GROWTH OF SPINACH (*Spinacia oleracea* L.)
AT DIFFERENT RATE OF SPENT
MUSHROOM COMPOST

ROZITA RONNIE

DISSERTION SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF BACHELOR OF AGRICULTURAL SCIENCE WITH HONOURS

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VERIFIED BY

1. Prof. Dr. Abd Rahman Milan
   SUPERVISOR

PROF. DR. ABD RAHMAN MILAN
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ABSTRACT

Spinacia oleracea L. or well known as spinach are widely grown as food source. Spent mushroom compost (SMC) and peat moss (PM) are used as a raw material for substrate. Eight different substrates were prepared either alone or combination of two materials. Peat moss was used as a control in this study. Each treatment had four treatments. These substrates were mixed with given ratio of peat moss and spent mushroom compost. The result of the study shows that there is a significance difference in plant height, number of branches per plant, diameter of stem per plant, fresh weight, dry weight and leaf area. The result obtained from the experiment in this study indicates that spent mushroom compost can be used as an alternative growing substrate with the best ratio of 1:1 of SMC and PM with NPK amendment. Thus, I recommend SMC: PM+NPK as a best treatment to be used.

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TUMBESARAN BAYAM (Spinacia oleracea L.) TERHADAP PERBEZAAN KADAR KOMPOS HAMPAS CENDAWAN

ABSTRAK

Spinacia oleracea L. atau lebih dikenali sebagai bayam sering ditanam sebagai sumber makanan. Kompos bongkah cendawan (SMC) dan tanah gambut (PM) digunakan sebagai bahan mentah untuk dijadikan substrat. 8 komposisi substrat yang berbeza akan disediakan sama ada bersendirian atau digabungkan. Tanah gambut (PM) adalah sebagai kawalan. Setiap rawatan mempunyai 4 replikasi. Bahan mentah ini dicampur dengan menggunakan nisbah yang tetap. Keputusan kajian ini menyatakan bahawa ada perbezaan ketara pada ketinggian tumbuhan, bilangan dahan pada pokok, ukur lilit pokok, berat kering, berat basah dan luas permukaan daun. Keputusan yang diperolehi melalui penyelidikan ini menyatakan bahawa kompos bongkah cendawan mampu menjadi substrat alternatif dengan ratio 1:1 kepada kompos bongkah cendawan (SMC) dan tanah gambut (PM) dengan penambahan NPK. Justeru itu, saya ingin mengesyorkan PM:SMC + NPK sebagai rawatan terbaik untuk digunakan.

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LIST OF SYMBOLS, UNITS AND ABBREVIATIONS

%	Percentage
ANOVA	Analysis of Variance
cm	Centimeter
cm^2	Centimeter square
CV	Coefficient of Variation
g	Gram
ha	Hectare area
K	Potassium
kg	Kilogram
LSD	Least Significant Difference
mt	Metric tonne
N	Nitrogen
°C	Degree Celcius
P	Phosphorus
PM	Peat Moss
SAS	Statistical Analysis of Software
SMC	Spent Mushroom Compost
WAT	Week After Transplant

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CHAPTER 1

INTRODUCTION

1.1 Background of Study

Spinach (*Spinacia oleracea L.*) is an eatable flowering plant which belongs in the family of Amaranthaceae. Spinach is commonly known as Spinach (English), Chhurika (Sanskrit), Palak (Hindi; Gujarati; and Marathi), Palakh (Kashmiri), Palang (Bangla), Pasalai (Tamil) and Mathubucchali (Telegu). It is categorized as an annual plant which able to grow up to 30 cm. Smaller leaves are found at top on the flowering stem and large leaves are found at the base of the plant (LeStrange *et al.*, 1999). Spinach contain a high nutritional value which is extremely rich in antioxidants, as consumed fresh, steamed or quickly boiled. It is an annual plant having medicinal property native to central and south-western Asia. It is rich with vitamin A (lutein), vitamin C, vitamin E, vitamin K, magnesium, manganese, folate, iron.

Besides high in nutritional value, it also undergo various biological activates such as virus inhibitor, anthelmintic, antioxidant, hepatoprotective and reducing risk of breast cancer. It has been used medicinally in treat of anaemia, night blindness, tooth disorder, urinary disorder, cancer and respiratory disorder. It also been practiced as antioxidant, anti-ageing agent, sun protective, antipyretic agent and anti-inflammatory. In 2005, studies showed that spinach was the most protective nutrition of all against cancer risks (Isoko *et al.*, 2005). It has the strongest effects on cancer cell proliferation. It was found that spinach could help to prevent breast cancers because of its high lutein and other carotenoids (Longnecker *et al.*, 1997).

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Scientific Classification

Kingdom: Plantae

Subkingdom: Tracheobionta

Superdivision: Spermatophyta

Division: Magnoliopsida

Subclass: Caryophyllidae

Order: Caryophyllales

Family: Chenopodiaceae

Genus: Spinacia L.

Species: *Spinacia oleracea L.*

1.2 Significance of the Study

Mushroom cultivation is a lucrative agribusiness. In last three decades, total mushroom production worldwide has increased more than 6-folds, from around 1.2 million metric tons in year 1980 to about 7.3 million metric tons in year 2010 (FAO, 2008). After different flushes of mushrooms have been harvested, there will be a leftover called as spent mushroom compost. They will be abandoned at the end of each production cycle which become the major environmental problems in the mushroom producing countries where every kilogram of mushroom produced followed with 5 kilogram of spent mushroom compost production (Sample *et al.*, 2001). Leftover spent mushroom compost are commonly sent to landfill or openly burnt at mushroom farms and could not be reused in the next cultivation due to the possibility of contamination which will consequently affect the mushroom production.

High demand in Malaysia resulted approximately 8.424 metric tons of mushrooms based on 324g intake of mushroom per person (Laupa, 2008) which generated an approximate value of 42,120 metric tons of spent mushroom compost. Even though spent mushroom compost is a waste but still it contains nutrients which could be used for plant's growth. It's generally non-toxic to plants thus could be used as soil amendment for different crops. Spent mushroom compost also claimed to be the source of humus formation, which responsible to improve soil water holding capacity, soil aeration, provides plant micronutrients and helps maintaining soil structure (Kadiri, 2010). It also been used in horticulture as a component of potting soil mixes, soil amendment to improve grass in wetlands for remediation of contaminated water,

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stabilizing severely disturbed soils, bedding for animals and control plant diseases (Danny, 2002).

Peat moss is the most widely used growing substrate for the pot culture (Rebeiro *et al.*, 2007). It is a non-renewable natural resource and continuously using resulted to diminishing availability and increasing price of peat moss. Due to this issue, finding for viable alternatives to substitute peat as a growth media component is crucial.

Thus, this study being done to see the potential of abandoned spent mushroom compost to be successfully used as medium if prepare in proper proportion indirectly can be a future replacement growth media for peat moss.

1.3 Objectives

Generally, objectives of this study are:

1. To determine the growth of *Spinacia oleracea at* different rate of spent mushroom compost.
2. To determine the suitable ratio for mixture of peat moss and spent mushroom compost on the performance of *Spinacia oleracea* L.

1.4 Hypothesis

H_0: There are no significant differences among the spent mushroom on the growth of spinach.

H_a: There are significant differences in the growth of spinach on different rate of spent mushroom compost.

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CHAPTER 2

LITERATURE REVIEW

2.1 Introductions to Spinach Crop

Spinacia oleracea L. which is commonly known as spinach belonging to family of Amaranthaceae and its native to central and south-western Asia. It has been used medicinally in treating anaemia, night blindness, and tooth disorder. It also used as anti-oxidant, anti-ageing agent, sun protective, anti-pyretic agent and anti-inflammatory (Kiritiker, 2005).

It is cultivated for the sake of it succulent leaves and was introduced in Europe in the 15th century. It is the favourite food among Indians in winter season (Guha, 2008). Spinach is regarded as a valuable dietary source of vitamin A, non heme iron, folate and lutein. It contains a number of antioxidants including carotenoids, polyphenols and flavonoids. Spinach is regarded as a valuable dietary source of vitamin A, non heme iron, filote and lutein (Pool-Zobel, 1997).

2.1.1 Botanical Descriptions

The stem can erect from 30-60 cm high with rounded, smooth, piped, succulent and sometimes with reddish colour. The leaves are alternative where the lower ones appear very long petiole and variously lobed with lobes of an acute triangular shape. It feels smooth on the both side. Spinach are flowering plant which produce both male and female flower. Male flowers exist on long terminal glomerate spikes and on shorter ones from the axial. Present in a very numerous amount and sessile. It has a calyx 4- parted and twin anthers. It usually very large in size.

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Female flowers are come in axillary, sessile and crowded. It has calyx 2-tipped with a projecting horn in each side which grow into spines when the seed is ripe. The styles are generally come in 4 with white tapering. It has a one celled capsule, one valve armed with two opposite short horns and crowned with the small remaining calyx (Kiritikar, 2005).

2.2 Chemical Constituents

2.2.1 Flavonoids

Spinacia oleracea are highly rich in flavonoids. There are various type of flavonoid reported to be present; querecetin, myricetin, kampeferol, apigenin, luteolin, patuletin, spinacetin,jaceidin,4'-glucuronide,5,3',4'-trihydroxy-3-methoxy-6:7-methylenedioxyflavone-4'-glucuronide, 5,4'-dihydroxy-3.3'-dimethoxy-6,7-methylene-dioxyflavone-4'glu-curonide, 5,4'-dihydroxi-3,3'-dimithoxi-6,7-methylene-dioxi-flavone ($C_{18}H_{14}O_8$), 3,5,7,3',4' pentahydroxi-6-methoxiflavone (Sultana, 2008).

2.2.2 Phenolic Compounds

The polyphenols that isolated from the plant are *para*-coumeric acid, ferulic acid, *ortho*-coumaric acid (Andjelkovic, 2008).

2.2.3 Carotenoids

Spinach contain different type of carotenoids such as lutein, beta-carotene, violaxanthin and 9'-(Z)-neoxanthin (Guha, 2008).

2.2.4 Vitamins

Spinach contains very high concentration of vitamin A, E, C and K along with folic acid and oxalic acid (U.S. Department of Agriculture, 2005).

2.2.5 Minerals

Some minerals also present with the various chemicals, they are; magnesium, manganese, calcium, phosphorus, iron, zinc, copper and potash (Anonymus, 2004).

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2.3 Pharmacological Activities

2.3.1 Antioxidant Activity

Grossman (2001) reported the chemical fraction of natural antioxidant (NAO) components in *Spinacia oleracea.* A study was demonstrated by using the extract of spinach leaves and for the first time the aqueous extract of spinach leaves are positive in both flavonoids and p-coumaric acid derivatives as antioxidant components.

2.3.2 Protection against Gamma Radiation

The radioprotective efficacy of spinach against radiation induced oxidative stress was studied by Verma and Bhatia (2003) where some of the Swiss albino male mice treated with *Spinacia oleracea* leaves alcoholic extract (SE) once daily for 15 days. Animals then exposed to single dose of gamma radiation which then being sacrificed and brain was removed to estimate LPO. The indication of possible role of *Spinacia oleracea* as radio protector to some extent if taken continuously will be shown in LPO value of brain in the study.

2.3.3 Anticancer Activity

This study called as spinach ethanol extract (SE) are used to investigate the inhibition of calf, DNA polymerase (pol). The activity of pol alpha with IC5o will be inhibited by the spinach glycoglycerolipid fraction dose dependent while the fat soluble fraction slightly inhibited the activity of pol alpha, even the water soluble fraction does not show any effect. This concluded that the spinach glycoglycerolipid fraction can inhibit mammalian pol activity, human cultured cancer cell growth and in vivo solid tumour proliferation with oral administration. Thus, this can help to prevent cancer and be a functional food for anticancer activity (Maeda, 2008).

2.3.4 Inhibition of Mammalian DNA polymerase

The major glycolipids being purified from the green vegetable spinach (*Spinacia oleracea* L.) and classified into the class of monogalactosyldiacylglycerol (MGDG), digalactosyldiacylglycerol (DGDG) and sulfoquinovosyldiacylglycerol (SQDG). It was

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reported that, MGDG can inhibit the growth of NUGC-3 human gastric cancer cell, but no cytoxic effect for both DGDG and SQDG. MGMG was found to prevent the cancer cell growth. It can inhibit the activities of the all mammalian DNA polymerases including repair-related activity (Mizushina, 2003).

2.3.5 Sulphite Oxidase Activity

Sulphite oxidase activity where the oxygen consumption and reduction of ferricyanide are occur which being possessed by the spinach chloroplasts. The isolation activity was insensitive towards radical scavengers and catalase, and was inhibited only with very high concentrations of superoxide dismutase. The kinetic parameters of thylakoid sulphite oxidase were measured and compared with those of others sulphite oxidases (Jolivet, 1995).

2.3.6 Hepatroprotective Activity

The amelioration of spinach leaves alcoholic extract (SE) against the hepatosuppression induced by carbon tetrachloride (CC14) were reported by Gupta and Singh 2006. The LPO was monitored in both serum and liver. Spinach has the potential of hepaprotective against hepatosuppression possibly involves mechanism which related to the ability to block the P-450 mediated CC14 bioactivation through selective inhibitors of ROS (reactive oxygen species). This shows that, spinach able to protect the liver and may prove as a rich source of antioxidants (Gupta, 2006).

2.3.7 CNS Depressant Effect

Result of decreasing in the locomotor activity, grip strength, increasing of pentobarbitone induced sleeping time could be happened after being treated with *Spinacia oleracea* extract (SO). SO responsible in increasing the serotonin level and reduce both norepinephrine and domanine levels in the cerebral cortex, cerebellum, caudate nucleus, midbrain and pons and medulla. The CNS depressive effect in PTZ of the SO are seizure by modulating the monoamines in different brain areas (Guha, 2008).

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2.4 Spinach Market Price in Malaysia

In Malaysia, spinach also become one of vegetables being consumed by the community. Below are the market price of spinach in Malaysia. The range price of spinach per 100 kilogram are from RM70 – RM500. The maximum price is RM500 per 100kg which centred in Sibu, Sarawak. The minimum price is RM70 per 100kg in Johor Bahru, Johor.

Table 2.4 Spinach market price in Malaysia

Centre	Grade	Unit/kg	Price/RM		
			Maximum	Average	Minimum
Sibu, Sarawak	F.A.Q	100	500	500	500
Kota Setar, Kedah	F.A.Q	100	250	200	150
Kinta, Perak	F.A.Q	100	200	200	200
Perlis, Perlis	F.A.Q	100	200	175	150
Kuching, Sarawak	F.A.Q	100	200	175	100
Kota Kinabalu, Sabah	F.A.Q	100	160	140	120
Seberang Perai Tengah, Pulau Pinang	F.A.Q	100	150	150	150
Melaka Tengah, Melaka	F.A.Q	100	130	120	110
Kota Bahru, Kelantan	F.A.Q	100	120	115	110
Miri, Sarawak	F.A.Q	100	100	100	100
Johor Bahru, Johor	F.A.Q	100	100	85	70

Report of Average Price for Spinach at Farm Level on Tuesday, 10th November 2015
Source: FAMA, 2015

2.5 Planting Area and Production of Spinach in Malaysia

Malaysia also have some planting area for the production of spinach. Table below shows the type of vegetables being planted with the planting area and production. The planting area for spinach in 2011 until 2013 increasing from 4,098 ha to 4,377 ha while the production reducing from 45,136 mt to 40,535 mt.

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Table 2.5 Planting Area and Production for Primary Vegetables According to Type (2011-2013)

Type of Vegetables	2011		2012		2013	
	Planting Area (ha)	Production (mt)	Planting Area (ha)	Production (mt)	Planting Area (ha)	Production (mt)
Spinach	4,098	45,136	4,355	40,333	4,377	40,535
Lady Finger	2,350	28,871	2,318	26,530	2,330	26,663
Chilli	2,597	28,766	3,001	29,834	3,016	29,983
Sweet Leaves	366	4,810	355	4,528	356	4,551
Leek	401	5,951	452	5,516	455	5,543
String bean	551	6,704	1,003	8,682	1,008	8,726
Long bean	3,663	42,623	3,607	39,802	3,625	40,001
Chinese kale	1,185	14,327	1,200	13,683	1,206	13,751
Water Spinach	6,015	74,063	6,454	59,173	6,486	59,469
Round cabbage	5,655	179,323	4,966	145,001	4,991	145,726
Cabbage flower	593	3,270	340	3,216	342	3,232

Agricultural Statistics (Agricultural Food Sub-Sector)
Source: Unit Perangkaan Bahagian Perancangan, 2013

2.6 Introduction to Spent Mushroom Compost

According to Daba (2006), mushroom have created interest in man early civilization. The Romans identified mushrooms as "food of the gods" and the Greeks believed that mushroom provided strength for the warriors in battles. The Pharaohs of ancient Egypt decreed that no commoner could ever touch them. The unique texture and flavour of mushroom has never been found in other food items and it has been used for medicinally purpose for about hundreds of year mainly in Latin America, Africa, Asia, Australia and

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Papua New Guinea (Kadiri *et al.*, 2003; Kadiri, 2005; Kadiri and Adegboye, 2006; Kadiri *et al.*, 2007).

Nowadays, many species of mushroom are cultivated world-wide. According to Chang (1999), global production increased to about 6.2 million tonnes in 1997, with more than 12% increase annually from 1981 to 1997. Chang also stated that seventy percent of the global mushroom production is derived from three mushroom groups, *Agaricus bisporus*, *Pleurotus* spp, and *Lentinula edodes*. Natural materials from agriculture, woodlands, animal husbandry and manufacturing industry are used to produce mushroom. Millions of tonnes of "spent" (used) mushroom compost are produced after mushroom crops are harvested.

After harvesting mushroom fruit bodies, there will be some substrate left behind known as spent compost or spent mushroom substrate (SMS) or spent mushroom compost (SMC). Spent mushroom compost contain lignocellulosic materials, hemicellulose, lignin, mushroom mycellal and products liberated into the compost by metabolic activities of the mycelia. It has become an effective soil manure or conditioner if added to agricultural or garden soil which considerably found to increase the yield of some vegetable crops.

Spent mushroom compost (SMC) when added to soils can have a number of beneficial effects. SMC contains valuable plant nutrients and organic matter that can improve soil fertility. It can supply plant nutrients and organic matter that can improve soil fertility. It can supply plant nutrients to the crop and thus replace inorganic fertilizer. Trials have shown that it is an excellent source of phosphorus, potassium and trace elements but needs supplementation with nitrogen for best results. Plant nutrient value of the SMC is examined by most of the researchers and it is indicated that the nutrient combination is similar to soil arrangements, based on the organic wastes, applied to the agricultural areas routinely such as cattle manure and compost (Stewart *et al.*, 1998; Kutuk *et al.*, 1998).

The phytonutritive capacity of compost has often been demonstrated to be analogous to that of manure; the same level of productivity, both quantitatively and qualitatively, can be maintained by replacing manure with the compost (Beyca *et al.*, 1993). However, SMC often contains high salt levels remaining from the fertilizer

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materials applied during mushroom cultivation. High or excessive soluble salts may be injurious to plants (Anonymous, 1994).

According to Chang and Yau (1981) and Iwase *et al.* (2000), spent mushroom compost of *Volvariella volvacea* on addition to soil has increased the yield of tomatoes 7 fold and the yields of soybean, lettuce and radish 2 fold each.

The yield production of cabbage, cauliflower, beans and celery are greater if applied with *Agaricus* spent compost to soil compared to addition of poultry manure to soil. Spent mushroom compost strongly believed to provide humus formation and humus which known as a source of micronutrients for plant's growth, improve soil aeration, soil-water holding capacity and help the maintenance of soil structure.

2.7 Introduction to Peat Moss

Wet condition with very high water table near to the soil surface are the natural state of peat soils itself. They have a soft texture as they are organic matter which made up of mainly rotting plant materials in nature. Thus, peat soils also known as organic soils. The bulk density of peat soils is also low as the other organic matter. Since peat soils has a high water table and soft thus it makes them soggy and a little bit difficult to walk on an undrained peat soils without sinking down.

In Malaysia, the area of virgin lowland peat are often flooded and swampy. As for this reason, most of the peat areas are marked as swamp areas. According to Paramanathan (2008) and Yew *et al.* (2010), the tropical peat has not been well studied. The estimation of CO_2 emissions are often extrapolated based on data from temperate peat. Thus, it is often doubted that this can be done since the factors of peat formation in temperate areas are different from that in tropical areas and the peat will be different morpho-genetically.

According to Wust (2003), temperate peat deposits are also known to be derived from bryophytes and small shrubs while tropical peat deposits are derived from various tree species with root penetration up to several meters. Veloo *et al.* (2014) obtained new information from the soil survey and soil classification research which carried put in Sarawak, Malaysia is confirming that tropical peat soils are indeed different from

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temperate peat soils with many of the tropical peat soils containing wood materials in the soil solum.

Just like temperate peat materials, tropical peat material can be differentiated based on the stage of decomposition. There are 3 stages of decomposition, namely sapric, hemic and fibric. Sapric is a peat which undergo very high stage of decomposition, hemic which undergo moderate stage of decomposition and fibric with the low stage of decomposition.

The presence of wood in tropical peats are the difference from temperate peat. As information, tropical woods are come from the range of hard, medium to soft. Thus, the process of breaking down or decaying rate are vary depending on the types of wood present. As for example, hard wood is very recalcitrant thus need a longer time to break down and emit CO_2 during the process.

According to Ongkili (2005), South East Asia region, Malaysia, is second to Indonesia in the abundance of peat ground. It has a total of 2.13 million hectares of peat lands in the states of Selangor, Johor, Perak, Pahang, Sabah and Sarawak. Sarawak is the largest area of more than 1.5 million hectares.

Peat is present in a colour of brownish-black with its natural state is composed of 90% water and 10% solid material. Peat formed when a partially decomposed organic matter accumulates over thousands of years due to the lack of oxygen under waterlogged conditions. It also defined as containing at least 65% organic matter (Soper *et al.,* 1922; Radforth, 1969; Babel, 1975; Stanek *et al.,* 1983; Moore, 19889; Van der Heijden *et al.,* 1994). The behaviour of peat found in different geographical areas are differ from one another due to the type and origin of the organic matter which emphasizing the need of careful geothechnical characterisation (Huat, 2004; Edil, 2003; Den Haan, 1997; Jarret, 1995; Landva, 1980).

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CHAPTER 3

METHODOLOGY

3.1 Site of Study

This study was conducted inside Rain Shelter No.8B at Faculty of Sustainable Agriculture, Sandakan Campus, Universiti Malaysia Sabah which located at Mile 10 with latitude of $5^0 55'$ N and longitude of $118^0 02'$ E. Temperature range in the main shelter was between 25^0C to 33^0C. Further analysis was carried out in PTA LAB 1.

3.2 Experimental Period

The study was conducted for 3 months. It started from August 2015 until November 2015.

3.3 Materials and Apparatus

3.3.1 Plant Material

Spinach (*Spinacia oleracea* L.) was the test crop in this study. The variety used was hybrid H153 green spinach which can matures within 40 days. The seeds were obtained from Hien Huat Seeds (Sabah) Sdn. Bhd, Malaysia.

3.3.2 Fertilizer

Chemical fertilizer NPK Green (15:15:15) was used in this study.

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