UNIVERSITI TEKNOLOGI MARA

THE EFFECTS OF DIFFERENT BIOFERTILIZERS ON THE VEGETATIVE GROWTH OF POTTED RICE PLANTS

IZZUDDIN BIN ALI

Final year project report submitted in partial fulfilment of
the requirements for the degree of
Bachelor of Science (Hons.) Plantation Technology and Management

Faculty of Plantation and Agrotechnology

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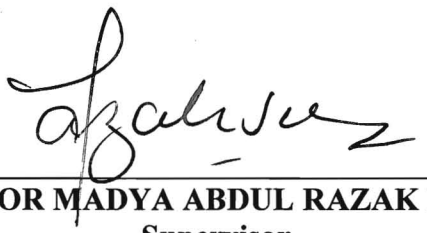
APPROVAL SHEET

This Final Year Project Report entitled "**The Effects of Different Biofertilizers on the Vegetative Growth of Potted Rice Plants**" was submitted by **Izzuddin Bin Ali**, in partial fulfilment of the requirement for the Degree of Bachelor of Science (Hons.) Plantation Technology and Management, in the Faculty of Plantation and Agrotechnology, and was approved by



PROFESSOR MADYA ABDUL RAZAK BIN BABA
Supervisor
Faculty of Plantation and Agrotechnology
Universiti Teknologi MARA
Jasin, Melaka

NUR FIRDAUS BINTI ABD RASHID
Co-supervisor
Faculty of Plantation and Agrotechnology
Universiti Teknologi MARA
Jasin, Melaka

WAN NATASYA BINTI WAN AHMED
Project Coordinator
BSc. (Hons) Plantation
Technology and Management
Faculty of Plantation and
Agrotechnology
Universiti Teknologi MARA
Jasin, Melaka

NORDIANA BINTI IBRAHIM
Head of Study Center
BSc. (Hons) Plantation
Technology and Management
Faculty of Plantation and
Agrotechnology
Universiti Teknologi MARA
Jasin, Melaka

Date:_____

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Name of Candidate	:	**IZZUDDIN BIN ALI**
Candidate's ID No.	:	2012240826
Programme	:	Bachelor of Sciences (Hons.) Plantation Technology and Management
Faculty	:	Plantation and Agrotechnology
Title	:	The Effect of Different Biofertilizers on the Vegetative Growth of Potted Rice Plants
Signature of candidate	:	
Date	:	30th January 2015

ABSTRACT

Application of biofertilizers are able to increase soil fertility and the effectiveness used of fertilizer. Biofertilizers give positive effects on paddy growth performance by increasing the nutrients available in the soil. Arbuscular mycorrhizal fungi and *Trichoderma spp.* give better growth performance by colonising rhizosphere of host plant and provide nutrient. Plant-microbes interaction will enhanced the fertilizer-use efficiency. The objective of this study was to evaluate the effects of different biofertilizers on paddy growth under pot conditions. Arbuscular mycorrhizal fungi and *Trichoderma spp.* were used in this study either alone or in combination. Two weeks old seedlings were transplanted to the field soil under a pot. Paddy that were inoculated with arbuscular mycorrhizal fungi had better plant height, number of tiller and phosphorus content after 9 weeks after sowing. Conversely, shoot and root biomass were not significantly influenced by AMF and *Trichoderma spp.* colonization under pot conditions. We conclude that the AMF inoculation during transplanting increased plant height, number of tiller and phosphorus content.

ABSTRAK

Penggunaan baja-bio meningkatkan kesuburan tanah dan keberkesanan penggunaan baja. Baja bio memberikan kesan positif terhadap pertumbuhan padi dengan cara meningkatkan nutrient tersedia dalam tanah untuk diserap oleh padi. Fungi Mikoriza Arbuskular dan *Trichoderma spp.* membantu tumbesaran dengan cara mengkoloni kawasan akar perumah dan menyalurkan nutrien. Interaksi antara tumbuhan dan mikrob akan menambah baik pengunaan baja lebih efisien. Objektif kajian ini dijalankan adalah untuk menilai kesan pelbagai jenis baja-bio terhadap pertumbuhan padi di dalam rumah hijau. Fungi Mikoriza Arbuskular dan *Trichoderma spp.* digunakan sepanjang kajian ini sama ada tunggal atau secara kombinasi. Anak benih berumur dua minggu ditransplan ke dalam bekas tanaman. Pokok padi yg diinokulasi dengan Fungi Mikoriza Arbuskular (AMF) menunjukkan kesan positive terhadap ketinggian pokok, bilangan anak pokok dan kandungan fosforus dalam tanah sepanjang 9 minggu selepas transplan. Sebaliknya, biomass pucuk dan akar tidak dipengaruhi oleh Fungi Mikoriza Arbuskular dan *Trichoderma spp.* Kesimpulannya, penggunaan Fungi Mikoriza Arbuskular semasa transplan meningkatkan kadar ketinggian pokok, bilangan anak pokok dan kandungan fosforus dalam tanah.

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My fellow friends should also be recognised for their support. My sincere appreciation also extends to all my colleagues and others who have provided assistance at various occasions. Their views and tips are useful indeed. Unfortunately, it is not possible to list all of them in this limited space. I am grateful to all my family members.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

AGR	Absolute Growth Rate
AMF	Arbuscular Mycorrhizal Fungi
ANOVA	One-Way Analysis of Variance
CFU	Colony Forming Unit
DAT	Date After Transplanting
Kg	Kilogram
P	Phosphorus
PGPF	Plant Growth Promoting Fungi

CHAPTER 1

INTRODUCTION

1.1 Background of Study

Biofertilizers are broadly used in several developed countries for various crop species. Paul *et al.* (2013) stated that biofertilizer contain free-living organisms which are capable to colonize the intercellular or intracellular spaces and devoid of damage to the host. They colonize the rhizosphere of the plant and increase the availability of primary nutrients and growth stimulus to the target crop. When it amended to seed, root or soil, it mobilizes the availability and utility of the microorganisms and thus improves the soil health.

According to Vessey (2003) defined biofertilizer as products having live or dormant strains of microbes, either bacteria or fungi that raise the nutrient availability and uptake of nutrient by the plant. Rice production in Malaysia usually used high of input chemical fertilizer, which can lead to soils being degraded, polluted and unproductive. According to Mishra and Dash (2014) increasingly extensive uses of chemical fertilizers in India assist soils to be degraded, polluted, less productive and environmental hazards.

Soil pollution is caused by the excessive application of chemical fertilizers. In other words, there is much concern to preserve environments through the use of less intensive and more sustainable agricultural practices by reducing the input of chemical fertilizers. Mishra and Dash (2014) stated that these issues can be solved by using biofertilizers to promote balanced ecosystem. Thus, it minimizes dependency of farmer on chemical fertilizers. These biofertilizers are not harmful to crops, but facilitate the unabsorbed nutrient in the soil to be

available to plant for growing. Recently, large scale farming be influenced by inorganic fertilizers to maintain large scale monocropping methods. Inappropriately, inorganic fertilizers are always excessive uses to the crop cultivated area. Truthfully, it is expected that only half of fertilizers applied are uptake by plants.

In this study, it is concerned about improving the soil fertility status to increase efficiency of nutrient absorption by the paddy plants using environmental friendly method such as biofertilizer. Two different biofertilizers were used. Paddy requires a sufficient amount of nutrients for healthy growing. Wan Yusoff *et al.* (2013) documented that four fungal (*Gliocladium virens, Trichoderma virens, Trichoderma harzianum* and *Aspergillus niger*) were examined for their effect on germination, root and shoot length and seedling weight of rice. The result suggested that seedling root length, shoot height and fresh weight of rice seedling were significantly increased. Therefore, biofertilizer is used to increase the soil nutrient availability for plant growth. In this context biofertilizers have gained prime importance. According to Smith (2003) arbuscular mycorrhizal (AM) fungi are vital component of nearly all terrestrial ecosystems, forming mutually beneficial (mutualistic) symbioses with the roots of around 80% of vascular plants and often increasing phosphate (P) uptake and growth. Hence, the current investigation was undertaken to examine the response of paddy to biofertilizers.

1.2 Problem Statement

There is much concern to preserve a healthy environment through less intensive and more sustainable agricultural practices. These can be achieved through less dependable on chemical input, instead using green technology. Biofertilizer

could give a positive impact on plant growth and farmers intensification of yields and output resources without compromising environmental quality. Whether application of biofertilizer on paddy can improve growth performance and soil fertility. In Malaysia, paddy is usually grown in nutrient deficient soils. This interpretation was confirmed by Vaishampayan *et al.* (2001) stated that the most important limiting factors in rice production is the amount of Nitrogen available in the soil. Application of biofertilizers helps to achieve the important phenomenon.

1.3 Significance of Study

This research study is suitable to be used by farmers, especially rice production in Malaysia for using biofertilizers to increase their paddy productivity and yield together maintaining their soil chemical and physical properties or soil fertility. Biofertilizer is green technology and environmental friendly. Thus, the environment will be preserved and becoming sustainable agriculture. The outcome of this study includes identifying the soil chemical and paddy physiology after it was treated with biofertilizers. Therefore, by using particular treatment and followed the proper methodology to conduct this experiment. Thus, precise conclusion can be made from the data collect and used as a solution and alternative by the farmers to increase their agricultural productivity. There are numerous farmers in Malaysia not being exposed regarding to benefit of biofertilizers which gives a positive impact on productivity of crop and the environment.

1.4 Scope of Study

Study of the effect of paddy growth performance by applying biofertilizers and a level of nitrogen and phosphorus. It is in the proper experimental methodology. The MR269 variety was used to determine the effect of biofertilizer on growth performance during vegetative phase, basic physiology traits and level of nitrogen and phosphorus.

1.5 Objective of Study

The research objectives of this study are as below:

1. To investigate the effect of different biofertilizer on the vegetative growth of paddy.
2. To analyse the effects of biofertilizer on soil fertility.

1.6 Hypothesis

H_0: There is no significant difference between different biofertilizers and rice growth.

H_1: There is a significant difference between different biofertilizers and rice growth.

H_0: There is no significant between biofertilizers and soil fertility.

H_1: There is significant between biofertilizers and soil fertility.

CHAPTER 2

LITERATURE REVIEW

2.1 Effect of Biofertilizer on Growth Parameters

Biofertilizer is a substance which contains living microorganisms. Rhizosphere colonizes to promote growth by increasing the availability of major nutrients. Nagananda *et al.* (2010) validates that the use of biofertilizer can increase yield of crops by using root nodule bacteria and fungi that are able to increase availability of nutrients from the soils. It stimulates microbial activity around the root system lead to increase the root mass and improving plant condition, increase the available nutrient for plants. Biofertilizer improve plants with increased nutrient content, improve seed germination and upsurge soil microorganism populations which in turn increase the uptake of nutrients from soil to plants (Ritika & Utpal , 2014). When biofertilizers are applied to the seed and increases the availability of the nutrient to the plant and increase the yield up to 10-20% without producing any adverse effect to the environment. Therefore, there is significant increase the plant growth parameter such as plant height, number of roots, length of root, length of shoot, dry matter accumulation in plant organs and vigour index. (Bhattacharjee & Dey, 2014). However, Arbuscular mycorrhizal (AM) fungi are ubiquitous component of most agroecosystems, where they provide several benefits to their host plant, including better phosphorus nutrition (Toro *et al.*, 1998).

In addition, Naderifar & Daneshian (2012) pointed out that nitrogen had a significant effect on the seed number per silques or seedpods, number of silques per plant, 1000 seeds weight and plant height. A vigorous increase in the growth

of plant height, number of branches and leaves were observed in the treatments. However, these effects were due to application of biofertilizer from 10 month onwards. There are increases in plant height, branches number and leaves number.

2.2 Effect of AMF on Paddy Growth

Sanni (1976) stated that paddy root system plays an vital role in the uptake of water and nutrients from the soil. Jeffries & Rhodes (1987) the mycorrhizal improvement of plant growth is mostly increased nutrient uptake particularly Phosphorus. An early study showed that there was a positive relationship between the presence of arbuscular mycorrhizal fungi (AMF), the number of spores in individual root system and the growth of rice. Besides that, Douds and Schenck (1990) stated that arbuscular mycorrhizae are present in the soils with high levels of extractable Phosporus. In addition, Smith and Read (1997) pointed out that Phosphorus (P) deficiency severely limits paddy production worldwide, while the colonization of plant roots with AMF often enhances Phosphorus uptake and plant growth. There is a significant importance that showed by previous study. Solaiman & Hirata (1997) reported that leaves and stem dry weights tended also to be greater for inoculated than non-inoculated plants. Moreover, leaf plus stem Nitrogen concentrations tended to be higher in the inoculated than in the non-inoculated plants at tillering stage of 52 days after transplanting. The inoculated seedlings had a higher total biomass than uninoculated seedlings at transplanting to the field. This indicates that seedlings benefited from mycorrhizal colonization prior to transplanting.

Arbuscular mycorrhizal fungi increased grain and straw yields of wetland paddy (Sivaprasad *et al.*, 1990) and increased the grain yield and phosphorus and zinc content in plants (Secilia and Bagyaraj, 1994). According to Solaiman & Hirata (1995) the grain harvested index of wetland rice in the mycorrhizal treatment tended to be higher than in the non mycorrhizal one under flooded conditions whereas under non-flooded condition. Chen *et al.* (2013) the biomass of the paddy root and shoot were significantly enhanced upon the inoculation of AMF. It has been shown that AMF increased the biomass of plant shoots as a result of root surface area increasing.

2.3 Effect of Trichoderma on Paddy Growth

Serna-Cock *et al.* (2011) reported that *Trichoderma spp.* increasing root growth and productivity of plant by creating a symbiotic rather than parasitic relationships. *Trichoderma spp.* also overcome stress condition and improving nutrient absorption in soil. Wan Mohtar *et al.* (2013) reported that many researchers found that ability of plant growth promoting fungi (PGPF) enhanced growth and yield. Four fungal such as *Trichoderma virens, Trichoderma harzianum, and Aspergillus niger* provides effect on germination, root and shoot length, and seedling weight of rice. The previous study shows inoculation of relevant microbes significantly increased the seedling emergence, seedling vigor and root length. According to Wan Yusoff *et al.* (2013) reported that PGPR are the rhizosphere bacteria that can improve plant growth by various mechanisms such as phosphate solubilization, siderophore production, nitrogen fixation and encouraging plant-microbe relationship. Wan Yusoff *et al.* (2013) identified that PGPF is a useful fungi that has capability to boost plant growth. It includes the

production of hormone, mineralization and suppression of harmful microorganism. *Trichoderma virens* and *Trichoderma harzianum* effected on germination, root and shoot length and seedling weight of paddy. According to Al-Taweil *et al.* (2009) application of *Trichoderma viride* to paddy seedling can improve seedlings growth. Khan, Sinha & Rathi (2005) suggested that *Trichoderma harzianum* give better seedling emergence, root and shoot length. The ability of PGPF to enhance growth and yield of paddy has been reported by many researchers. The results suggested that seedling root length, shoot height and fresh weight of rice seedling were significant increased (Mishra and Sinha, 2000). Saravanakumar *et al* (2013) revealed that *Trichoderma spp.* it ability to establish mycorrhizal-like association with plants, control of root and foliar pathogens, change the micro-floral composition in roots, enhance nutrient uptake, enhance root development, increase root hair formation, aid the plant in acquiring systemic resistance, degrade cellulose, solubilize phosphate and produce siderophore.

CHAPTER 3

METHODOLOGY

3.1 Experimental Site

The study was conducted from August to December in the greenhouse, Faculty of Plantation and Agrotechnology, Universiti Teknologi Mara Jasin, Melaka. A completely randomized design with four treatments and eight replications was used for this experiment.

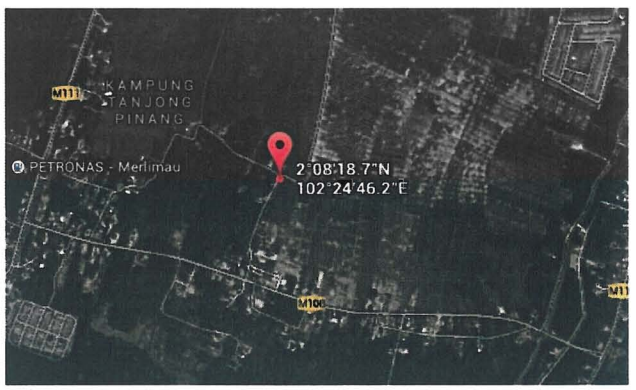

Figure 3.1 The location of experimental soil

3.2 Soil Preparation

Pot experiment was conducted in the greenhouse using paddy field soil as the growth medium. The geographical location of the soil samples is latitude, N 02.13854°, longitude E 102.41283°. Before setting up experiment, four soil samples were collected from experimental soil plot at a depth of 0-15cm. The samples were air dried, ground, and passed through a 2mm sieve (Cong *et al.*, 2009). This soil was used without sterilization in the pot experiment in the greenhouse. Each pot contains 1.5 kilograms of soil (Ferreira *et al.*, 2013). Particle size analysis was carried out by using hydrometer method. The

experiment soil is sandy clay loam and pH 3.6 (distilled water). Soil pH (1:5, soil: water ratio) was measured by using glass electrode (Peech, 1965). Liming was done until pH 6.5. Soil bulk density is 1.12g/cm^3 . Before planting, the soil was ensured to be moist and wet enough for paddy seedling.

3.3 Transplanting of Seedlings

Rice seeds were provided by the Department Of Agriculture. Fifteen-day-old rice seedlings (*Oryzae sativa* MR269) of uniform height (15cm) were selected and transplanted with one seedling per pot. Plants were grown in plastic pots with a diameter of 17cm and a depth of 15cm, and assumed that were competed intra varieties in growing conditions. During the growth period, the plants were maintained under greenhouse condition and watered twice daily. The plants were watered daily with deionized water (Solaiman & Hirata, 1995). Flooding level was maintained at 2cm level from the soil surface and actively aerated by physically disturbing and breaking-up the soil surface once every 10 days (Doni *et al.,* 2014a).

3.4 Fertilizer Application

Plants were grown for 2 months in a greenhouse. Complete fertilization recommended by the Malaysian Agricultural Research and Development Institute (MARDI) was applied to paddy plant. 10 days before transplanting 865.31mg/pot of organic fertilizer were added, and after 15 days, 692.25mg/pot of PADI 1 (N:P:K 17.5 : 15.5 : 10) were applied. 50 days after transplanting, 865.31mg/pot of PADI 1 (N:P:K 17.5 : 15.5 : 10), 173.06mg/pot of urea and 865.31mg/pot of compound (N:P:K 120 : 56 : 80) were applied.

3.5 Inoculum Application

Twenty grams of inoculum containing AMF (*Acaulospora spp., Gigaspora spp., Glomus spp.* and *Scutellospora spp.*) and *Trichoderma spp. (Trichoderma viride* and *Trichoderma harzianum)* were added uniformly as a thin layer in each pot which contained 1.5 kg of soil. A total of two fungus were used for the experiment which include Arbuscular mycorrhizal fungi (AMF), *Trichoderma spp.* respectively. 20gram inocula per plant was applied. 10 days after transplanting, a 20gram of each biofertilizer was inoculated into soil around the rice plant in each pot of inoculated treatment (Chen *et al.*, 2013).

Table 3.1 *The Description of Product Used*

Name Product	**MYCOgold®**	**TRICHOgold®**
Active Ingredient	*Acaulospora spp.* *Gigaspora spp.* *Glomus spp.* *Scutellospora spp.*	*Trichoderma viride* *Trichoderma harzianum*
Concentration	Spore AMF ≥ 250 Spore / 50g	1.0 x 10^7 Colony Forming Unit (CFU) per gram dry weight

3.6 Treatments

A pot experiment was performed with different combinations of biofertilizers treatment. Paddy variety MR269 was used in the experimentation released by Malaysian Agricultural Research and Development Institute (MARDI). The seeds were obtained from The Department of Agriculture Kuala Muda station.

A paddy plant per pot. The experiment consisted of the following treatments. There are:

T1-Inoganic fertilizer only (Control)

T2-Inoganic fertilizer + Arbuscular mycorrhizal fungi (AMF)

T3-Inoganic fertilizer + *Trichoderma spp.*

T4-Inoganic fertilizer + AMF + *Trichoderma spp*

3.7 Experimental Design and Arrangement

The experiment was assigned in completely randomize design (CRD) with 4 treatments and 8 replications. The experimental layout as shown on Figure 3.2.

T1	T3	T3	T4	T2	T3	T3	T4
T2	T4	T1	T3	T2	T3	T1	T4
T3	T2	T4	T2	T3	T4	T2	T2
T4	T1	T2	T1	T4	T1	T1	T1

Figure 3.2 Layout of the Experiment

3.8 Harvesting of the Plants and Analysis

Paddy plants were harvested after 60 days of transplanting through the separating of plants from the soil. The plant was washed through dipping into a vessel. Plant height (cm plant^{-1}), number of tiller and biomass of each plant were recorded. Eight replicates were measured after transplanting. Rice seedlings growth component were measured after 15 days after transplanting (Doni *et al.*, 2014b).

3.9 Collection of Experimental Data

3.9.1 Plant Height

The plant height measured from the base of the plant to the terminal growing point of the main stem without including the length of awn every weeks interval until 60 days after Day After transplanting (DAT) or measured from ground level to the tip of the longest leaf (Doni *et al.,* 2014c). The average plant height was expressed in centimeters. It is generally expressed as cm/day of plant height. Sritarapipat, Rakwatin & Kasetkasem (2014) rice crop height is an important agronomic trait linked to plant type and yield potential. Height for all treatments were taken using a measuring tape by measuring the height of the plant from the base of the soil to the tip of the longest leaf (Jeyanny *et al.*, 2007).

3.9.2 Absolute Growth Rate (AGR)

It is the increasing in height per unit time (cm/day) and was calculated by using the following formula (Dube, 2010).

$$\text{AGR} = \frac{H_2 - H_1}{t_2 - t_1}$$

Where,

H_1 = height of the plant at time t1

H_2 = height of the plant at time t2

t_2-t_1 = Time interval in days

Absolute growth rate (AGR) is the total increase in plant height within a specific time interval. It is commonly stated as cm/day height per plant per day.

3.9.3 Number of Tiller Per Hill

It was measured at the 5th leaf stage begin from the first tiller is developed and appears from the axillary bud. The number of tiller was counted weekly. Most tillers arise between the main stem and leaf. The productivity of the rice plant is greatly dependent on the number of productive tillers (tillers which bears panicle) rather than total tiller numbers (Hasanuzzaman *et al.*, 2010).

3.9.4 Dry Weight of Stem and Leaves

To determine the dry weight of leaves and stem, the samples were air dried for 6 to 8 hours. Leaves and sterns were then packed in a separate brown paper bag and were oven dried for 72 Hours at 85± 5°C. Dry weight of leaves and stems were noted down by using an electrical balance. Dry weight of leaves and stems were altogether regarded as total dry matter. Dry weight of the stem and leaves were taken at the end of the experiment (Hossain, Sarkar and Paul, 2011).

3.9.5 Root Biomass

During the end of the experiment, plant was measured on growth of root. Rice root fresh weight (g) measurements, the rice plants were separated carefully from soil. The rice plants were uprooted gently without causing any damage to the root and shoot systems and washed well with water. Root fresh weight was measured using digital scale. Rice root dry weight (g) measurement was done after rice roots were dried in the oven at a temperature of 60°C for 48 hours, roots and plant part were weighed to measure plant biomass (Chiangmai and Yodmingkhwan, 2011).

3.10 Soil Analysis

Soil samples were collected from each treatment before and after experiment. To maintained uniformity, the soil was taken from 0-15cm from soil surface The soil samples were dried, crushed, and passed through a 2mm sieve. These soil samples were then analyzed (Tallapragada & Seshachala, 2012). The soil analysis done to determine the macronutrinet content such as nitrogen, phosphorus, potassium in soil after applying biofertilizer.

3.10.1 Acid Digestion Method

0.5g oven dried soil was added with 10ml of 1.0 M HCl, 10ml of 1.0 M HNO_3 and 10ml Milli-Q water. The mixture heated over a hot plate at 90°C for two hours to digest the soil. The mixture was cooled and filtered through a Whatman no. 1 filter paper into 50 ml volumetric flasks and made up to the mark with Milli-Q water. Then, the solutions were analyzed by using inductively coupled plasma optical emission spectrometry (ICP-OES) to determine the rate of phosphorus, potassium, zinc and magnesium in soil (Tallapragada & Seshachala, 2012).

3.10.2 Kjeldahl Analysis

Nitrogen in the soil was determined by a Kjeldahl analysis. A working manual for soil analysis refer in soils and analytical services manual number 6. It was done by Division of Agriculture at Ayer Hitam, Ministry of Agriculture and Rural Development Malaysia.

3.11 Statistical Analysis

The experiment was arranged in a completely randomized design (CRD). The data were analysed by one-way analysis of variance (ANOVA). Prior to analysis, data were checked for normality and homogeneity of variances. Significant differences between means were compared using a tukey multiple range test at $p \leq 0.05$. Statistical analysis was performed by using Minitab Statistical Analysis software version 16.

3.12 Schedule of Work

Table 3.2 *Schedule of Study Starting March 2014- December 2014*

Year	2014									
Month	Mar	Apr	May	Jun	Jul	Aug	Sept	Oct	Nov	Dec
ACTIVITIES										
PROPOSAL										
Title										
Introduction										
Literature review										
Research methodology										
Expected output										
Power point presentation										
Submission proposal										
THESIS										
Received material										
Seed sowing										
Data collection										
Data analysis and Discussion										
Report writing										
Presentation										
Submission										

CHAPTER 4

RESULTS

4.1 Plant Height

Table 4.1 *ANOVA Table for Plant Height*

Source	Deg of Freedom	Sum of Square	Mean of Square	F	P
Treatment	3	55.34	18.45	5.61	0.004
Error	28	92.01	3.29		
Total	31	147.35			

The P value for the height parameter was 0.004 which is less than 0.05. There is a significant difference between the treatments towards the height of paddy.

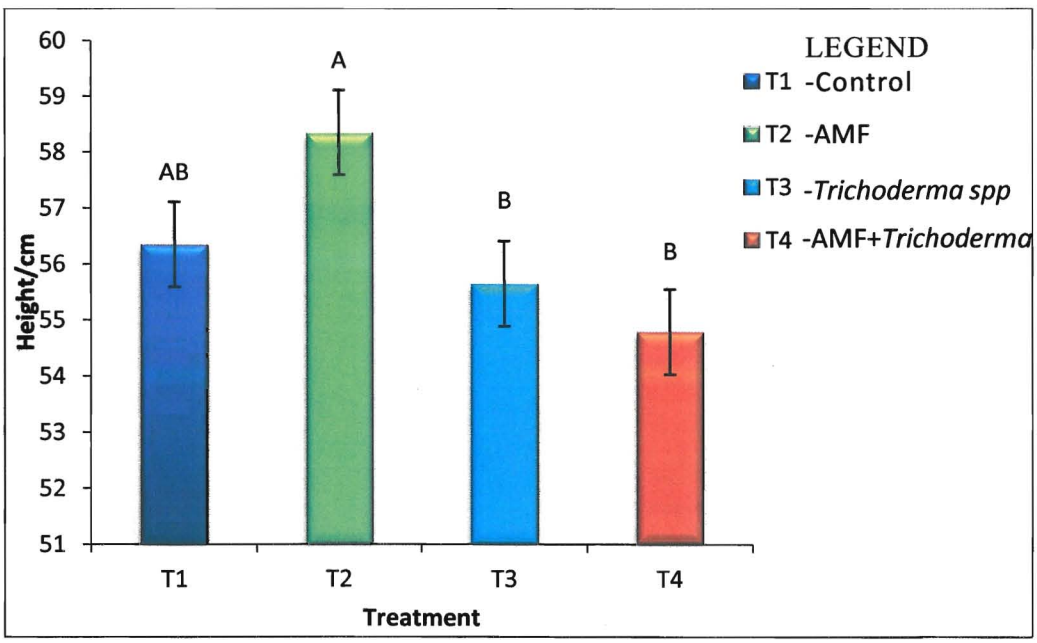

Figure 4.1 The Effect of Treatment on Plant Growth in Height

Treatment 2 shows the highest height of paddy which is 58.3cm. This method shows that treatment 2 has the highest plant height compared to the other

treatment. There is a significant difference between four treatments. However, treatment 2 shows the highest plant height among other treatment. The lowest plant height shows on treatment 4 which is inoculate with AMF and *Trichoderma spp* .

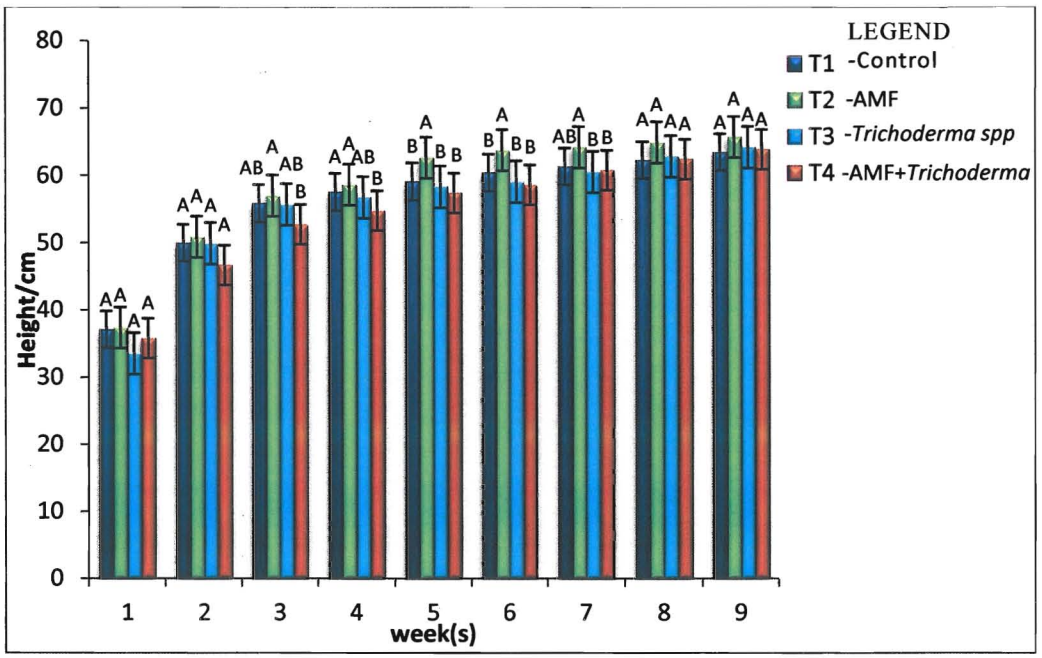

Figure 4.2 Plant Height in Rice Plant by Weeks

The plant height was different significantly at 3rd, 4th and 7th weeks after transplanting. At 3rd weeks after transplanting, the plant height was significantly higher for the AMF application (57.0cm) over without biofertilizers application (55.9 cm). At 7th weeks after transplanting shows significantly higher in paddy height for treatment 2 (AMF) which is (64.2cm) over all other treatments followed by treatment 1(non-inoculated) which is 61.34 cm. Whereas, lower plant height was recorded in inoculated with *Trichoderma spp.* which is 60.53cm.

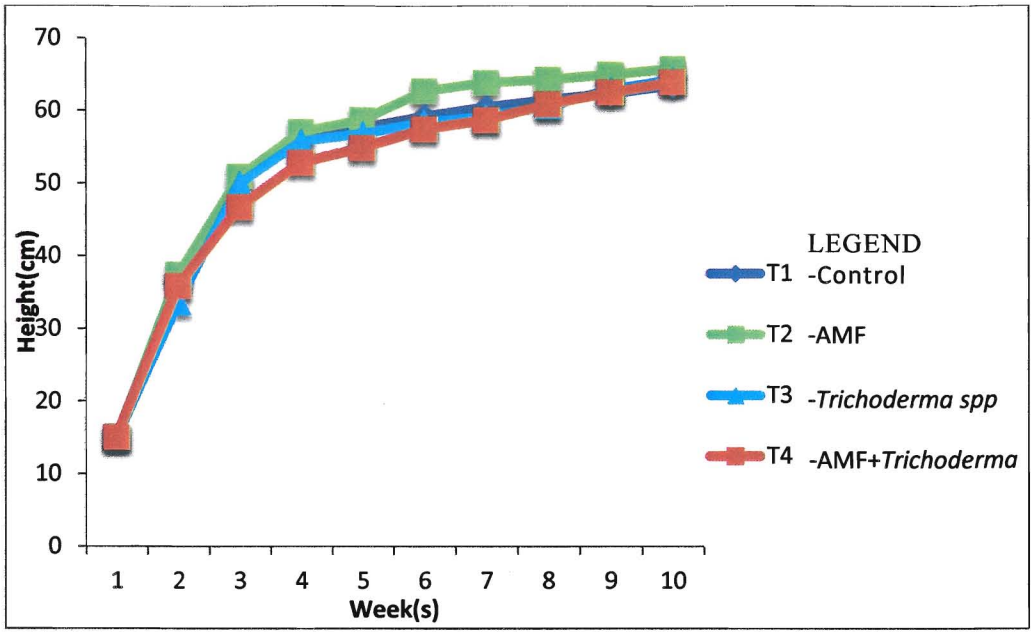

Figure 4.3 Trend of Paddy Height by Weeks

The plant height increased continuously from 1st week after transplanting to 9th weeks after DAT in all the treatments of both with and without biofertilizers. The treatments inoculated with AMF was found to be significantly superior over other treatments in respect of plant height at all the stages.

4.2 Absolute Growth Rate (AGR)

Table 4.2 *ANOVA Table for Absolute Growth Rate*

Source	Deg of Freedom	Sum of Square	Mean of Square	F	P
Treatment	3	55.34	18.45	5.61	0.004
Error	28	92.01	3.29		
Total	31	147.35			

The P value for absolute growth rate was 0.004> 0.05. There is a significant different between treatments towards absolute growth rate.

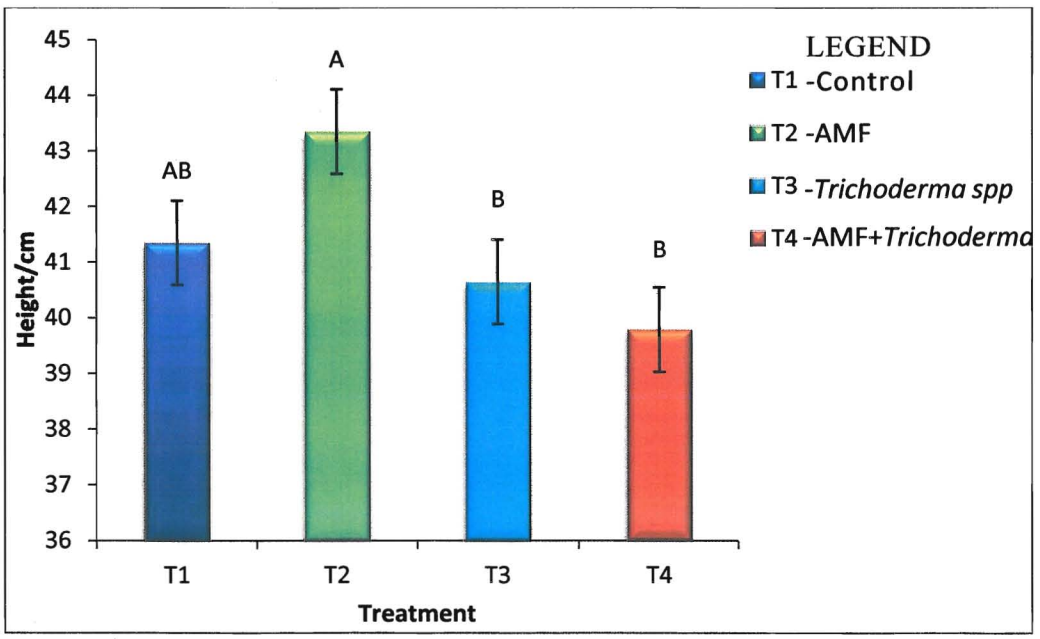

Figure 4.4 The Effect of Treatment on Absolute Growth Rate

There is a significant difference between four treatments. However, treatment 2 shows the highest absolute growth rate among other treatment. The lowest absolute growth rate shows on treatment 4 which is AMF mixed with *Trichoderma spp.*

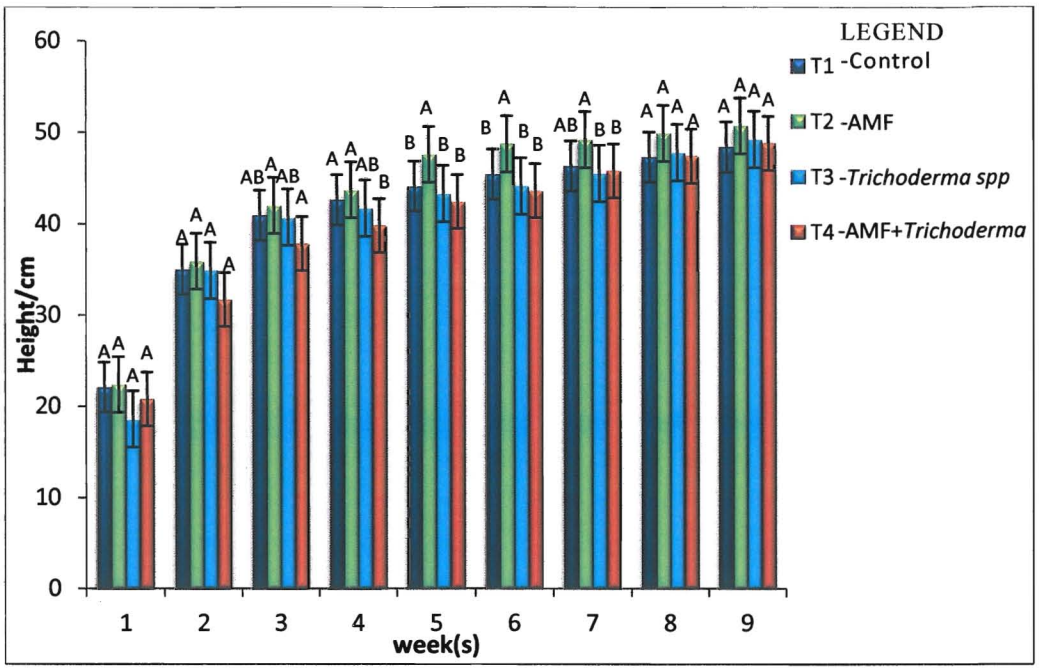

Figure 4.5 The Effect of Treatment on Absolute Growth Rate

Third weeks shows significantly difference between treatment 3 and treatment 4 but not between treatment 1 and treatment 2. For the fifth week, there is significant difference between treatment 1, treatment 2 and treatment 3 but not treatment 4. Seventh week shows significant differences between treatment 4, treatment 1 and treatment 2 but not treatment 3. At the eighth and ninth weeks, there is no significant difference between the treatment.

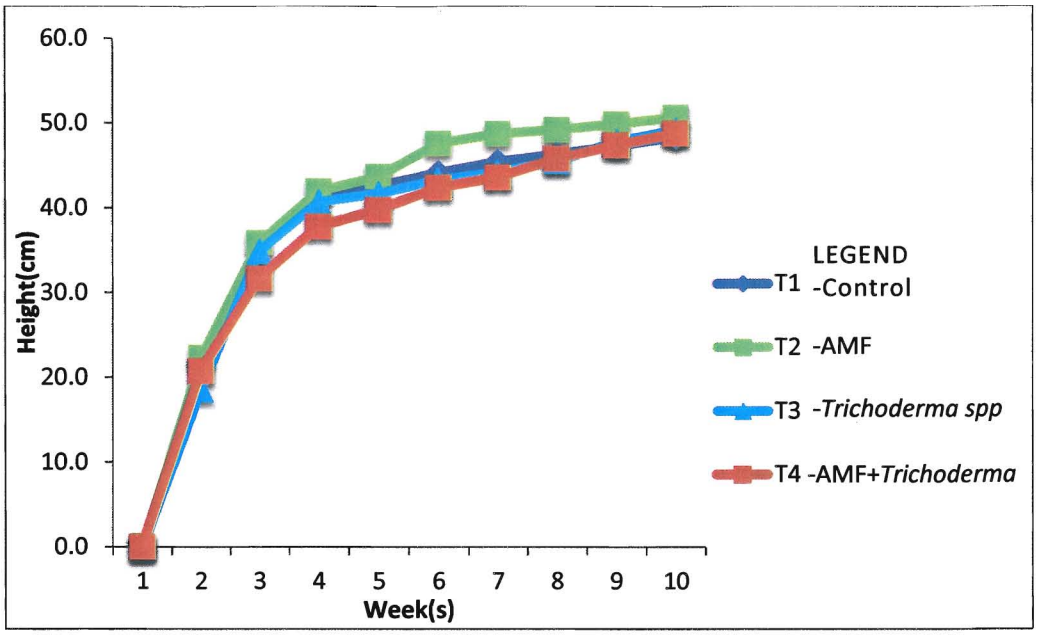

Figure 4.6 Trend of Absolute Growth Rate by Weeks

For the first forth weeks show tremendous development in plant absolute growth rate. However, third week shows slight increase in growth for treatment 2 that inoculated with mycorrhizae. Then, all treatment shows slow in growth.

4.3 Number of Tiller

Table 4.3 *ANOVA Table for Number of Tiller*

Source	Deg of Freedom	Sum of Square	Mean of Square	F	P
Treatment	3	73.42	24.47	6.44	0.002
Error	28	106.38	3.80		
Total	31	179.80			

The P value for number of tiller was 0.002 < 0.05. There is a significant difference between the treatments towards number of tillers produced. By using Tukey's comparison, it shows that the treatments 3 has no significantly difference as compared to other treatment. This method shows that the treatment 2 has the highest number of tillers(25 unit) compared to the rest.

Table 4.4 *Tukey Method and 95% Confidence*

Treatment	Number	Mean	Grouping
T2	8	25.14	A
T1	8	23.96	A
T3	8	23.03	AB
T4	8	21.00	B

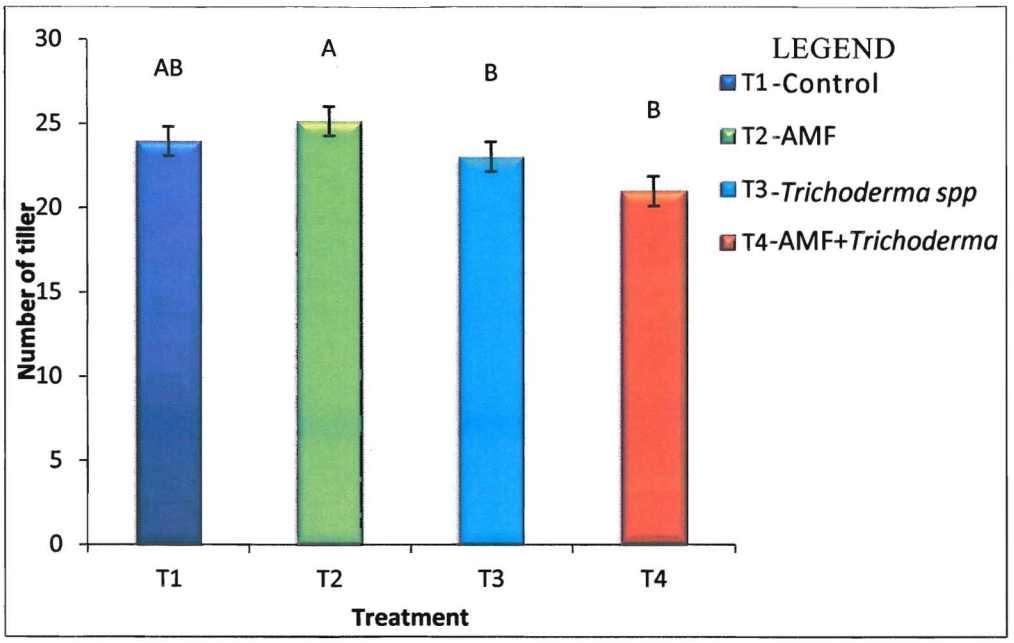

Figure 4.7 The Effect of Treatment on Tiller Production

The number of tillers were significantly higher in Arbuscular Mycorrhizal Fungi application (25 unit) over without biofertilizers application (24unit). Treatment 4 recorded lower number of tiller was 21 tillers.

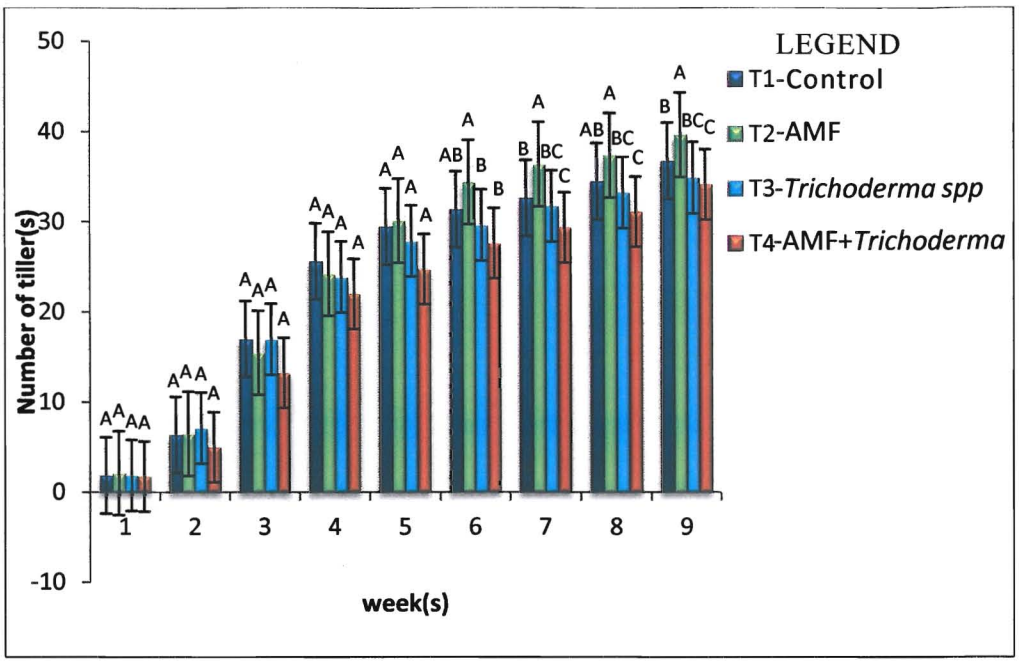

Figure 4.8 Effect of Treatment on Tiller Production by Weeks

The data on number of tillers at different growth stages as influenced by different types of biofertilizers. The number of tiller was differed significantly at 6^{th} , 7^{th} , 8^{th} and 9^{th} weeks after transplanting. The number of tillers increased continuously from 1^{st} week after transplanting to 9^{th} weeks after transplanting in all the treatments of both with and without biofertilizers. Among the treatments with AMF treatment was found to be significantly superior over without biofertilizers treatments in respect of the number of tillers. At last fourth weeks after transplanting, the number of tiller was significantly higher in inoculated with mycorrhizae over treatment 1, treatment 3 and treatment 4. At the 9^{th} weeks, it shows significantly higher tillers number was observed in treatment 2 which used AMF (39unit) over all other treatments followed by treatment 1 (non-inoculated) (36unit). Whereas, lower tiller number was recorded in *Trichoderma spp* mixed with AMF (34unit).

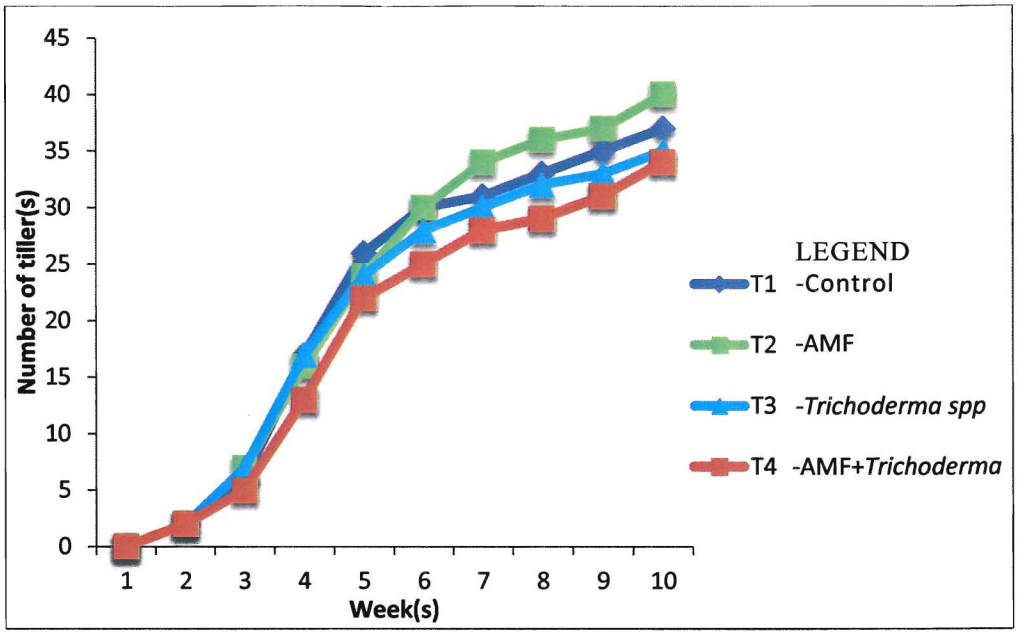

Figure 4.9 Trend of Number of Tiller Production by Weeks

The number of tillers increased continuously from 1st week after transplanting to 9th weeks after DAT in all the treatments of both with and without biofertilizers. The treatments inoculated with AMF was found to be significantly superior over other treatments in respect of tiller number. All treatment shows slightly increased by production of tillers. The treatments inoculated with *Trichoderma spp* mixed with AMF was found to be the lowest over other treatments in respect of tiller number.

4.4 Dry Weight of Stem and Leaves

Table 4.5 *ANOVA Table for Dry Weight of Stem and Leaves*

Source	Deg of Freedom	Sum of Square	Mean of Square	F	P
Treatment	3	44.8	14.9	0.61	0.615
Error	28	687.5	24.6		
Total	31	732.3			

The P value for the dry weight of stem and leaves parameter was 0.615 which is more than 0.05. There is no significant difference between the treatments towards dry weight of stem and leaves of paddy.

Table 4.6 *Tukey Method and 95% Confidence*

Treatment	Number	Mean	Grouping
T1	8	35.13	A
T3	8	33.08	A
T2	8	32.70	A
T4	8	31.93	A

By using Tukey's comparison, it shows that all of the treatments have belonged to the same group which is group A. This method shows that the treatment 1 has the highest dry weight and follow by treatment 3.

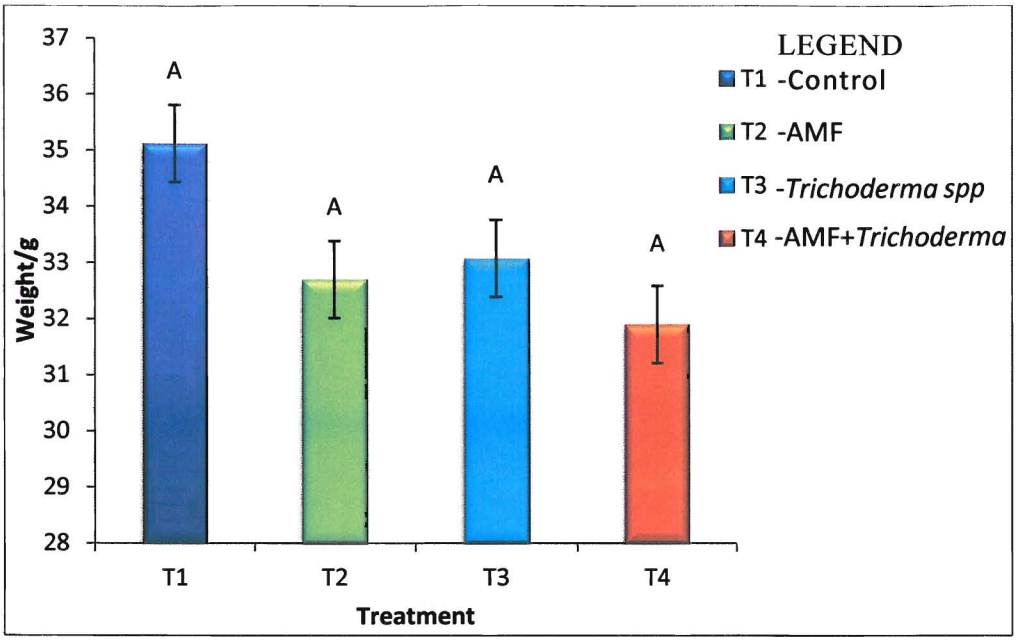

Figure 4.10 The Effect of Treatment on Dry Weight of Stem and Leaves

In this results, the table shows that group A contains all treatment . It indicates that all treatment have no significantly mean. Non inoculated paddy shows the highest dry matter or biomass of stem and leaves which is 35.1gram among other treatments. Treatment 4 which is inoculated with AMF and *Trichoderma spp*. shows the lowest biomass accumulation compared to others

4.5 Root Biomass

Table 4.7 *ANOVA Table for Root Biomass*

Source	Deg of Freedom	Sum of Square	Mean of Square	F	P
Treatment	3	1995	665	1.48	0.242
Error	28	12608	450		
Total	31	14603			

The P value for the height parameter was 0.242 which is more than 0.05. There is no significant difference between the treatments towards root biomass of paddy.

Table 4.8 *Tukey Method and 95% Confidence*

Treatment	Number	Mean	Grouping
T1	8	84.45	A
T3	8	71.38	A
T4	8	70.90	A
T2	8	62.35	A

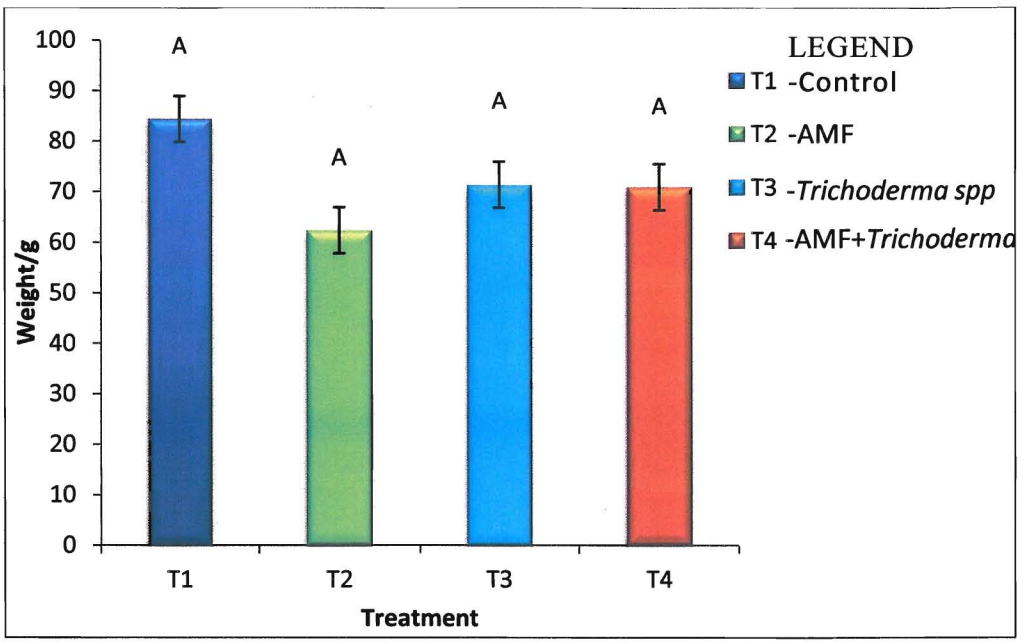

Figure 4.11 The Effect of Treatment on Root Biomass

In this result, it shows that group A contains all treatment . It indicates that all treatment have no significantly mean. Non inoculated paddy shows the highest biomass of root which is 84.45gram among the other treatments. Treatment 2 which is inoculated with AMF shows the lowest biomass accumulation compared to others.

4.6 Total Biomass

Table 4.9 *ANOVA Table for Total Biomass*

Source	Deg of Freedom	Sum of Square	Mean of Square	F	P
Treatment	3	2525	842	1.46	0.246
Error	28	16110	575		
Total	31	18634			

The P value for total dry matter was 0.246 > 0.05. There is no significant difference between the treatments towards plant biomass. By using Tukey's comparison, it shows that all of the treatments have belonged to the same group which is group A. This method shows that the treatment 1 has the highest biomass and follow by treatment 3.

Table 4.10 *Grouping Information Using Tukey Method*

TREATMENT	Number	Mean	Grouping
T1	8	119.58	A
T3	8	104.45	A
T4	8	102.82	A
T2	8	95.05	A

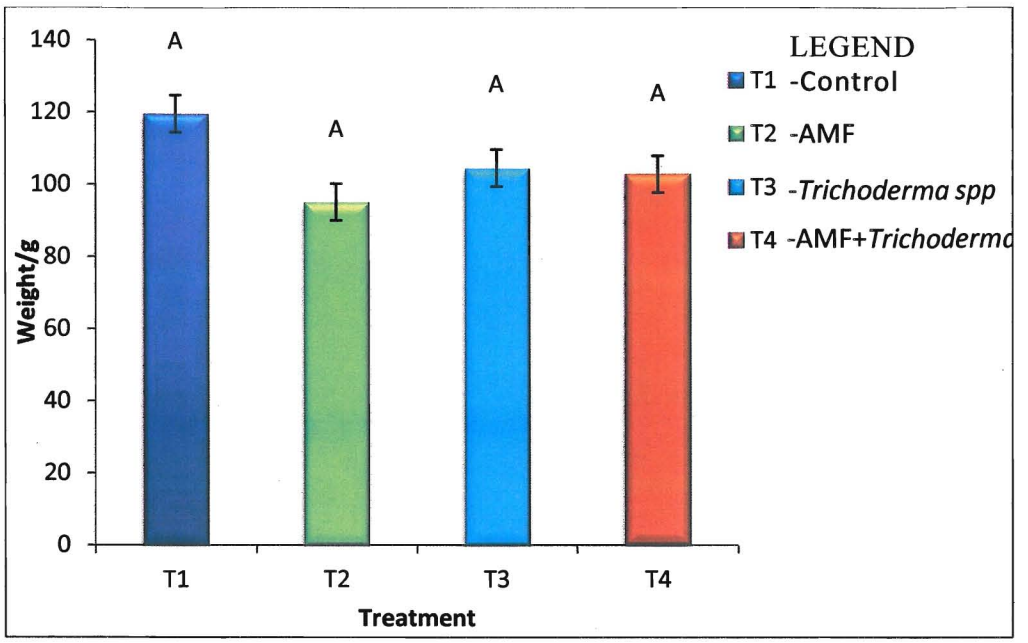

Figure 4.12 The Effects of Treatment on Total Biomass

In this result, the table shows that group A contains all treatment . It indicates that all treatment have no significantly mean. Non inoculated paddy shows the highest total dry matter or biomass, which is 119.58gram among other treatments. Treatment 2 which is inoculated with AMF shows the lowest biomass accumulation compared to others.

4.7 Nutrient Analysis

Table 4.11 *The Effect of Treatment on Level of Nutrient Before and After*

Nutrient	T1	T2	T3	T4
	Different	Different	Different	Different
N (%)	-0.014	-0.040	-0.088	0.065
P (mg/L)	3.189	4.866	0.960	1.169
K (mg/L)	-2.291	-2.246	4.773	4.275
Mg (mg/L)	1.562	7.465	30.108	32.955
Ca (mg/L)	5.713	20.272	11.431	8.655
Zinc (mg/L)	-0.014	0.023	0.029	0.001

The percentage of total nitrogen shows decreasing total nitrogen among the treatment. However , treatment 4 show increasing total nitrogen percentage during 9^{th} week after transplanting. The treatments inoculated with AMF mixed with *Trichoderma spp.* was found to be increased at the end of the experiment. All treatment shows slightly decreased of total nitrogen percentage. The amount of nutrient availability in soil analysis shows an increasing amount of nutrient such as phosphorus, potassium, magnesium, calcium and zinc. However, treatment 4 shows the highest amount of magnesium .

CHAPTER 5

DISCUSSION

The application of beneficial microorganism is encouraged in agriculture because of their potential in improving plant disease resistance and to increase crop production in an environmental friendly way. For the paddy height, by applying AMF only or treatment 2 is significantly different from other treatments. The expected result is reached because mycorrhizae are able to increase nutrient absorption through paddy roots. Thus, the paddy growth development are maximized. Based on previous studies, Smith *et al,* (2003) documented that AMF colonization takes up mainly the phosphorus via mycorrhizal pathway. Chalot *et al,* (2006) revealed that the AM fungal hyphae extend into the rhizosphere and thereby improve the absorption of water and nutrients such as phosphate and nitrogen, which are two of the three major nutrients from the soil through arbuscules.

In addition, with regard to that the calcium level in plant cells responds to AM fungi, a fungal signal molecule was reported to cause transient calcium elevation in plant cells. Inorganic and organic nutrients are uptaken by extraradical hyphae from soil via fungal specific transporters of phosphate (Maldonado-Mendoza *et al.,* 2001) and zinc (Gonzalez-Guerrero *et al.,* 2005). Especially in the case of phosphate, plant roots often create a phosphate-depleted zone close to the root surface, because the level of uptakes is much higher than the rate of diffusion in the soil (Bucher, 2007).

However, it is a great advantage of AM symbiosis that extraradical hyphae elongate beyond the depleted zone to reach a fresh phosphate pool. Then, the

absorbed phosphate and nitrogen nutrients are converted to polyphosphate and arginine respectively, in the extraradical hyphae.

In earlier studies Solaiman and Hirata (1998) performed similar experiments and confirmed that the increases percent of AM fungi colonization on roots paddy increases the phosphorus uptake by plant bodies. Therefore, Smith and Read (2008) revealed that it is true that mycorrhizae, not roots, but it is the chief organs of nutrient uptake by plants. Plants in a symbiotic relationship with AM fungi utilize precipitated inorganic phosphate such as Fe-phosphate, more efficiently than nonmycorrhizal ones. This is due to exploitation of the soil by the fine hyphae of AM fungi.

Based on Smith and Read (1997) the AM fungus-plant association is a mutually beneficial occurrence which are the plant provides the fungus with carbon while fungus assists the plant uptake of phosphate from the soil. Adesemoye and Kloepper (2009) reported that the plant-microbes interaction in enhanced fertilizer-use efficiency.

However, it is not influence on plant biomass accumulation. AMF inoculation ineffective to stimulate shoot dry matter production and root biomass. The earlier study showed, there is no increase in plant biomass. Solaiman and Hirata (1995) also documented that AMF inoculation resulted in the decrease of dry matter production of shoots and roots. They also documented that the amount of shoot (leaves and stem) dry matter at the maturation stage has a tendency to decline due to AMF inoculation, root biomass keep on unchanged because of the carbohydrate translocation from shoots to roots for AMF utilization.

Combination of AMF and *Trichoderma spp.* or Treatment 4 has worst growth performance because synergistic and antagonistic interactions give impact to the establishment of the AM symbiosis. The most important aspect in the life cycle of AM fungi for the establishment of the AM symbiosis are propagule germination, germ tube elongation and contact with the root surface of a mycotrophic plant .

Jansa and Grydler (2010) revealed that during these first events, AM fungi are particularly sensitive to the presence of other microorganisms. Martinez *et al.* (2004) reported that synergistic and antagonistic interactions give impact to the establishment of the AM symbiosis. They also carries out several studies that verified the role of soluble exudate and volatile substances produced by *Trichoderma* species. These substances lead to inhibit the development of the AM fungi presymbiotic phase. De Jaeger *et al.* (2010) reported that mycoparasitic nature of *Trichoderma spp.* represent an important element of the decline in AM fungal spore viability.

The earlier studies by McAllister *et al.* (1994) reported that *Trichodema spp.* was able to reduce the AM root colonization when it inoculated before or at the same time to the AM fungi. The reduction in AM root colonization was not to a direct effect of *Trichoderma harzianum* on the intraradical mycelium but to a harmful effect on the AM presymbiotic development of the fungus. Mycorrhizal fungi contribute to soil structure by growth of external hyphae into the soil to form a skeletal structure that holds soil particles together. Secondly, it increases soil structure formation by external hyphae that are contributed for the formation of micro-aggregates. Thirdly, binding of micro aggregates by external hyphae and roots to create macro aggregates. Fourthly,

the directly tapping carbon resources of the plant to the soils. Mycorrhizal impacts on soil aggregate stability can also influence soil physical condition. Andrade *et al.* (1998) have shown that the root and component of mycorrhizae enhance aggregates stability. Under suitable condition, the spores of AM fungi germinate, and the elongation of the hyphae stops repeatedly if they do not receive any plant signal (Logi *et al.*, 1998).

In contrast, the respiration of hyphae is activated in the presence of compound(s) secreted from plant roots (Tamasloukht *et al.*, 2003). The mature stages of arbuscules typically continue growing for 2-3 days only (Smith and Read, 2008). Then, the arbuscules shrink quickly and the fungus forming many septa in the collapsing branches (Javot *et al.*, 2007).

The whole arbuscule cycle was estimated to be 7-8days (Smith and Read, 2008). Within arbuscules, the polyphosphate granules are hydrolyzed to orthophosphate (Funamoto *et al.*, 2007) and arginine is decomposed to ammonium through the urea cycle (Cruz *et al.*, 2007). Then, phosphate and ammonium are exported into the periarbuscular space. AM fungi totally depend on the host plants for their carbon sources sucrose, which is the most common photosynthate form transported from shoots to AM roots, is hydrolyzed mainly in the periarbuscular space by either sucrose synthase or invertase (Schaarschmidt *et al.*, 2007).

The resulting hexoses mainly glucose are thought to be imported into AM fungi primarily through arbuscules. Then, the hexoses are converted to lipid bodies mainly composed of triacylglycerol or polysaccharides such as glycogen for long distance translocation and storage (Bago *et al.*, 2002). Then,

the lipid and polysaccharides are digested to supply energy and carbon skeletons of organic compound where needed.

For treatment 3 which was used *Trichoderma spp.* give moderate performance. The earlier studies by Kleifeld and Chet (1992) reveal that the growth response of plants caused by *Trichoderma spp.* be influenced by the ability of the fungus to survive and mature in the rhizosphere. A mechanism for increasing plant growth over the stimulation of nutrient from soil to the root, as *Trichoderma spp.* can colonize the roots interior.

CHAPTER 6

CONCLUSION

The present study concludes that AMF significantly enhance rice growth development and improve on soil fertility. In overall , the inoculation of paddy plants with AMF may increase plant height, number of tiller and phosphorus content in soil. However, it gives no effect on plant biomass. AMF inoculation failed to promote shoot dry matter production. Thus, objectives of this study are achieved. The arbuscular mycorrhizal fungi show a positive effect on the vegetative growth of paddy. It has increased nutrient availability in the soil and enhance grow the development of plant height. There is a significant when applied of arbuscular mycorrhizal fungi only rather than mixed with *Trichoderma spp.* When inoculated by using arbuscular mycorrhizal fungi, there is increased production or the number of tillers per hill. This shows that arbuscular mycorrhizal fungi able to increase nutrient absorption through paddy roots. AMF and *Trichoderma spp.* must not be mixed together because it suppresses or inhibit the development of the AM fungi during presymbiotic phase.

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APPENDICES

APPENDIX A

PLANT HEIGHT

Trt	No.	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9
		Height (cm)	Height (cm)	Height (cm)	Height (cm)	Height (cm)	Height (cm)	Height (cm)	Height (cm)	Height (cm)
Trt 1	1	28.50	47.10	55.50	56.50	57.20	58.20	59.10	60.00	61.50
	2	32.00	45.20	52.10	55.10	56.10	59.20	60.20	61.90	63.30
	3	36.50	45.00	50.00	54.00	58.10	59.60	60.20	62.20	64.40
	4	37.10	52.00	58.00	60.00	61.20	62.00	63.40	64.00	64.60
	5	45.20	55.00	59.00	60.00	60.20	61.40	62.20	63.20	65.00
	6	40.50	53.10	56.20	56.80	59.40	60.20	61.40	62.00	62.60
	7	37.20	52.50	55.00	56.00	57.60	58.20	59.20	59.80	60.20
	8	40.00	50.00	61.20	62.00	63.20	64.40	65.00	65.20	65.80
Trt 2	9	41.00	52.10	57.00	59.00	63.20	64.60	65.20	65.80	66.40
	10	39.50	54.00	58.00	59.00	61.80	63.20	64.00	64.60	65.80
	11	33.20	50.20	57.00	58.00	62.20	63.50	64.10	64.60	65.00
	12	31.50	50.60	55.00	57.20	63.60	64.80	65.00	65.60	66.00
	13	39.50	53.50	58.00	59.00	60.20	61.80	62.20	63.40	64.50
	14	38.50	46.50	53.00	57.00	58.40	58.80	59.00	59.80	60.80
	15	39.50	52.10	59.00	60.00	65.40	66.20	67.00	67.60	68.80
	16	36.50	48.20	59.00	60.00	66.30	67.00	67.10	67.80	68.20
Trt 3	17	28.00	48.20	52.00	53.80	54.40	56.40	58.20	65.10	68.10
	18	32.10	49.50	57.00	58.00	59.60	60.00	63.40	65.20	66.20
	19	29.00	50.00	56.00	57.00	58.20	59.00	60.80	62.80	64.50
	20	30.50	48.50	56.50	57.00	58.50	59.10	59.60	60.00	60.80
	21	37.10	53.00	57.00	58.00	59.80	60.00	60.80	62.20	63.00
	22	35.50	46.10	55.00	57.00	58.70	59.00	59.40	60.00	60.50
	23	39.00	51.20	55.00	55.20	58.20	59.20	59.80	62.60	64.50

	24	37.20	53.00	57.00	57.80	59.10	59.80	62.20	64.60	65.80
Trt 4	25	29.00	47.10	50.00	54.00	55.60	60.20	62.40	63.20	64.40
	26	36.50	42.20	54.00	55.20	56.60	57.00	58.80	60.00	61.50
	27	30.50	41.00	47.00	54.00	58.50	59.00	60.20	61.40	62.10
	28	33.50	46.00	54.00	55.00	56.40	57.60	58.80	60.00	61.20
	29	40.50	52.00	56.00	57.00	58.20	59.00	60.20	62.60	63.00
	30	34.50	47.50	52.00	52.00	59.40	60.00	62.80	66.80	69.00
	31	40.10	48.00	55.00	56.00	58.40	59.00	63.80	64.40	66.40
	32	42.00	49.50	54.00	55.00	56.20	57.00	59.40	60.80	63.00

Absolute growth rate

TREATMENT	NO. OF SAMPLE	ABSOLUTE GROWTH RATE(cm)								
		WEEK 1	WEEK 2	WEEK 3	WEEK 4	WEEK 5	WEEK 6	WEEK 7	WEEK 8	WEEK 9
TRT 1	1.0	1.9	2.7	1.2	0.1	0.1	0.1	0.1	0.1	0.2
	2.0	2.4	1.9	1.0	0.4	0.1	0.4	0.1	0.2	0.2
	3.0	3.1	1.2	0.7	0.6	0.6	0.2	0.1	0.3	0.3
	4.0	3.2	2.1	0.9	0.3	0.2	0.1	0.2	0.1	0.1
	5.0	4.3	1.4	0.6	0.1	0.0	0.2	0.1	0.1	0.3
	6.0	3.6	1.8	0.4	0.1	0.4	0.1	0.2	0.1	0.1
	7.0	3.2	2.2	0.4	0.1	0.2	0.1	0.1	0.1	0.1
	8.0	3.6	1.4	1.6	0.1	0.2	0.2	0.1	0.0	0.1
TRT 2	9.0	3.7	1.6	0.7	0.3	0.6	0.2	0.1	0.1	0.1
	10.0	3.5	2.1	0.6	0.1	0.4	0.2	0.1	0.1	0.2
	11.0	2.6	2.4	1.0	0.1	0.6	0.2	0.1	0.1	0.1
	12.0	2.4	2.7	0.6	0.3	0.9	0.2	0.0	0.1	0.1
	13.0	3.5	2.0	0.6	0.1	0.2	0.2	0.1	0.2	0.2
	14.0	3.4	1.1	0.9	0.6	0.2	0.1	0.0	0.1	0.1
	15.0	3.5	1.8	1.0	0.1	0.8	0.1	0.1	0.1	0.2
	16.0	3.1	1.7	1.5	0.1	0.9	0.1	0.0	0.1	0.1
TRT 3	17.0	1.9	2.9	0.5	0.3	0.1	0.3	0.3	1.0	0.4
	18.0	2.4	2.5	1.1	0.1	0.2	0.1	0.5	0.3	0.1
	19.0	2.0	3.0	0.9	0.1	0.2	0.1	0.3	0.3	0.2
	20.0	2.2	2.6	1.1	0.1	0.2	0.1	0.1	0.1	0.1
	21.0	3.2	2.3	0.6	0.1	0.3	0.0	0.1	0.2	0.1
	22.0	2.9	1.5	1.3	0.3	0.2	0.0	0.1	0.1	0.1
	23.0	3.4	1.7	0.5	0.0	0.4	0.1	0.1	0.4	0.3
	24.0	3.2	2.3	0.6	0.1	0.2	0.1	0.3	0.3	0.2

TRT 4	25.0	2.0	2.6	0.4	0.6	0.2	0.7	0.3	0.1	0.2
	26.0	3.1	0.8	1.7	0.2	0.2	0.1	0.3	0.2	0.2
	27.0	2.2	1.5	0.9	1.0	0.6	0.1	0.2	0.2	0.1
	28.0	2.6	1.8	1.1	0.1	0.2	0.2	0.2	0.2	0.2
	29.0	3.6	1.6	0.6	0.1	0.2	0.1	0.2	0.3	0.1
	30.0	2.8	1.9	0.6	0.0	1.1	0.1	0.4	0.6	0.3
	31.0	3.6	1.1	1.0	0.1	0.3	0.1	0.7	0.1	0.3
	32.0	3.9	1.1	0.6	0.1	0.2	0.1	0.3	0.2	0.3

Number of tiller

TREATMENT	NO. OF SAMPLE	NUMBER OF TILLER								
		W1	W2	W3	W4	W5	W6	W7	W8	W9
TRT 1	1	1	3	16	23	24	26	31	33	35
	2	1	3	12	22	24	27	30	33	35
	3	2	7	17	26	31	33	33	34	37
	4	3	8	20	28	33	35	31	33	36
	5	2	9	20	26	31	30	34	35	37
	6	2	7	17	21	25	28	32	34	36
	7	2	8	20	30	34	36	36	38	40
	8	2	6	14	29	34	36	34	36	38
TRT 2	9	2	7	17	26	29	33	36	39	41
	10	2	6	12	33	41	34	39	38	39
	11	2	7	16	20	27	35	37	37	39
	12	2	7	16	24	27	36	36	38	40
	13	3	7	11	21	27	35	35	37	39
	14	1	4	11	21	26	33	35	36	40
	15	2	7	20	25	32	37	37	38	40
	16	3	7	21	24	32	32	36	36	39
TRT 3	17	2	7	14	20	24	30	31	32	34
	18	2	7	16	26	32	29	32	34	35
	19	2	9	17	21	25	29	33	35	36
	20	2	7	19	23	26	30	33	34	35
	21	3	5	15	27	29	30	31	32	34
	22	2	9	16	29	30	31	32	33	34
	23	2	7	20	23	30	29	32	34	36
	24	0	6	19	22	27	29	30	32	35
TRT 4	25	2	6	12	20	25	27	29	30	32
	26	2	3	10	20	23	25	27	28	33

	27	1	3	10	20	24	26	28	30	35
	28	2	7	13	25	27	30	31	34	36
	29	1	4	13	21	22	25	26	28	32
	30	1	4	13	17	18	22	25	27	30
	31	2	4	19	27	30	34	35	36	38
	32	3	9	16	26	29	32	34	36	37

Biomass

Treatment	No. Of Sample	Dried Weight/G		
		Stem And Leaves	Root	Total
TRT 1	1	29.6	69.8	99.4
	2	24.2	94.4	118.6
	3	44.0	94.2	138.2
	4	41.6	127.4	169.0
	5	32.0	49.2	81.2
	6	39.2	109.2	148.4
	7	36.8	66.4	103.2
	8	33.6	65.0	98.6
TRT 2	9	35.2	44.8	80.0
	10	29.8	49.0	78.8
	11	27.6	41.4	69.0
	12	33.6	57.2	90.8
	13	30.6	66.8	97.4
	14	34.0	98.8	132.8
	15	31.0	53.2	84.2
	16	39.8	87.6	127.4
TRT 3	17	33.6	68.8	102.4
	18	37.6	109.6	147.2
	19	34.0	80.6	114.6
	20	35.0	84.4	119.4
	21	30.2	55.2	85.4
	22	38.0	54.0	92.0
	23	26.6	53.4	80.0
	24	29.6	65.0	94.6

TRT 4	25	37.2	107.8	145.0
	26	39.2	64.2	103.4
	27	23.0	64.0	87.0
	28	28.8	71.2	100.0
	29	31.2	58.6	89.8
	30	32.0	50.4	82.4
	31	33.0	80.8	113.8
	32	31.0	70.2	101.2

Nutrient level

	T1		T2		T3		T4	
	Before	**After**	**Before**	**After**	**Before**	**After**	**Before**	**After**
Nitrogen (%)	0.220	0.206	0.240	0.200	0.260	0.172	0.160	0.225
Phosporus (mg/L)	3.049	6.238	3.441	8.307	3.263	4.223	3.058	4.227
Potassium (mg/L)	0.735	-1.556	1.082	-1.164	0.980	5.753	0.869	5.144
Magnesium (mg/L)	2.735	4.297	3.085	10.550	3.172	33.280	2.650	35.605
Calcium (mg/L)	9.522	15.235	7.738	28.010	7.564	18.995	11.720	20.375
Zinc (mg/L)	0.039	0.026	0.042	0.065	0.028	0.057	0.047	0.048

CURRICULUM VITAE

A. Personal Profile

Name	: Izzuddin Bin Ali
Address	: Blok B Sawah Sempadan, 45500 Tanjung Karang, Selangor Darul Ehsan
Ic Number	: 911101-10-5635
Date of Birth	: 01/11/1991
Place of Birth	: Tanjung Karang
Gender	: Male
Citizenship	: Malaysian
Telephone No. (Hp)	: 012-2714009
Email Address	: adeadam1991@gmail.com

B. Hobbies and Interest

Playing swimming and Badminton

C. Academic Qualification

Level	: Bachelor of science(Hons.) Plantation Technology and Management
Name of Institution	: Universiti Teknologi MARA Jasin, Melaka.
Level	: Diploma in Planting Industry Management
Name of Institution	: Universiti Teknologi MARA Arau, Perlis.