	2.7400(*)	.57280	.000	1.5115	3.9685
	6.00	.0700	.57280	.904	-1.1585	1.2985

* The mean difference is significant at the .05 level.

ANOVA for wt. of 1000 grains (gm) in the pot

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.991	6	.165	.668	.677
Within Groups	3.460	14	.247		
Total	4.451	20			

Multiple LSD comparisons of means on dependent variable wt. of 1000 grains (gm) in the pot.

(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1.00	2.00	-.5300	.40590	.213	-1.4006	.3406
	3.00	-.1800	.40590	.664	-1.0506	.6906
	4.00	-.3900	.40590	.353	-1.2606	.4806
	5.00	-.4500	.40590	.286	-1.3206	.4206
	6.00	-.6000	.40590	.161	-1.4706	.2706
	7.00	-.6500	.40590	.132	-1.5206	.2206
2.00	1.00	.5300	.40590	.213	-.3406	1.4006
	3.00	.3500	.40590	.403	-.5206	1.2206
	4.00	.1400	.40590	.735	-.7306	1.0106
	5.00	.0800	.40590	.847	-.7906	.9506
	6.00	-.0700	.40590	.866	-.9406	.8006
	7.00	-.1200	.40590	.772	-.9906	.7506
3.00	1.00	.1800	.40590	.664	-.6906	1.0506
	2.00	-.3500	.40590	.403	-1.2206	.5206
	4.00	-.2100	.40590	.613	-1.0806	.6606
	5.00	-.2700	.40590	.517	-1.1406	.6006
	6.00	-.4200	.40590	.318	-1.2906	.4506
	7.00	-.4700	.40590	.266	-1.3406	.4006
4.00	1.00	.3900	.40590	.353	-.4806	1.2606
	2.00	-.1400	.40590	.735	-1.0106	.7306
	3.00	.2100	.40590	.613	-.6606	1.0806
	5.00	-.0600	.40590	.885	-.9306	.8106
	6.00	-.2100	.40590	.613	-1.0806	.6606
	7.00	-.2600	.40590	.532	-1.1306	.6106
5.00	1.00	.4500	.40590	.286	-.4206	1.3206
	2.00	-.0800	.40590	.847	-.9506	.7906
	3.00	.2700	.40590	.517	-.6006	1.1406
	4.00	.0600	.40590	.885	-.8106	.9306
	6.00	-.1500	.40590	.717	-1.0206	.7206
	7.00	-.2000	.40590	.630	-1.0706	.6706
6.00	1.00	.6000	.40590	.161	-.2706	1.4706
	2.00	.0700	.40590	.866	-.8006	.9406
	3.00	.4200	.40590	.318	-.4506	1.2906
	4.00	.2100	.40590	.613	-.6606	1.0806
	5.00	.1500	.40590	.717	-.7206	1.0206
	7.00	-.0500	.40590	.904	-.9206	.8206
7.00	1.00	.6500	.40590	.132	-.2206	1.5206
	2.00	.1200	.40590	.772	-.7506	.9906
	3.00	.4700	.40590	.266	-.4006	1.3406
	4.00	.2600	.40590	.532	-.6106	1.1306
	5.00	.2000	.40590	.630	-.6706	1.0706
	6.00	.0500	.40590	.904	-.8206	.9206

ANOVA for grain yield (gm)/pot in the pot

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	159.040	6	26.507	77.974	.000
Within Groups	4.759	14	.340		
Total	163.799	20			

Multiple LSD comparisons of means on dependent variable grain yield (gm)/pot in the pot.

(I) Treatment	(J)Treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1.00	2.00	-7.0600(*)	.47606	.000	-8.0810	-6.0390
	3.00	-4.6900(*)	.47606	.000	-5.7110	-3.6690
	4.00	-6.2200(*)	.47606	.000	-7.2410	-5.1990
	5.00	-6.6300(*)	.47606	.000	-7.6510	-5.6090
	6.00	-8.2300(*)	.47606	.000	-9.2510	-7.2090
	7.00	-8.9600(*)	.47606	.000	-9.9810	-7.9390
2.00	1.00	7.0600(*)	.47606	.000	6.0390	8.0810
	3.00	2.3700(*)	.47606	.000	1.3490	3.3910
	4.00	.8400	.47606	.099	-.1810	1.8610
	5.00	.4300	.47606	.382	-.5910	1.4510
	6.00	-1.1700(*)	.47606	.028	-2.1910	-.1490
	7.00	-1.9000(*)	.47606	.001	-2.9210	-.8790
3.00	1.00	4.6900(*)	.47606	.000	3.6690	5.7110
	2.00	-2.3700(*)	.47606	.000	-3.3910	-1.3490
	4.00	-1.5300(*)	.47606	.006	-2.5510	-.5090
	5.00	-1.9400(*)	.47606	.001	-2.9610	-.9190
	6.00	-3.5400(*)	.47606	.000	-4.5610	-2.5190
	7.00	-4.2700(*)	.47606	.000	-5.2910	-3.2490
4.00	1.00	6.2200(*)	.47606	.000	5.1990	7.2410
	2.00	-.8400	.47606	.099	-1.8610	.1810
	3.00	1.5300(*)	.47606	.006	.5090	2.5510
	5.00	-.4100	.47606	.404	-1.4310	.6110
	6.00	-2.0100(*)	.47606	.001	-3.0310	-.9890
	7.00	-2.7400(*)	.47606	.000	-3.7610	-1.7190
5.00	1.00	6.6300(*)	.47606	.000	5.6090	7.6510
	2.00	-.4300	.47606	.382	-1.4510	.5910
	3.00	1.9400(*)	.47606	.001	.9190	2.9610
	4.00	.4100	.47606	.404	-.6110	1.4310
	6.00	-1.6000(*)	.47606	.005	-2.6210	-.5790
	7.00	-2.3300(*)	.47606	.000	-3.3510	-1.3090
6.00	1.00	8.2300(*)	.47606	.000	7.2090	9.2510
	2.00	1.1700(*)	.47606	.028	.1490	2.1910
	3.00	3.5400(*)	.47606	.000	2.5190	4.5610
	4.00	2.0100(*)	.47606	.001	.9890	3.0310
	5.00	1.6000(*)	.47606	.005	.5790	2.6210
	7.00	-.7300	.47606	.147	-1.7510	.2910
7.00	1.00	8.9600(*)	.47606	.000	7.9390	9.9810
	2.00	1.9000(*)	.47606	.001	.8790	2.9210
	3.00	4.2700(*)	.47606	.000	3.2490	5.2910
	4.00	2.7400(*)	.47606	.000	1.7190	3.7610
	5.00	2.3300(*)	.47606	.000	1.3090	3.3510
	6.00	.7300	.47606	.147	-.2910	1.7510

* The mean difference is significant at the .05 level.

ANOVA for straw yield (gm)/pot in the pot

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	75.451	6	12.575	24.768	.000
Within Groups	7.108	14	.508		
Total	82.559	20			

Multiple LSD comparisons of means on dependent variable no. of panicles/pot in the pot.

(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1.00	2.00	-4.9000(*)	.58180	.000	-6.1478	-3.6522
	3.00	-3.9400(*)	.58180	.000	-5.1878	-2.6922
	4.00	-4.4100(*)	.58180	.000	-5.6578	-3.1622
	5.00	-4.7200(*)	.58180	.000	-5.9678	-3.4722
	6.00	-5.7900(*)	.58180	.000	-7.0378	-4.5422
	7.00	-6.2400(*)	.58180	.000	-7.4878	-4.9922
2.00	1.00	4.9000(*)	.58180	.000	3.6522	6.1478
	3.00	.9600	.58180	.121	-.2878	2.2078
	4.00	.4900	.58180	.414	-.7578	1.7378
	5.00	.1800	.58180	.762	-1.0678	1.4278
	6.00	-.8900	.58180	.148	-2.1378	.3578
	7.00	-1.3400(*)	.58180	.037	-2.5878	-.0922
3.00	1.00	3.9400(*)	.58180	.000	2.6922	5.1878
	2.00	-.9600	.58180	.121	-2.2078	.2878
	4.00	-.4700	.58180	.433	-1.7178	.7778
	5.00	-.7800	.58180	.201	-2.0278	.4678
	6.00	-1.8500(*)	.58180	.007	-3.0978	-.6022
	7.00	-2.3000(*)	.58180	.001	-3.5478	-1.0522
4.00	1.00	4.4100(*)	.58180	.000	3.1622	5.6578
	2.00	-.4900	.58180	.414	-1.7378	.7578
	3.00	.4700	.58180	.433	-.7778	1.7178
	5.00	-.3100	.58180	.603	-1.5578	.9378
	6.00	-1.3800(*)	.58180	.033	-2.6278	-.1322
	7.00	-1.8300(*)	.58180	.007	-3.0778	-.5822
5.00	1.00	4.7200(*)	.58180	.000	3.4722	5.9678
	2.00	-.1800	.58180	.762	-1.4278	1.0678
	3.00	.7800	.58180	.201	-.4678	2.0278
	4.00	.3100	.58180	.603	-.9378	1.5578
	6.00	-1.0700	.58180	.087	-2.3178	.1778
	7.00	-1.5200(*)	.58180	.020	-2.7678	-.2722
6.00	1.00	5.7900(*)	.58180	.000	4.5422	7.0378
	2.00	.8900	.58180	.148	-.3578	2.1378
	3.00	1.8500(*)	.58180	.007	.6022	3.0978
	4.00	1.3800(*)	.58180	.033	.1322	2.6278
	5.00	1.0700	.58180	.087	-.1778	2.3178
	7.00	-.4500	.58180	.452	-1.6978	.7978
7.00	1.00	6.2400(*)	.58180	.000	4.9922	7.4878
	2.00	1.3400(*)	.58180	.037	.0922	2.5878
	3.00	2.3000(*)	.58180	.001	1.0522	3.5478
	4.00	1.8300(*)	.58180	.007	.5822	3.0778
	5.00	1.5200(*)	.58180	.020	.2722	2.7678
	6.00	.4500	.58180	.452	-.7978	1.6978

* The mean difference is significant at the .05 level.