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BORANG PENGESAHAN STATUS TESIS

JUDUL : **EFFECT OF ENHANCING BIOFERTILIZER CONTAINING K SOLUBILIZER ON PATCHOULI GROWTH**

SESI PENGAJIAN: **2008/2009**

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EFFECT OF ENHANCING BIOFERTILIZER CONTAINING K SOLUBILIZER ON PATCHOULI GROWTH

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A desertation submitted in partial fulfillment
of the requirements for the award of the degree of
Bachelor of Chemical Engineering (Biotechnology)

Faculty of Chemical & Natural Resources Engineering
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APRIL 2009

I declare that this dissertation entitled "Effect of Enhancing Biofertilizer Containing K Solubilizer on Patchouli Growth" is the result of my own research except as cited in references. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree."

Signature :.....

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Date : 30 April 2009

Special Dedication to my family members,
DayangRafikaAtiqah, Block I members, my friends, my fellow colleague
and all faculty members

For all your care, support and believe in me.

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ABSTRACT

Biofertilizers are products containing living cells of different types of microorganisms, which have an ability to convert nutritionally important elements from unavailable to available form through biological processes. However, the application of biofertilizer in practice, somehow, has not achieved constant effects on the plants growth. The objectives of this paper are to study the effect of biofertilizer enhance with K solubilizer inoculums on plants growth and making a comparison on physical appearances of patchouli growth between modified biofertilizer, biofertilizer, organic fertilizer and chemical fertilizer. In order to achieve the objectives, this study is devided into two part which are the K solubilizer bacteria cultivation and pots experiment. The cultivation of K solubilizer bacteria was carried out on 72 hour of fermentation at 28°C and at the speed of 200 rpm. The K solubilizer bacteria growth curve and their glucose consumption were determined at 3 hour interval for the first 24 hour and 6 hour interval for the next 48 hour. The result showed that K solubilizer bacteria is in active phase in its first 40 hour of cultivation. The pots experiment were carried out by eight treatments with duplication. The modifications of biofertilizer were coded as BF5 for 5% of inoculums, BF10 for 10% of inoculums, BF15 for 15% of inoculums and BF20 for 20% of inoculums. Another four treatments were set up as comparison including chemical fertilizer, sterilized organic fertilizer, biofertilizer and no fertilizer (control). Data were taken after 6 weeks of seedling. The result showed that after 6 weeks of seedling, the enhanced biofertilizer are capable of showing outcome as best as chemical fertilizer in terms of physical appearences and potassium content in the soil. Therefore, the application of the enhance biofertilizer containing beneficial microbes showed a promoting effect on the growth of plant and improvement of soil properties.

ABSTRAK

Baja bio-organik merupakan sejenis produk baja yang mengandungi pelbagai jenis mikoorganisma yang berupaya untuk mengubah elemen nutrisi penting melalui proses biologi. Namun begitu, penggunaan baja bio-organik dalam bidang pertanian tidak memberi kesan yang konsisten kepada tanaman. Objektif kajian ini adalah untuk mengkaji kesan baja bio-organik yang telah ditambahbaik dengan inokulum K solubilizer pada pertumbuhan tanaman dan untuk membuat perbandingan secara fizikal pokok nilam di antara baja bio-organik yang telah diubahsuai, baja bio-organik, baja organik dan baja kimia. Untuk mencapai objektif tersebut, gerak kerja kajian ini telah dibahagikan kepada dua bahagian iaitu pengkulturan bakteria K solubilizer dan eksperimen pasu. Pengkulturan bakteria K solubilizer telah dijalankan selama 72 jam pada suhu 28°C dan kelajuan 200 rpm. Profil pertumbuhan K solubilizer dan kadar penggunaan glukosa dianalisa setiap 3 jam untuk 24 jam pertama dan setiap 6 jam pada 48 jam yang seterusnya. Hasil dapatan menunjukkan bakteria K solubilizer berada dalam fasa aktif pada 40 jam pertama pengkulturan. Eksperimen pasu dijalankan dengan menggunakan lapan jenis rawatan di mana setiap satu pasu diduplikasikan. Pengubahsuaian pada baja bio-organik telah dilabel sebagai BF5 untuk inokulum sebanyak 5% , BF10 untuk inokulum sebanyak 10% , BF15 untuk inokulum sebanyak 15% dan BF20 untuk inokulum sebanyak 20% bagi empat rawatan yang pertama. Manakala baki empat rawatan lagi adalah termasuk baja kimia, baja organik yang telah disanitasi, baja bio-organik dan tiada baja (kawalan). Data diambil selepas 6 minggu penanaman. Hasil pemerhatian menunjukkan, selepas 6 minggu penanaman, baja bio-organik yang telah ditambahbaik berupaya memberi hasil yang sejajar dengan baja kimia dari segi rupa fizikal dan kandungan kalium di dalam tanah. Justeru itu, aplikasi baja bio-organik yang telah diubahsuai dan mengandungi mikroorganisma yang berguna menunjukkan kesan positif pada pertumbuhan tanaman dan penambahbaikan sistem nutrisi tanah.

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LIST OF SYMBOLS/ABBREVIATIONS

DNS	-	Di-Nitro Salicylic Acid
Glu.	-	Glucose
KSB	-	K solubilizer bacteria
g	-	gram
h	-	hour
ppm	-	part per million
mL	-	mililiter
rpm	-	radius per minutes
v/v	-	volume per volume
%	-	percentage
°C	-	degree Celsius

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CHAPTER 1

INTRODUCTION

1.1 Background of study

Since the beginning of the "Green Revolution" in the early 1970's, which focused on food crop productivity, through high-yielding varieties, agrochemicals and irrigation system, chemical fertilizers were extensively used throughout most of agricultural Asia. In fact, Asia is the world's largest user of chemical fertilizers, consuming around 40% of the global total each year. The emphasis on chemical fertilizers, which sometimes led to injudicious application, has meant that the soil be regarded as an inert substrate for plant roots, instead of a living biosphere, the rhizosphere, containing a myriad of organisms. It is now realized that in agricultural lands under intensive monoculture system, including paddy rice, which receive heavy applications of chemical fertilizers alone, productivity is slowly declining, and environmental quality is deteriorating too. In the light of these problems, the use of organic fertilizers, biofertilizers and other microbial products is crucial in the current attempt to make the agriculture industry a viable component of a healthy and pleasant ecosystem (www.fnca.mext.go.jp).

A healthy plant usually has a healthy rhizosphere which should be dominated by beneficial microbes (Chen, 2008). Rhizosphere of the plant is clearly depicted in Figure 1.1. Conversely in unhealthy soil, it is dominated by pathogenic microbes that can obstruct optimum plant growth.

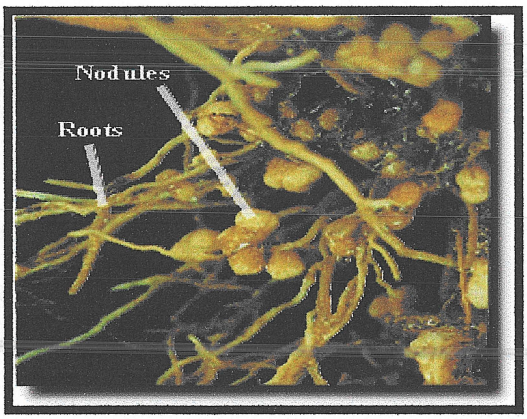


Figure 1.1: Rhizosphere of plant (www.wikipedia.com)

There are plentiful of microorganisms thriving in soil (Wu *et al.*, 2003). It is well known that a considerable number of bacterial and fungal species possess a functional relationship and constitute a holistic system with plants. They are able to exert beneficial effects on plant growth (Vessey, 2003; Wu *et al.*, 2003). Application of beneficial microbes in agricultural practices started 60 years ago and now there is increasing evidence that these beneficial microbial populations can also enhance plant resistance to adverse environmental stresses such as water and nutrient deficiency and heavy metal contamination (Shen, 1997; Wu *et al.*, 2003). Moreover, the implementation of beneficial microbes to the crop is the best answer to void the excess application of chemical fertilizer to crop land that can causes ecological problems such as pollution. Therefore, biofertilizer has been identified as an alternative source rather than using chemical fertilizer to raise soil fertility and yield production in sustainable farming.

Nowadays, biofertilizers are considered the most advanced biotechnology that can increase the production, improve the quality and developing an organic, green and non-poluted agriculture. In addition, biofertilizers contain a variety of beneficial microorganisms and enzymes which accelerate and improve plant growth and protect plants from pests and diseases. Completely fermented organic matters resulted in biofertilizers which improve the physical properties of soils, enrich air aeration, water and nutrient retention capacity. Biofertilizers provide the cultivated plants with the macro as well as micronutrients, required for healthy growth, therefore, improve yield and quality of agricultural crops, and reduce the overall cost of chemical fertilizers as pesticide application.

1.2 Problem Statement

Biofertilizers are products containing living cells of different types of microorganisms, which have an ability to convert nutritionally important elements from unavailable to available form through biological processes (Hegde *et al.*, 1999; Vessey, 2003; Wu *et al.*, 2003) In recent years, biofertilizers have emerged as an important component of the integrated nutrient supply system and hold a great promise to improve crop yields through environmentally better nutrient supplies (Wu *et al.*, 2003). However, farmers are prefer to use chemical fertilizer rather than biofertilizer because of the efficiency of biofertilizer is depends on the components available in raw materials as well as contribution from living microorganisms in them (Hasarin and Viyada, 2008). Moreover, their performance also depends on whether the environments they are introduced to are conducive. Therefore, the application of biofertilizer in practice, somehow, has not achieved constant effects. The mechanisms and interactions among these microbes still are not well understood, especially in real applications.

1.3 Objectives

Objectives of this study were highlighted such as follow:

i. To study the effect of biofertilizer enhance with K solubilizer inoculums on plants growth
ii. To make a comparison on physical appearances of patchouli growth between modified biofertilizer, biofertilizer, organic fertilizer and chemical fertilizer.

1.4 Scopes of Study

This study consists of several scopes of studies which are the cultivation of 5%,10%,15% and 20% K solubilizer bacteria (KSB) inoculum onto nutrient broth. Then is the mixing of K solubilizer bacteria cultivated with peat moss and biofertilizer for 6 weeks pot experiment. Finally is the observation on seedling height, leaf diameter and leaves colour as well as determining the potassium content in soil at the end of pot experiment

CHAPTER 2

LITERATURE REVIEW

2.1 Biofertilizer

Biofertilizer is a fertilizer that is 100% environmental friendly compared to conventional fertilizer. Therefore, the use of biofertilizer can help to provide and keep in all the nutrients and microorganisms in the soil required for the benefits of the plants. Biofertilizer are the source of microbial inoculants, which have brought hopes for many countries both economically and environmentally. Therefore, in developing countries like Malaysia, biofertilizer can solve problems of high cost of fertilizers and thus can save the economy of the country. Biofertilizers, in strict sense, are not fertilizers which directly give nutrition to crop plants. These are cultures of microorganisms like bacteria, fungi, packed in a carrier material. Thus, the critical input in biofertilizers is the microorganisms. They help the plants indirectly through better Nitrogen (N) fixation or improving the nutrient availability in the soil.

Biofertilizers can be defined as "*microbial inoculants which contain live or latent cells of selected strains of nitrogen fixing, phosphate solubilizing microorganisms used for application to seed, soil or composting areas to accelerate certain microbial processes; thus augmenting the availability of nutrients in an easily assimilable forms to plants.*" (Vibhas and Gurdeep, 2006). In other word, biofertilizer is a substance which contains living microorganisms and it is a combination between benefit biological microorganisms with organic fertilizer and it is known to help with expansion of the root system and better seed germination.

The commercial history of biofertilizers began with the launch of '*Nitragin*' by Nobbe and Hiltner, a laboratory culture of *Rhizobia* in 1895, followed by the discovery of *Azotobacter* and then the blue green algae and a host of other micro-organisms. *Azospirillium* and *Vesicular-Arbuscular Mycorrhizae* (VAM) are fairly recent discoveries (Khairuddin, 2002). However in Malaysia, the industrial scale microbial inoculants started in the late 1940's and peaking in the 1970's, with *Bradyrhizobium* inoculation on legumes, especially leguminous cover crops (LCC) taking precedent and the most accepted biofertilizer product nowadays is the *mycorrhiza* inoculum (Khairuddin, 2002).

2.1.1 Types of biofertilizer

Every microorganism in biofertilizer has a specific capability and function. There are two types of biofertilizers typically known and detail of important microbes used in each of biofertilizer discussed as follows:

2.1.1.1 Nitrogen-fixing biofertilizers

Rhizobium

Rhizobium is a group of bacteria that fixes nitrogen in association with the roots of leguminous crops. *Rhizobia* can fix 40-120 kg of nitrogen per acre annually depending upon the crop, *rhizobium* species and environmental conditions. They help improve soil fertility, plant nutrition and plant growth and have no negative effect on soil or the environment. Every leguminous crop requires a specific *rhizobium* species.

Azotobacter

Azotobacter is also a group of nitrogen-fixing bacteria but unlike *rhizobia*, they do not form root nodules or associate with leguminous crops. They are free-living nitrogen fixers and can be used for all types of upland crops but cannot survive in wetland conditions. In soils of poor fertility and organic matter, *azotobacter* needs to be regularly applied. In addition to nitrogen-fixation, they also produce beneficial growth substances and beneficial antibiotics that help control root diseases.

Azospirillium

Similar to *azotobacter*, *azospirillum* species also do not form root nodules or associate with leguminous crops. They are however not free-living and live inside plant roots where they fix nitrogen, and can be used in wetland conditions. This group of microorganisms also produces beneficial substances for plant growth, besides fixing atmospheric nitrogen. *Azospirillum* does well

in soils with organic matter and moisture content, and requires a pH level of above 6.0.

Blue-green algae

Blue-green algae or *cyanobacteria*, they are free-living nitrogen-fixing photosynthetic algae that are found in wet and marshy conditions. Blue-green algae are so named for their colour but they may also be purple, brown or red. They are easily prepared on the farm but can be used only for rice cultivation when the field is flooded and do not survive in acidic soils.

Azolla

Azolla, are a free-floating water fern that fixes nitrogen in association with a specific species of *cyanobacteria*. *Azolla* is a renewable biofertilizer and can be produced in such a mass amount on the farm like blue-green algae. It is a good source of nitrogen and on decomposition as well a source of various micronutrients. Its ability to multiply fast means it can stifle and control weeds in (flooded) rice fields. *Azolla* is also used as a green manure and a high-quality feed for cattle and poultry.

2.1.1.2 Phosphorus mobilising biofertilizers

Phosphate-solubilizing microorganisms are a group of bacteria and fungi that are capable of breaking down insoluble phosphates to make them available to crops. Their importance lies in the fact that barely a third of phosphorous in the soil is

actually available to the crop as the rest is insoluble. They require sufficient organic matter in the soil to be of any great benefit. Microorganisms used for phosphate solubilizer are *Bacillus*, *Pseudomonas* and *Aspergillus niger*:

Mycorrhiza is a sweeping term for a number of species of fungi which form a symbiotic association with the plant root system. Of these, the most important in agriculture is *vesicular-arbuscular mycorrhiza* or VAM. Plants with VAM colonies are capable of higher uptakes of soil and nutrients and water. VAM strands acts as root extensions and bring up water and nutrients from lateral and vertical distances where the plant root system does not reach. Some of the species categorized as VAM fungi are *Glomus* and *Gigaspora.*

2.1.2 Preparation of biofertilizer

Biofertilizers are typically prepared as carrier-based inoculants containing effective microorganisms. It is because of microorganisms in carrier material are easy-handling, long-term storage and high effectiveness of biofertilizers. Among various types of biofertilizers, bacterial inoculant is one major group which includes *rhizobia*, nitrogen-fixing *rhizobacteria*, plant growth-promoting *rhizobacteria*, phosphate-solubilizing bacteria, and so on. Basically, the carrier-based inoculant of these bacteria can be prepared by mixed well the beneficial bacteria with the carrier.

The most common way of inoculation is "seed inoculation", in which the inoculants (bacteria-carrier mixture) is mixed with water to make slurry-form, and then mixed with seeds. In this case, the carrier must be a form of fine powder. To achieve the tight coating of inoculant on seed surface, use of adhesive, such as gum arabic, methylethylcellulose, sucrose solutions, and vegetable oils, is recommended.

However seed inoculation may not always be successful, it is because of the inoculation resulted in low nodule occupancy of the inoculated *rhizobial* strain, or low establishment of the inoculated *rhizobacterial* strain. This might be due to low population and low survival of the inoculated bacterial strain on the seed surface and in the soil. In such case in point, "soil inoculation" will be adopted, whereby a large population of a bacterial strain can be introduced into the soil. For soil inoculation in general, granular inoculant is placed into the furrow under or alongside the seed. This enhances the chance for the inoculated strain to be in contact with plant roots (Vessey, 2003)

2.1.2.1 Carrier material

Variety types of material are used as carrier for seed or soil inoculation. For preparation of seed inoculant, the carrier material is milled to fine powder with particle size of 10 -40 μm. The properties of a good carrier material for seed inoculation are non-toxic to inoculant bacterial strain, good moisture absorption capacity, easy to process and free of lump-forming materials, easy to sterilize by autoclaving or gamma-irradiation, available in adequate amounts, inexpensive, good adhesion to seeds, good pH buffering capacity and non-toxic to plant.

Peat is the most frequently used carrier material for seed inoculation. Peat-based rhizobial inoculant is already used in many countries and a number of information is available on the properties and effect of the inoculant.

For soil inoculation, carrier material with granular form (0.5 – 1.5 mm) is generally used. Granular forms of peat, perlite, charcoal or soil aggregates are suitable for soil inoculation. Various types of material used or tested as carrier for bacterial inoculant (mostly *Rhizobia*) is listed in Table 2.1.

Table 2.1: Carrier material used for biofertilizer

Carrier material	**Inoculant bacterium**	**Characteristics**
Sterilized oxalic acid industrial waste	*Rhizobium*	- seed inoculation - *Rhizobium* multiplication in carrier in ambient temperature up to 90 days. - Carrier sterilization contributed significant increase in grain yield, nodule number and nitrogen content.
Alginate-perlite dry ganule	*Rhizobium*	- soil inoculation - *Rhizobium* strains survived in dry granules beyond 180 days. - The inoculants can be stored in a dry state without losing much viability.
Cheese whey grown cells in peat	*Rhizobium meliloti*	- seed inoculation - Better survival at various temperature during storage, even under desiccation
Mineral soils	*Rhizobium*	- seed inoculant - *Rhizobium* survived better at 4°C than at higher temperature
Coal	*Rhizobium*	- seed inoculant - Seven among eight tested coals supported the growth and survival of *R. phaseoli* strains. Most contained more than 107 rhizobia per g after 12 months
Perlite	*Rhizobium, Bradyrhizobium, Bacillus*	- seed inoculant - Combination of a sucrose adhesive with the perlite carrier gave better survival of bacteria on seeds - Produced similar number of nodules, nodule dry weight, crop yield and nitrogen content as peat-based inoculants
Wastewater sludge	*Sinorhizobium meliloti*	- seed inoculant - Result showed the suitability of using sludge as a carrier because it had the same or a higher potential than peat to support survival of *S. meliloti.*
Nutrient supplemented pumice	*Rhizobium*	- seed inoculant - Good storage and handling properties and could be mixed directly with the seeds during the sowing process

2.1.3 Biofertilizers effect on plant growth.

Beneficial microbes play an important role to enhance healthy of rhizosphere of a plant and increase crop production. Basically, the evaluation of effect of biofertilizer on plant growth due to the physical appearances parameter like plant height, plant stem base diameter, number of leaves and plant root length.

From the previous studies, Wu et al. (2003) have evaluated the effects of four biofertilizers containing an arbuscular mycorrhizal fungus (*Glomus mosseae* or *Glomus intraradices*) with or without N-fixer (*Azotobacter chroococcum*), P solubilizer (*Bacillus megaterium*) and K solubilizer (*Bacillus mucilaginous*) on soil properties and the *Zea mays* as the test crop. From their study, the fertilizer effect much more obvious after inoculation of the fertilizer with beneficial bacteria. The used of biofertilizer (*G. mosseae* and three bacterial species) resulted in the highest biomass and seedling height. They also stated that half of the amount of biofertilizer application had similar effects when compared with organic fertilizer or chemical fertilizer treatments. Microbial inoculums not only increased the nutritional assimilation of plant (total N, P and K), but also improved soil properties, such as organic matter content and total N in soil.

From Hossain *et al.* studied on *azola* as biofertilizer and BR 26 rice as the test crop. Their study were divided into six treatments which are control (T0), chemical fertilizer (T1), biofertilizer containing 0.1 kg m^{-2} *azola* inoculums (T2), biofertilizer containing 0.1 kg m^{-2} *azola* inoculums left growing (T3), biofertilizer containing 0.2 kg m^{-2} *azola* inoculums (T4), biofertilizer containing 0.2 kg m^{-2} *azola* inoculums left growing (T5). After four month of treatment, they found that the plant heights were influenced by biofertilizer containing *azola* compare to the control. They conclude that the usage of biofertilizer containing *azola* resulted to a better gorwth plant. Their study also showed that the effect of using biofertilizer containing *azola* and chemical fertilizer are almost same.

In other research of effect of biofertilizer on plant growth, Nuruzzaman *et al.* (2003) investigated the effect of biofertilizers on morpho-physiological characters of okra as the test crop by applying nine treatments such as T0 (control), T1 (*Azotobacter* biofertilizer), T2 (*Azospirillum* biofertilizer), T3 (*Azotobacter* + *Azospirillum* biofertilizers), T4 (*Azotobacter* + Cowdung), T5 (*Azospirillum* + cowdung), T6 (*Azotobacter* + *Azospirillum* + cowdung), T7 (cowdung), and T8 (60% Nitrogen fertilizer). From their studied, they claims that number of leaves per plant, stem base diameter, root length, root dry weight, leaf area index, and crop growth rate were larger in T4, T5, T6, and T8 than the others. In all the parameters, T8 gave the similar result with biofertilizers in combination with cowdung treatments, and T7 was identical with T0 (control).

2.1.4 Effect of biofertilizer enhancement on nutrient uptake by plant.

The efficiency of biofertilizer is depends on the components available in raw materials as well as contribution from living microorganisms in them (Hasarin and Viyada, 2008). Moreover, their performance also depends on whether the environments they are introduced to are conducive. Because of this issue, the improvement of biofertilizer is needed to achieve constant effect like chemical fertilizer.

There are many types of biofertilizer enhancement were studied such as increasing the quantity of beneficial microbes in the biofertilizer, combining more than one type of beneficial microbes in the biofertilizer and combining biofertilizer with chemical fertilizer.

From the studied done by Han and Lee (2005), they reported that by combining beneficial bacterial inoculation (phosphorus solubilizer bacteria and

kalium solubilizer bacteria) with chemical fertilizer (in red box) can increases nutrient uptake by the plant compare to the using biofertilizer itself. Detail of their finding shown in Table 2.2.

Table 2.2: Effect of enhancing biofertilizer on nutrient uptake for eggplant growth (Han and Lee, 2005)

Treatment	Shoot (mg plant^{-1})			Root (mg plant^{-1})		
	N	P	K	N	P	K
Control	16.4	5.04	30.7	5.36	0.86	8.92
Rock P	16.9	5.35	31.3	5.72	0.89	9.02
Rock K	16.8	5.09	32.0	5.43	0.83	9.54
Rock (P+K)	17.2	5.33	32.7	5.55	0.91	9.51
PSB	17.5	5.48	31.9	5.74	0.88	9.15
KSB	17.0	5.11	32.2	5.66	0.85	9.52
(P+K)SB	17.8	5.50	32.8	5.80	0.92	9.66
Rock P+PSB	18.1	6.07	33.0	5.91	0.96	9.76
Rock K+KSB	17.8	5.53	34.1	5.85	0.89	10.18
Rock (P+K)+(P+K)SB	18.7	6.16	34.9	5.94	0.98	10.83
$LSD_{0.05}$	1.5	0.9	2.3	0.5	0.06	1.0

The studied done by Wu et al. (2003) is combining two different types of beneficial microbes to the biofertilizer and their effect to the plant nutrient uptake. They state that, dual inoculation of biofertilizer is the most effective to improve plant nutrient uptake. Figure 2.1 and Figure 2.2 showed the detail of their report. From the figure, it is clearly show that the combination of dual microbes in the biofertilizer (BOM-GM and BOM-GI) can increase the nutrient uptake. The type of microbes used in their studies as shown at Table 2.3.

Table 2.3: The pot experiment design of *Zea mays* (Wu et al., 2003).

Treatment	1	2	3	4	5	6	7	8	9
With bacteria inoculation	no	no	no	no	no	yes	yes	yes	yes
Mycorrhizzal fungi species added	none	none	none	[a]GM	[b]GI	GM	GI	GM	GI
Fertilizer used	-	CF[c]	OM[d]	OM	OM	OM	OM	OM	OM
Fertilizer level	0%	100%	100%	100%	100 %	100%	100%	50%	50%
Code	control	Chemical fertilizer	OM	OM+GM	OM+ GI	BOM+ GM	BOM+ GI	50% BOM+ GM	50% BOM+ GI

[a]GM – *G mosseae*
[b]GI – *G intraradices*
[c]CF – chemical fertilizer
[d]OM – organic fertilizer (autoclaved fertilizer)

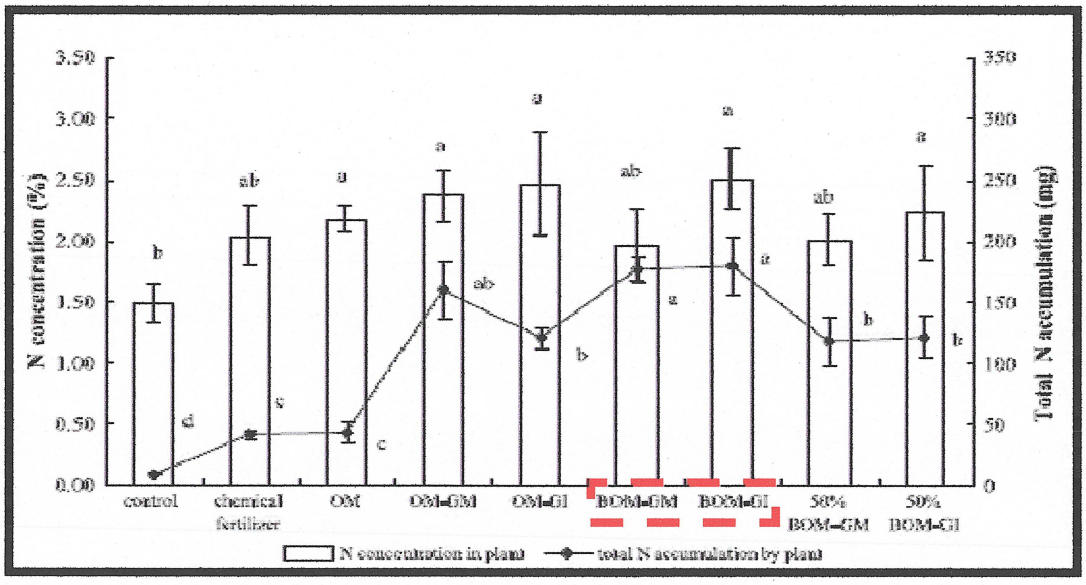


Figure 2.1: Nitrogen uptake of the plant. (Wu *et al.*, 2003)

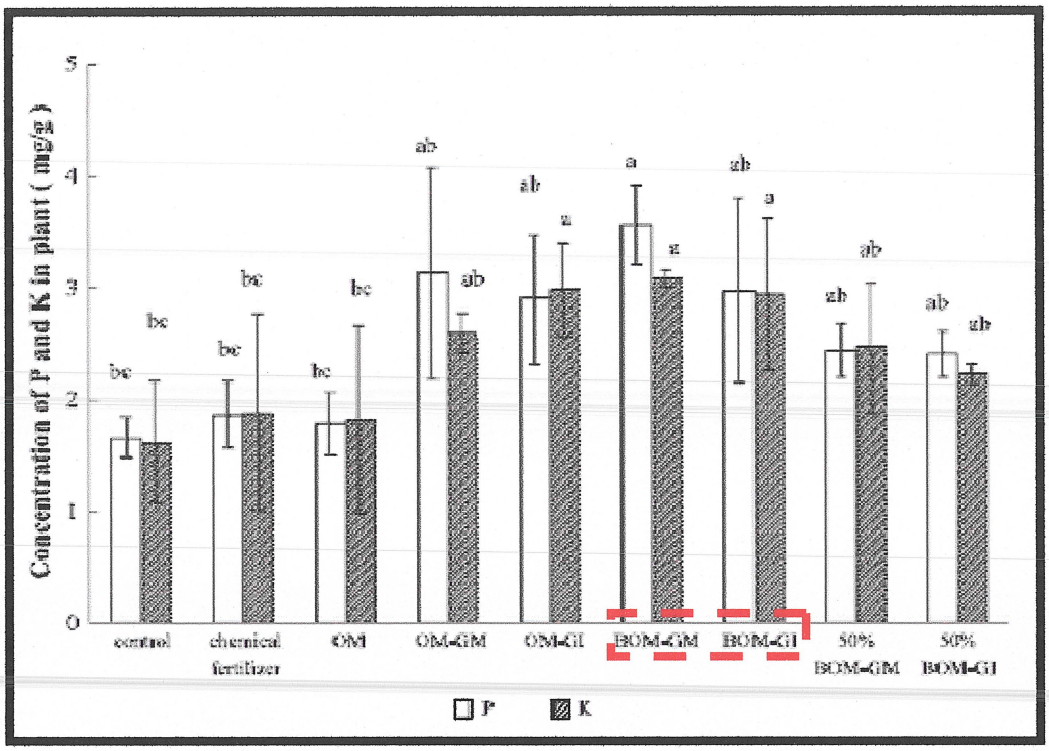


Figure 2.2: Phosphorus and potassium uptake of the plant (Wu *et al*., 2003)

2.1.5 Critical factors responsible for effectiveness of biofertilizer

There are several factors that influence effectiveness of a particular biofertilizer.First and foremost is the suitability of the strain in which there are specific strains of *Rhizobium* for different leguminous species like Cowpea, Red gram, Soybean, Alfalfa etc. Biofertilizer of specific culture should be used for specific crop. The identification of strains as suited to the agro-eco system , particularly the soil pH and moisture conditions also capable of influencing the effectiveness of biofertilizer. Through research, specific strains as suited to a particular soil and environmental conditions are usually identified and pure mother cultures are maintained in research labs for supply to the commercial manufacturers.

Some other factors that played crucial roles in influencing the effectiveness of biofertilizer are the aseptic conditions of manufacturing, the cell count of living organism present in the carrier material, purity and level of contamination, the conditions of carrier material in which the culture is packed and the quality of the packing material, which determine the shelf life, the conditions, in which the packed materials are stored, distributed and kept with the farmers before it is applied as well as oil conditions particularly pH, organic matter content, moisture level and agronomic practices.

2.2 Potassium (K)

Potassium (K) is one of sixteen essential nutrients required for plant growth and reproduction. It is classified as a macronutrient, as are nitrogen (N) and phosphorus (P). The chemical symbol for potassium is "K." It is taken up by plants in its ionic form (K+). The word potassium translates from the Latin or German word, Kalium. The term "potash" comes from the colonial practice of burning wood in large pots and using the ashes as fertilizer and making soap, gunpowder and glass. "Potash" is defined as K_2O and is used to express the content of various fertilizer materials containing potassium such as muriate of potash (KCl), sulfate of potash (K_2SO_4), double sulfate of potash and magnesium (K_2SO_4 $2MgSO_4$), and nitrate of potash (KNO_3). Frequently, the expressions "K" and "K_2O" are used interchangeably, although technically incorrectly.

2.2.1 Potassium in plant

While potassium is not a constituent of any plant structures or compounds, it plays a part in many important regulatory roles in the plant. It is essential in nearly all processes needed to sustain plant growth and reproduction. Potassium plays a vital role in photosynthesis, translocation of photosynthates, protein synthesis, control of ionic balance, regulation of plant stomata and water use, activation of plant enzymes and, many other processes (www.rainbowplantfood.com).

There are at least sixty enzymes involved in plant growth which might be its most important function in the plant (www.rainbowplantfood.com). Plants deficient in potassium are less resistant to drought, excess water, and high and low temperatures. They are also less resistant to pests, diseases and nematode attacks. Potassium is also known as the quality nutrient because of its important effects on quality factors such as size, shape, color, taste, shelf life, fiber quality and other quality measurements.

2.2.2 Potassium deficiency symptom

Plants absorb potassium as the potassium ion (K+). Potassium is a highly mobile element in the plant and is translocated from the older to younger tissue. Consequently, potassium deficiency symptoms usually occur first on the lower leaves of the plant and progress toward the top as the severity of the deficiency increases. One of the most common signs of potassium deficiency is the yellow scorching or firing (chlorosis) along the leaf margin. In severe cases of potassium deficiency the fired margin of the leaf may fall out. However, with broadleaf crops, such as soybeans and cotton, the entire leaf may shed resulting in premature defoliation of the crop.

Potassium deficient crops grow slowly and have poorly developed root systems. Stalks are weak and lodging of cereal crops such as corn and small grain is common. Legumes are not strong competitors for soil potassium and are often crowded out by grasses in a grass-legume pasture. When potassium is not sufficient, winter-killing of perennial crops such as alfalfa and grasses can occur. Seeds from potassium deficient plants are small, shriveled, and are more susceptible to diseases. Fruit is often lacking in normal coloration and is low in sugar content. Vegetables and fruits deteriorate rapidly when shipped and have a short shelf life in the market. Figure 2.3 is shown the effect of potassium deficiency symptom to the plants.

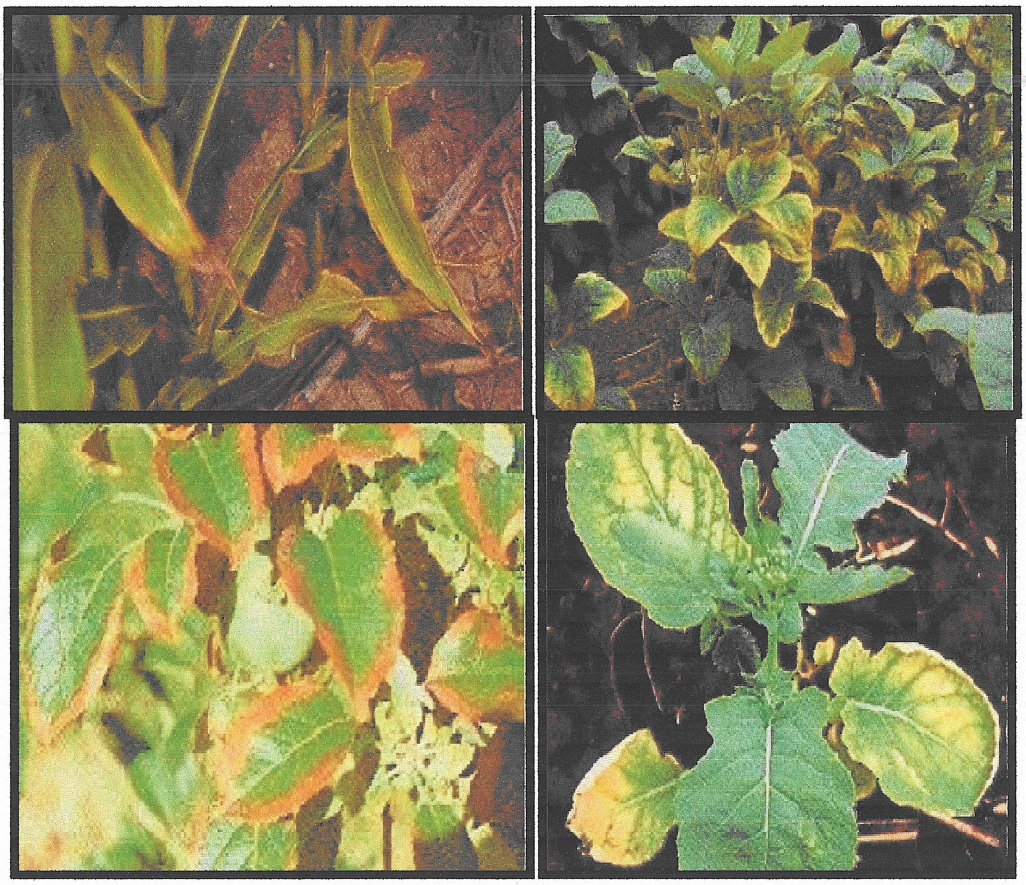


Figure 2.3: Potassium deficiency symptom of plant (www.rainbowplantfood.com)

2.2.3 K solubilizer bacteria (KSB)

K Solubilizer bacteria are able to solubilize potassium mineral powder such as micas, illite and orthoclasses through production and excretion of organic acid. It also increased potassium (K) availability in soils and increased mineral content in the plant. Most common K solubilizer is *Bacillus megaterium.* Figure 2.4 is shown the image of *Bacillus megaterium.* From the morphology of the bacteria, it is clear that *Bacillus megaterium* came from *coccus sp.* This is due to the spherical appearance observed on the agar plate.

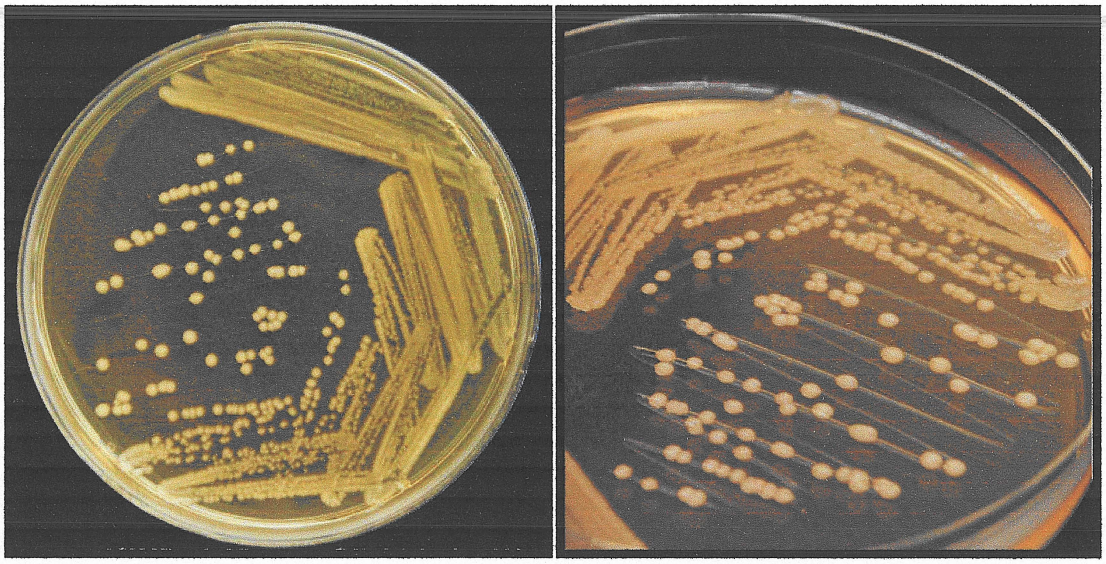


Figure 2.4: Image of *Bacillus megaterium*(www.microbelibrary.org)

2.3 Patchouli

Patchouli is a bushy herb of the mint family, with erect stems, reaching two or three feet (about 0.75 metre) in height and bearing small pale pink-white flowers. The plant is native to tropical regions of Asia and is now extensively cultivated in

Caribbean countries, China, India, Indonesia, Malaysia, Mauritius, Philippines, West Africa and Vietnam.

2.3.1 Application of patchouli oil

The plant and oil have a number of claimed health benefits in herbal folk-lore, and its scent is used with the aim of inducing relaxation. Chinese medicine uses the herb to treat headaches, colds, nausea, diarrhea and abdominal pain. Patchouli oil can be purchased from mainstream Western pharmacies and alternative therapy sources as aromatherapy oil. Patchouli is also in widespread use in modern industry. It is a popular component in perfumes, including more than half of perfumes for men. Patchouli is also an important ingredient in East Asian incense. It is also used as a scent in products like paper towels, laundry detergents, and air fresheners. Two important components of the essential oil are patchoulol and norpatchoulenol.

2.4 Cultivation of patchouli plant

Patchouli grows well in warm to tropical climates. It thrives in hot weather but not direct sunlight. If the plant withers due to lack of watering it will recover well and quickly once it has been watered. The seed-bearing flowers are very fragrant and bloom in late fall. The tiny seeds may be harvested for planting, but they are very delicate and easily crushed. Cuttings from the mother plant can also be rooted in water to produce further plants.

CHAPTER 3

RESEARCH METHODOLOGY

3.1 Introduction

This study consist two part of experiment. Part I is cultivation of K solubilizer bacteria (KSB). The main purpose of cultivation of KSB is to well understanding on the bacteria growth curve and as well as their glucose consumption. Part II is pot experiment that generally including the preparation of enhances biofertilizer, data collection from the growth of patchouli which is number of leaves, seedling height, the leaves colour and analysis of potassium content in the soil.

3.2 Materials and method

3.2.1 Medium preparation

3.2.1.1 Nutrient agar

Nutrient agar (NA) was prepared by dissolving 23 g of nutrient agar powder in 1 liter distilled water in a flask. Then the mixture was boiled and stirred until it reached homogeneous state. After that, the medium solution was sterilized in an autoclave for 15 minutes at 121°C. Next, the sterile medium was poured into petri plates in laminar flow hut and was cooled at room temperature to make the medium solidify. The solidified nutrient agar medium was stored in the freezer at 4°C.

3.2.1.2 Nutrient broth (NB)

For preparation of nutrient broth (NB), 8.0 grams of nutrient broth powder was dissolved in 1.0 liter distilled or deionized water in a 2.0 liter flask. Then, the mixture was boiled until it reached homogeneity and stirred by using magnetic stirrer. The pH of the nutrient broth was measured before being dispensed into medium bottle. The nutrient broth solution was autoclaved at 121°C for 15 to 25 minutes. After the nutrient broth had cooled to room temperature, it was stored at 4°C.

3.2.2 Culture condition

The KSB stock culture obtained from Faculty of Chemical & Natural Resources Engineering laboratory, Universiti Malaysia Pahang. The KSB were cultured in solid agar and they incubated at 28°C temperature. A single colony of KSB was transferred in to 250 ml of nutrient broth for 3 days of cultivation in the incubator shaker for 72 hour at 28 °C.

On activation process, 10% v/v of the cultured liquid media was introduced into a 100 ml of fresh liquid media and incubated for 30 hour at 28°C and 200 rpm. This process was duplicated.

Then the inoculation process were done in which 10% v/v of activation liquid media were introduced into a 100 ml of fresh liquid media. The same procedure were repeated for duplication. Both flask were incubated for 18 hour at 200 rpm and 28 °C.

Then, 10% v/v of this inoculum was tranfered into fresh nutrient broth for KSB fermentation. The working volume is 100 ml and the fermentation was done for 72 hour at 200 rpm and 28 °C.

3.2.3 Preparation of Biofertilizer

The biofertilizer preparation was conducted after the growth curve of KSB was obtained. The fermentation was stop at maximum growth rate obtained and thebroth is taken for inoculation of KSB into carrier. In this study, peat moss was

used as the carrier. With the aid of growth profile obtained from Part 1, it was supposed that after 20 hour of cultivation the KSB is in active atmosphere due to the fact that the stationary phase for KSB was around 40 hour. The 5% , 10% , 15% and 20% of inoculation were taken from cultivation liquid media respectively and mixed with 1kg of sterilized peat moss and 1kg of biofertilizer. The mixtures were packed and left for three day for the KSB adaptation. The biofertilizer and peat moss were obtained from Faculty of Chemical and Natural Resources Engineering laboratory, Universiti Malaysia Pahang.

3.2.4 Pot experiment

The pot experiment was conducted at Kolej Kediaman Kedua, Universiti Malaysia Pahang. There were eight treatments of pot experiment and it were duplicated for each treatment to make 16 pots in total. The modifications of biofertilizer were coded as BF5 for 5% of inoculums, BF10 for 10% of inoculums, BF15 for 15% of inoculums and BF20 for 20% of inoculums. Another four treatments were set up as comparison including chemical fertilizer, sterilized organic fertilizer, biofertilizer and no fertilizer (control). The detail is shown in Table 3.1. The organic fertilizer and chemical fertilizer were purchased from Premium Agro Product Sdn. Bhd., Selangor.

Table 3.1: Design of pot experiment

Treatment	**1**	**2**	**3**	**4**	**5**
With bacteria	-	-	-	Yes (not modified)	Yes (modified)
Fertilizer used	-	Chemical fertilizer	Organic fertilizer	Organic fertilizer	Organic fertilizer
Code	Control	Chemical fertilizer (CF)	Organic fertilizer (OF)	Biofertilizer (BF)	Biofertilizer (BF5, BF10, BF15, BF20)

The soil used in this study was obtained from Universiti Malaysia Pahang land field. The exactly type of the soil were unknown because of it have been randomly collect from the land field. The method used in this pot experiment was soil inoculation whereby a large population of a bacterial strain was introduced into the soil. Thus 0.5 kg of sterilized soil was well mixed with each of 16 treatments and left for one day in the polybag separately except treatment for control. The ratio of soil to fertilizer is 3:1. Whereas on the control, it was consisted entirely soil. Figure 3.1 shown is the soil used in this study.

Figure 3.1: The soil used. (from the author camera, 2009)

Each of 16 treatment stated before was seeded with patchouli plant. The seedling of patchouli plant were carried out in a polybag and randomly place. The plantation were in 6 weeks and the treatment was watered twice a day with deionized water to maintain the moisture of the soil.

3.3 Data analysis

Data analyses were divided into two part which are Part I and Part II. Part I was quantitative analysis through analytical method to obtain the KSB cell concentration, glucose consumption and potassium content while part II was qualitative analysis through physical observation of several parameters which were seedling height, leaf diameter and leaf colour.

3.3.1 DNS method for glucose assay

DNS reagent was prepared using 10g sodium hydroxide, 200g sodium potassium tartarate, 2g phenol, 0.5g sodium sulphite and 10g dinitrosalicylic acid. The ingredient was dissolved in 500mL distilled water before being top up to 1 liter in amber bottle or enwrapped medium bottle. Then it was left overnight to be stirred.

Glucose was detected by adding 0.4 ml of DNS reagent to 0.2 ml of glucose sample in a lightly capped test tube. Then, 2 ml deionized water was added to the mixture. In order to avoid the loss of liquid due to evaporation, the test tube was covered with a piece of paraffin film if a plain test tube is used. The mixture was heated at 90° C for 5 minutes to develop the red-brown color. The absorbance was

recorded with a spectrophotometer at 550 nm after cooling to room temperature using cold water bath. The glucose concentration were determine based on the standard glucose curve obtain in Appendix A.

3.3.2 Growth observation

The KSB growth observation were determine using UV Visible Spectrophotometer (UV-VIS) at 600 nm in 3 hour interval for the first 24 hour and 6 hour interval for the next 48 hour.

3.3.3 Potassium determination

Potassium content in the biofertilizer were determine using Polarized Zeeman Atomic Absorption Spectrophotometer (Model Z-5000 Series). On the initial and the end of pot experiment, 50 g of soil were taken from each pot. The soil was dissolved in water before being filtered using filter paper of 100 circles. Then, the samples were being filtered once again using membrane filter of 0.45 μm. After completing the two stage filter, the samples were being tested using atomic absorption spectrometer (AAS) at 766.5 nm and 12 mA potassium lamp. All proceduere were duplicated.

3.3.4 Physical observation

The observations were made within 6 week of treatment and the physical observations were made based on total of leaf, colour of leaf and seedling height of the patchouli plant in every 2 weeks interval on control, modification biofertilizer, biofertilizer, organics fertilizer and chemical fertilizer.

3.4 Summary of methodology

This section is to summarize the experimentation process. Figure 3.2 shown the summary procedure of Part I and Figure 3.3 is shown the summary procedure of Part II.

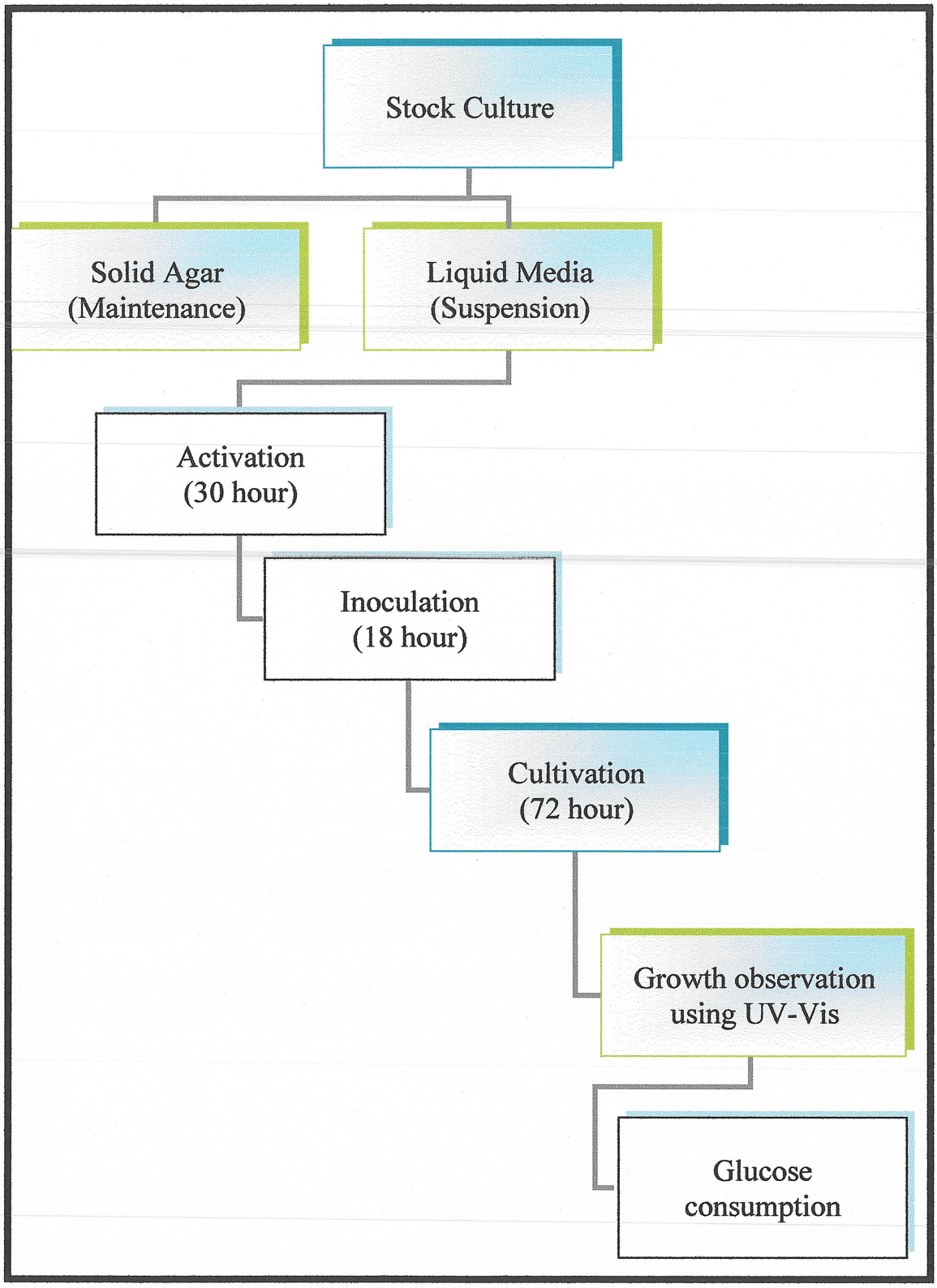


Figure 3.2: A methodology summary of Part I

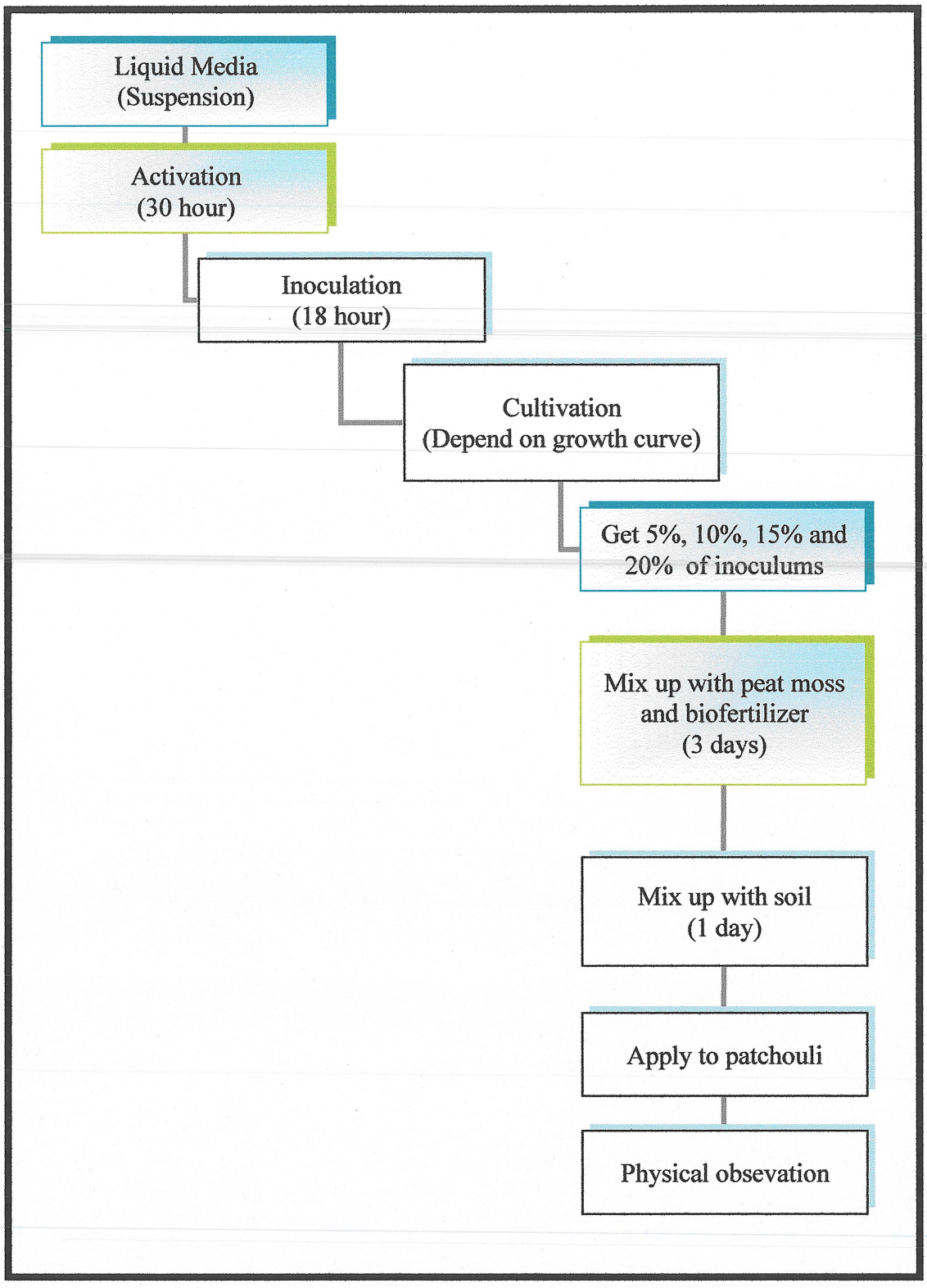


Figure 3.3: A methodology summary of Part II

CHAPTER 4

RESULTS AND DISCUSSION

4.1. Introduction

This chapter depicts the results and observation obtained from the entire study that has been carried out. Most of the results obtained from this study were based on observations, measurement of the optical density and detection of potassium using Atomic absorption spectrometry (AAS). Throughout the duration of this study, a total of two trials have been carried out for Part I and one trial for Part II. Part I was a study on K solubilizer cultivation and Part II was on pot experiment. A batch of aerobic fermentation was carried out for Part I using incubator shaker at 200 rpm and 28°C. Meanwhile for Part II, the pot experiment contained 8 different treatments and each treatment was duplicated. Data on physical appearance of patchouli plant were collected every 2 weeks interval and there were a total of 6 weeks of experiment. The physical observations were made based on total of leaf, colour of leaf and seedling height of the patchouli plant. Moreover, the potassium content for in biofertilizer before and after each treatment was detected using AAS. The details on the result obtained were discussed in the following sections.

4.2. Cultivation of K solubilizer (Part I)

The growth curve for K solubilizer bacteria (KSB) is a key since it helped to understand the KSB growth itself. Figure 4.1 shown is the growth curve glucose consumption of KSB.

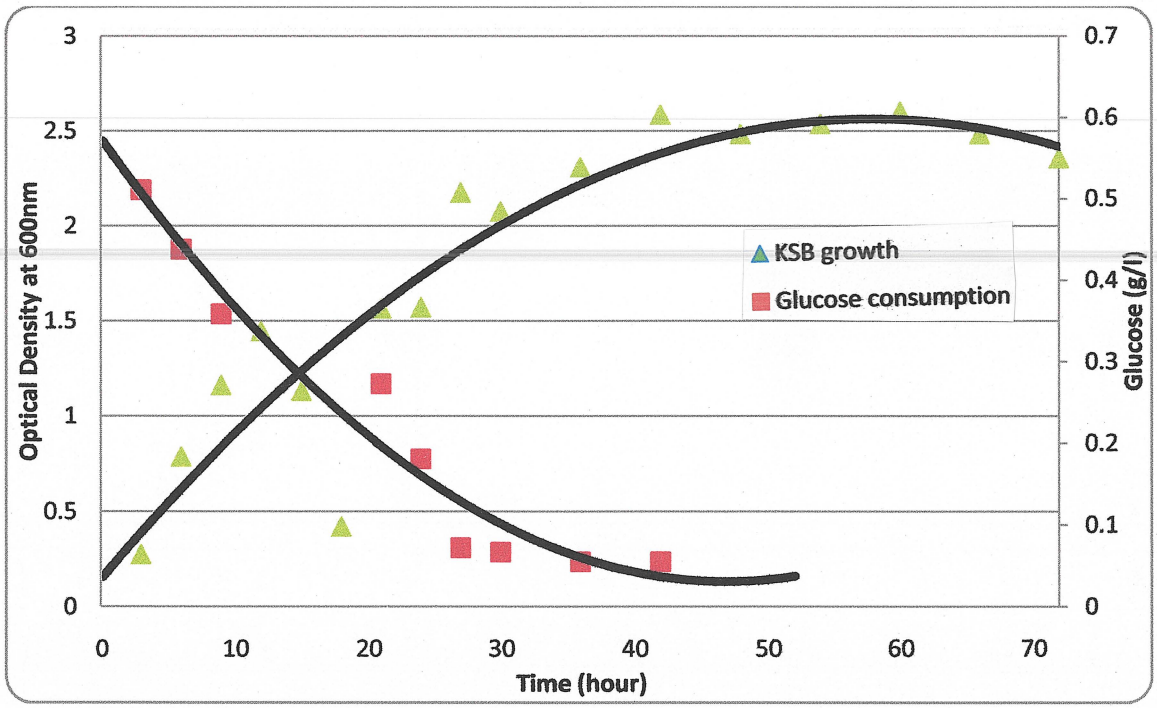


Figure 4.1: K solubilizer bacteria growth curve.and glucose consumption.

As observed in the curve shown in Figure 4.1, KSB growth followed the standard growth curve and the KSB growth curve were obtained from the first run of the fermentation process from two run of fermentation done. This is due the best data collected from both run. Although in this case, there was no lag phase, the data plotted showed it has exponential and stationary phase. This is due to the fact that this study contained activation process and inoculation process as stated on previous chapter. The activation and inoculation process were the key process in minimizing the lag phase of bacterial growth curve. Provided with this information on KSB growth curve, it is safe to conclude that KSB was in active phase in it first 40 hour of cultivation. It was during this time that cultivation of the bacteria to be mix in the soil

is being made. The curve also shown that the data were decreased after 12 hours. This is due to the contamination occur from the blank sample (broth without KSB) during obsevation using UV-Vis spectrophotometer. By the way, the problem were solve by changing with the new blank sample. Consequencely, the data after that were back to the desired result.

From figure 4.1 also shown the KSB glucose consumption curve. was constructed based on the glucose standard calibration curve. The glucoce consumption curve were obtained after second run of the fermentation done. This is because of the glucose determination method from the first run of fermentation were completely wrong due to human error. From the graph plotted, it is clear that the amount of glucose depleted as fermentation goes on. This implied to the fact that glucose act as carbon source in the fermentation. As the bacteria replicate themselves, they feed on glucose. Thus, the amount of glucose decreased over time until there were insufficient amount of glucose left. Since after 42 hours the growth data was in stationary phase, there are no data on glucose consumption were taken during that phase.

4.3. Pot experiment (Part II)

4.3.1. Patchouli plant physical appearances

The pot experiment contained 8 different treatments and each treatment was duplicated. That made 16 pots in total. The parameters that were observed on the patchouli plant growth were the number of leaves, the diameter of leaves and the seedling height of patchouli plant. Table 4.1 summarized the physical appearances of patchouli plant in 6 week of seedling. The data obtained from the table is an average value of each treatment.

Table 4.1: The physically appearances on patchouli plant in 6 weeks of seedling.

	Week	0	2	4	6
CONTROL	Number of Leaves	2.5	5	7.5	7.5
	Leaves Diameter (cm)	3.2	3.25	4	4
	Seedling Height (cm)	3.285	4.4	5	5
	Leaves Colour	light green	green	green	green + yellow
CHEMICAL FERTILIZER	Number of Leaves	2	4.5	10	15
	Leaves Diameter (cm)	3.15	4.5	6.5	6.5
	Seedling Height (cm)	4.18	6.5	9.5	10.65
	Leaves Colour	light green	green	green	green
ORGANIC FERTILIZER	Number of Leaves	3	5	10	10
	Leaves Diameter (cm)	3.26	3	5.75	5.75
	Seedling Height (cm)	3.23	6.5	8.75	8.75
	Leaves Colour	light green	green	green	green
BIOFERTILIZER	Number of Leaves	2.5	5.5	9.5	9.5
	Leaves Diameter (cm)	3.13	4.5	5.75	5.75
	Seedling Height (cm)	3.675	7	12	12.1
	Leaves Colour	light green	green	green	green
BIOFERTILIZER + 5% KSB	Number of Leaves	2	6	8	8
	Leaves Diameter (cm)	3.36	5	6.5	6.5
	Seedling Height (cm)	3.94	7	11	11.65
	Leaves Colour	light green	green	green	green
BIOFERTILIZER + 10% KSB	Number of Leaves	2.5	6.5	12.5	12.5
	Leaves Diameter (cm)	3.23	5.5	6.5	6.5
	Seedling Height (cm)	4.88	7	14.5	14.6
	Leaves Colour	light green	green	green	green
BIOFERTILIZER + 15% KSB	Number of Leaves	3	5.5	12	12
	Leaves Diameter (cm)	3.17	6	7	7
	Seedling Height (cm)	4.37	6.5	10	10.45
	Leaves Colour	light green	green	green	green
BIOFERTILIZER + 20% KSB	Number of Leaves	2	6.5	8	8
	Leaves Diameter (cm)	3.15	6	6	6
	Seedling Height (cm)	4.95	7	14	14.6
	Leaves Colour	light green	green	green	green

Theoretically, potassium is known as the quality nutrient because of its important effects on quality factors such as size, shape, color, taste, shelf life, fiber quality and other quality measurements. Referring to the Table 4.1, it is shown that the highest number of patchouli leaves of 15 leaves obtained was on the treatment using chemical fertilizer compared to other treatment after 6 weeks of seedling. In

term of patchouli leaves diameter, biofertilizer contained 15% of KSB inoculums resulted to the highest diameter value which is 7 cm after 6 weeks of seedling. The highest seedling height was obtained at the treatment of biofertilizer contained 20% of KSB inoculums and biofertilizer contained 10% of KSB inoculums with both resulted to 14.6 cm after 6 weeks of seedling. Figure 4.2 shows the difference in colour for each treatment after 6 weeks of seedling. From this figure, it is clear that the sign of potassium deficiency occurred in the control treatment. One of the most common signs of potassium deficiency is the yellow scorching or firing (chlorosis) along the leaf margin (www.rainbowplantfood.com). This sign of potassium deficiency is visible after 6 weeks of seedling. Due to the potassium deficiency, the crops grew slowly and this symptom usually occurred first on the lower leaves of the plant and progressed upward the top as the severity of the deficiency increases.

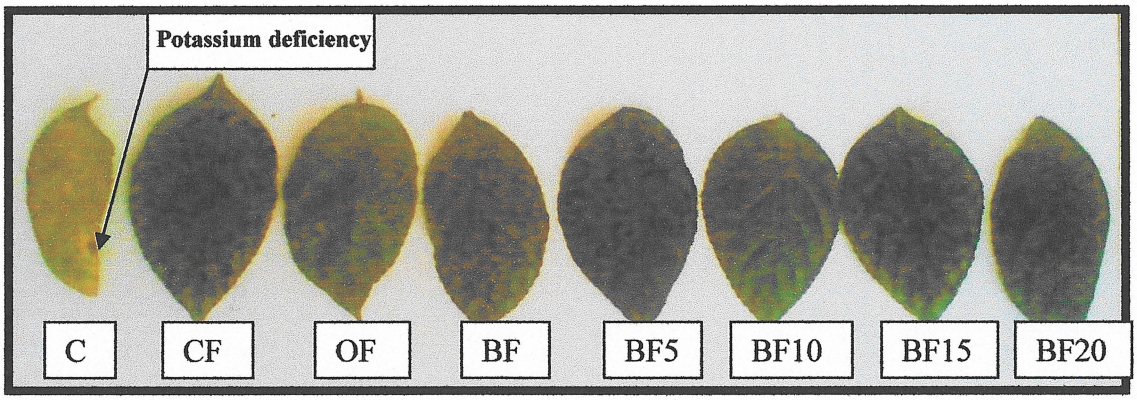


Figure 4.2: Comparison of patchouli leaves colour after 6 weeks seedling.

Figure 4.3 depicts the comparison on each treatment in term of seedling height and leaves diameter. From the figure it shows the difference in terms of seedling height and leaves diameter on each treatment. The treatment without fertilizer (control) resulted to very poor physical appearances of the patchouli plant. In addition, sterilization of the soil killed the native microflora which assisted plant growth and nutrient uptake (Wu *et al.*, 2003). This figure also shows that the application of biofertilizer itself gave the similar effect to the physical appearances of patchouli plant with chemical fertilizer. Apart from that, biofertlilizer showed a

better result than organic fertilizer in terms of seedling height and leaves diameter. At the same time with the enhancement of biofertilizer, it resulted to better effect compared to other fertilizer especially chemical fertilizer in terms of height though it gave similar result in terms of leaves diameter. This is because of organic matter content in the rhizosphere in biofertilizer was mainly influenced on plant growth, especially root exudates through the root metabolism and physiological activities (Wu *et al.*, 2003). This figure also clearly shown that biofertilizer with 10% of KSB were give the best result in term of seedling height and leaves diameter than other enhance biofertilizer.

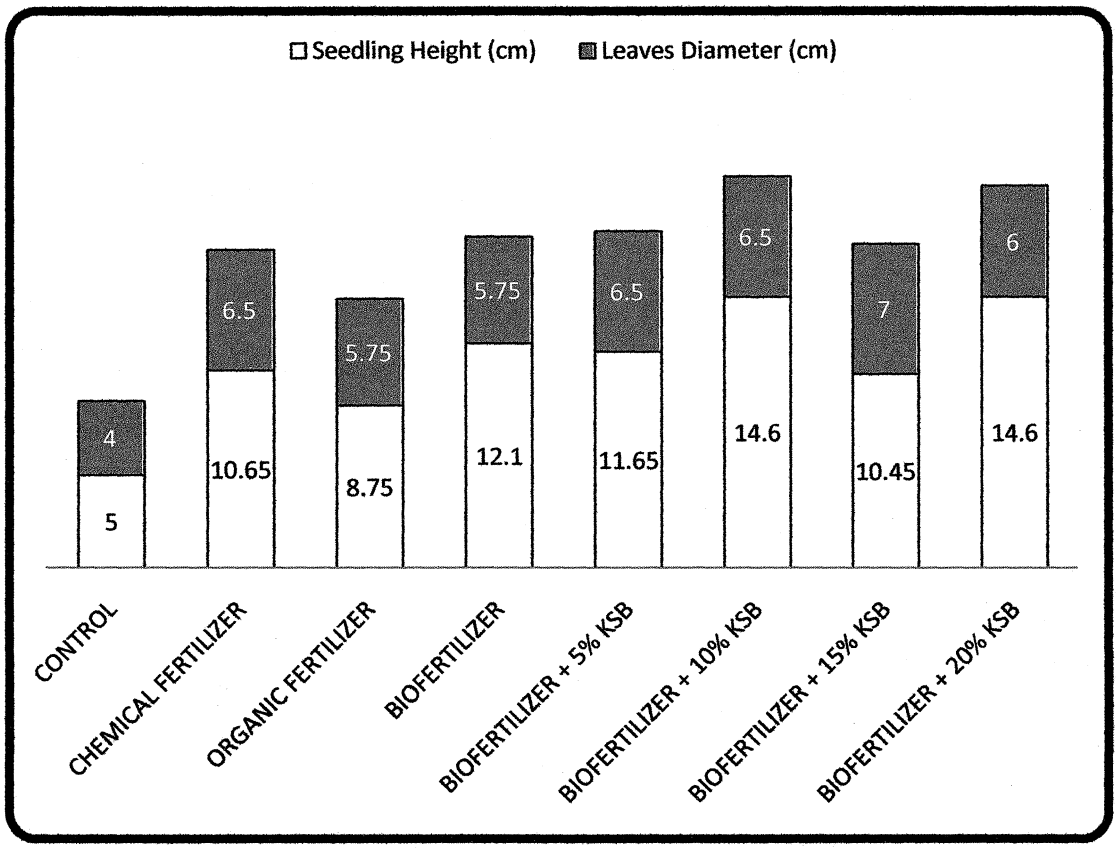


Figure 4.3: Comparison of patchouli leaves diameter and seedling height after 6 weeks of seedling.

Comparison of patchouli leaves numbers after 6 weeks of seedling. was shown in Figure 4.4. This figure depicts the comparison of number of patchouli plant leaves between all treatments after 6 weeks seedling. From the figure, a clear

indication was observed that the total number of leaves from chemical fertilizer treatment gave the highest number among all treatment with the value of 15 in average. This is the best result obtained than other treatment. Nevertheless, two of enhancement biofertilizer which were biofertilizer contained 10% of KSB inoculums and biofertilizer contained 15% of KSB inoculums followed closely with 12.5 and 12 in average number of patchouli plant leaves. This phenomena obviously showed that by enhancing the biofertilizer, one can achieve the physical effect similar to the one treated with chemical fertilizer.

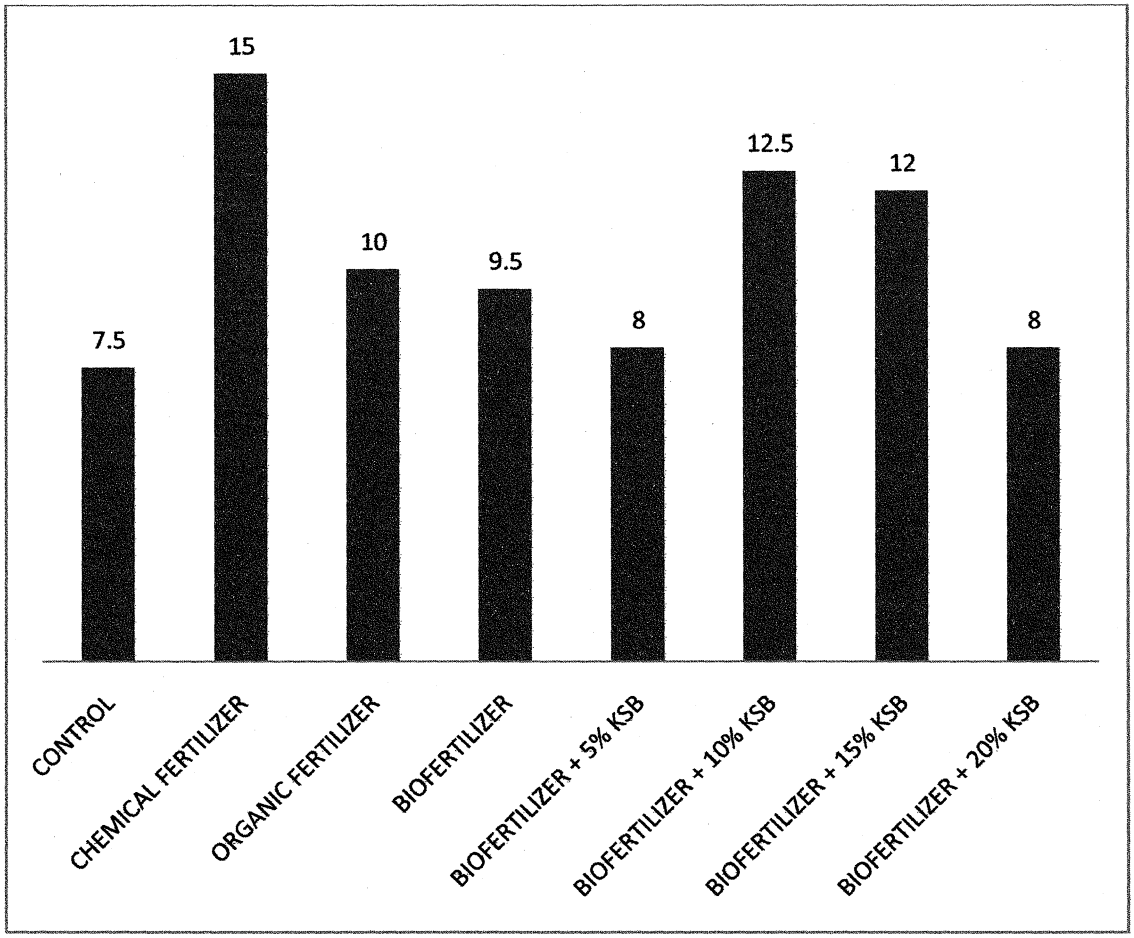


Figure 4.4: Comparison of patchouli leaves numbers after 6 weeks of seedling.

4.3.2. Potassium content in biofertilizer

The potassium content analysis was carried out using soil from the initial of seedling to after 6 weeks of seedling. Figure 4.5 is shown the comparison of potassium content in soil between all treatments.

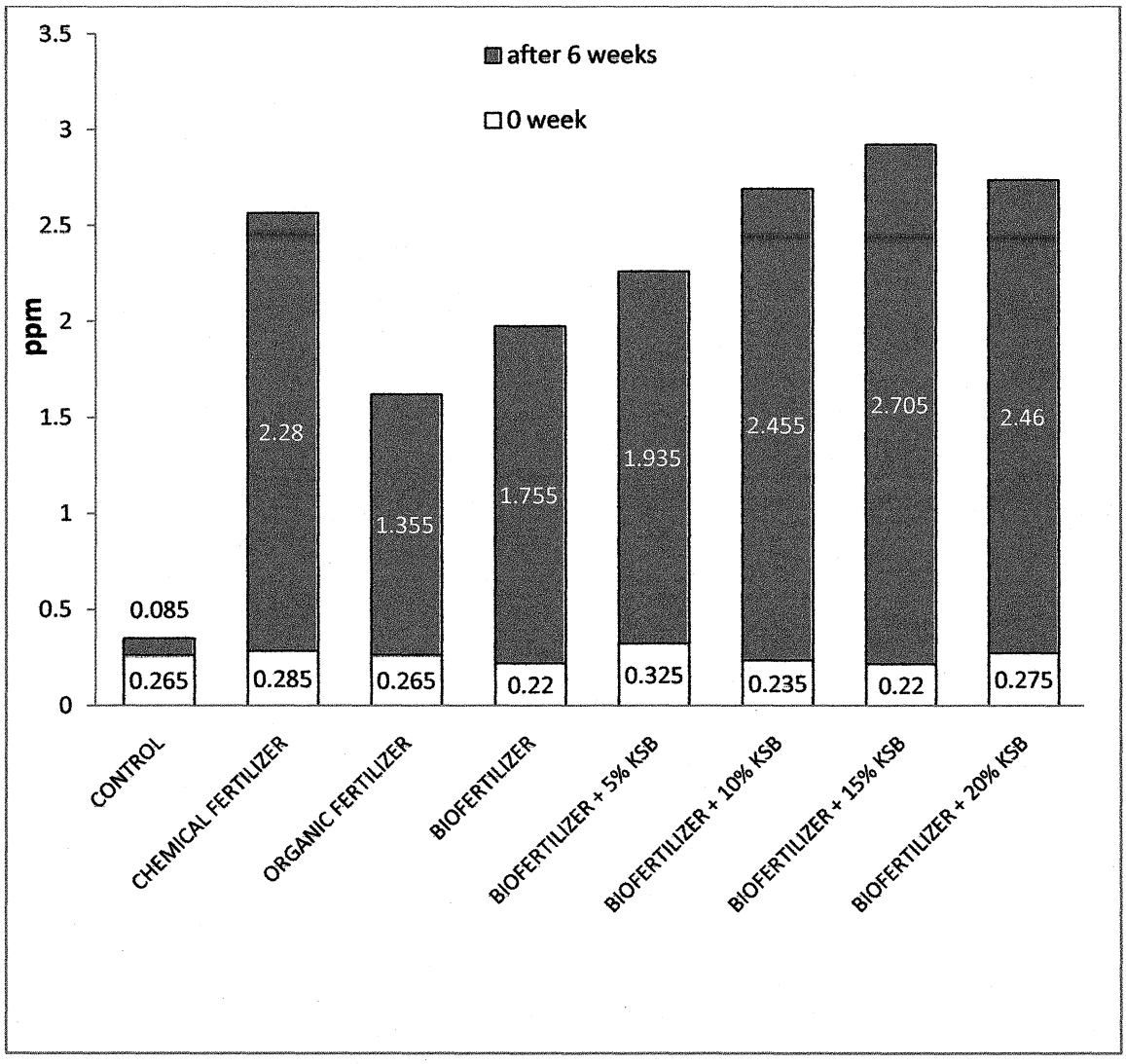


Figure 4.5: Comparison of potassium content in soil for each treatment after 6 week seedling

From the figure, the initial potassium content in the soil (0 week) for all treatment were almost same and after 6 weeks plantation, it is clearly shows that the

enhanced biofertilizer was able to achieved the same mineral content as in chemical fertilizer. It is because the addition of KSB in the treatment were able to solubilized the potassium in the soil after 6 weeks of plantation. This is important because of normally the usage of potassium is high at early growth stage of a plant than nitrogen and phosporus due to potassium play a vital role in protein synthesis, control of ionic balance, regulation of plant stomata and activation of plant enzymes (www.rainbowplantfood.com). From the figure also showed that the best result of potassium content in the plant for enhanced biofertilizer occur on biofertilizer with 15% of KSB followed by biofertilizer with 20% of KSB, biofertilizer with 10% of KSB and finally biofertilizer with 5% of KSB. This may because of the quantity of KSB in the biofertilizer were effect the amount of potassium solublized in the soil.

CHAPTER 5

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

The growth curve that obtained in part I of this study shows that KSB is in its active phase at the first 40 hours of fermentation at 20°C and 200 rpm agitation. The 20th hour of KSB cultivating were take in the biofertlizer preparation since it is best to cultivate the KSB in its active phase in order for the biofertilizer to give better result. This is due to the first 20 hour is in the log phase of the KSB active phase that was obtained in this study there were 40 hour.

The application of the enhanced biofertilizer containing beneficial microbes showed a promoting effect on the growth of patchouli and improvement of soil properties through 6 weeks of study (part II). The difference percentage of KSB inoculation can significantly give the similar effect like chemical fertilizer in terms of physical appearances and potassium content in the soil. This is being done by revising the results obtained from this study in which it showed that biofertilizer containing 10% of KSB, the plant showed a remarkable appearance in height, colour, number and diameter of leaves. Apart from that, biofertilizer containing 15% of KSB was give the best result in term of potassium content in the soil.

With the provided result, the best amount of KSB needed to enhanced the growth of the plants are between 10% to 15%. However excessive KSB content resulted to potassium deficiency symptom due to too much of potassium uptake by the patchouli plant. This can be seen from the dull appearance of the leaf and its dual tone colour of the leaf. The presence of KSB were proved able to solubilized the potassium content in the soil due to the potassium content that obtained in this study.

5.2 Recommendations

In order to improve this research, there are several things should be considered in the future. Firstly, reduce the range of percentage for KSB inoculation from 5% to 20% turn into 10% to 15% . This focusing range is required to achieve the best possible of plant growth and also the potassium content in the soil due to these range were provide the best result in this study. Furthermore, the additonal parameter also needed to keep on this study other than the physical appearences of the plant which are temperature and excess water effect to the plant. This is because of plants deficient in potassium are less resistant to drought, excess water, and high and low temperatures. (www.rainbowplantfood.com). From this additional parameter, it can show that whether the enhance biofertilizer containing KSB can give the same effect to the plant or not as this study. The recommended temperature is in range of ±10 from the normal Malaysia average termperature which is 33 ^{o}C (www.bbc.co.uk) and 50% of excess water from the normal water supply to the plant. Apart from that, to improve the undestanding on the potassium content in the plant, the detemination of potassium content on plant leave is needed rather than soil only because most of potassium uptake by the plant is come into view of the plant leaves whether the uptake it is high or not due to potassium is a vital component to their protein synthesis (www.rainbowplantfood.com). Therefore a better understanding on the effect of ehanced biofertilizer containing KSB in term of potassium content from the plant.

REFERENCES

Chen Zixi, Shengwu Ma and Lily(Lili) Liu (2008). Studies on phosphorus solubilizing activity of a strain of phosphobacteria isolated from chestnut type soil in China. *Bioresource Technology,* 99 (2008): 6702–6707

Han H.S and Lee K.D. (2005). Phosphate and Potassium Solubilizing Bacteria Effect on Mineral Uptake, Soil Availability and Growth of Eggplant. *Research Journal of Agriculture and Biological* 1(2): 176-180

Hasarin Ngampimol and Viyada Kunathigan (2008). The Study of Shelf Life for Liquid Biofertilizer from Vegetable Waste. *Assumption University Journal of Technology*, 11(4): 204-208

Hegde, D.M., Dwived, B.S. and Sudhakara, S.N., 1999. Biofertilizers for cereal production in India - a review. *Indian J. Agric.* Sci., 69:73– 83.

Hossain M.B.,Mian M.H.,Hashem M.A.,Islam M.Z. and Shamsuddoha A.T.M. (2001). Use of *Azola* as biofertilizer for cultivation of BR 26 rice in Aus season. *Online Journal of Biological Science,* 1(12): 1120 – 1123

http://www.fnca.mext.go.jp/english/bf/country_img/malaysia.pdf (4 March 2009)

http://www.microbelibrary.org/Culture%20Media/details.asp?id=2357&Lang= (25 February 2009)

http://www.rainbowplantfood.com/agronomics/efu/potassium.pdf (25 February2009)

http://www.bbc.co.uk/weather/world/city_guides/results.shtml?tt=TT002590. (25 April 2009)

Khairuddin Abdul Rahim (2002). Biofertilizer in Malaysia agriculture: perception, demand and promotion. *FNCA Joint Workshop on Mutation Breeding and Biofertilizer*: August 20-23, Beijing, China.

Nuruzzaman M., Ashrafuzzaman M., Islam M. Z. and Islam M. R., 2003. Field efficiency of biofertilizers on the growth of okra (*Abelmoschus esculentus* [(L.) Moench]). *Journal of Plant Nutrition and Soil Science*, 166 (6): 764-770

Shen, D., 1997. Microbial diversity and application of microbial products for agricultural purposes in China. *Agric. Ecosyst Environ,* 62: 237–245.Vessey, J.K., 2003. Plant growth promoting rhizobacteria as biofertilizers. *Plant Soil,* 255: 571–586.

Vessey, J.K., 2003. Plant growth promoting rhizobacteria as biofertilizers. *Plant Soil,* 255: 571–586.

Vibhas Kumar and Gurdeep Singh (2006). Biofertilizer: An eco-friendly fertilizer for the reclamation of overburden dumps.

Wu S.C, Cao Z.H., Li Z.G., Cheung K.C. and Wong M.H. (2003). Effects of biofertilizer containing N-fixer, P and K solubilizers and AM fungi on maize growth: a greenhouse trial. *Geoderma,* 125 (2005): 155–166

APPENDIX A

STANDARD CALIBRATION

Table A1: Standard calibration of glucose data

GLUCOSE CONCENTRATION (g/l)	OPTICAL DENSITY (A)	OPTICAL DENSITY (B)
0	0	0
0.1	0.074	0.075
0.2	0.237	0.237
0.3	0.392	0.392
0.4	0.52	0.515
0.5	0.663	0.663
0.6	0.755	0.775
0.7	0.894	0.894
0.8	1.026	1.02
0.9	1.154	1.146
1	1.231	1.221

Figure A1: Standard calibration curve of glucose

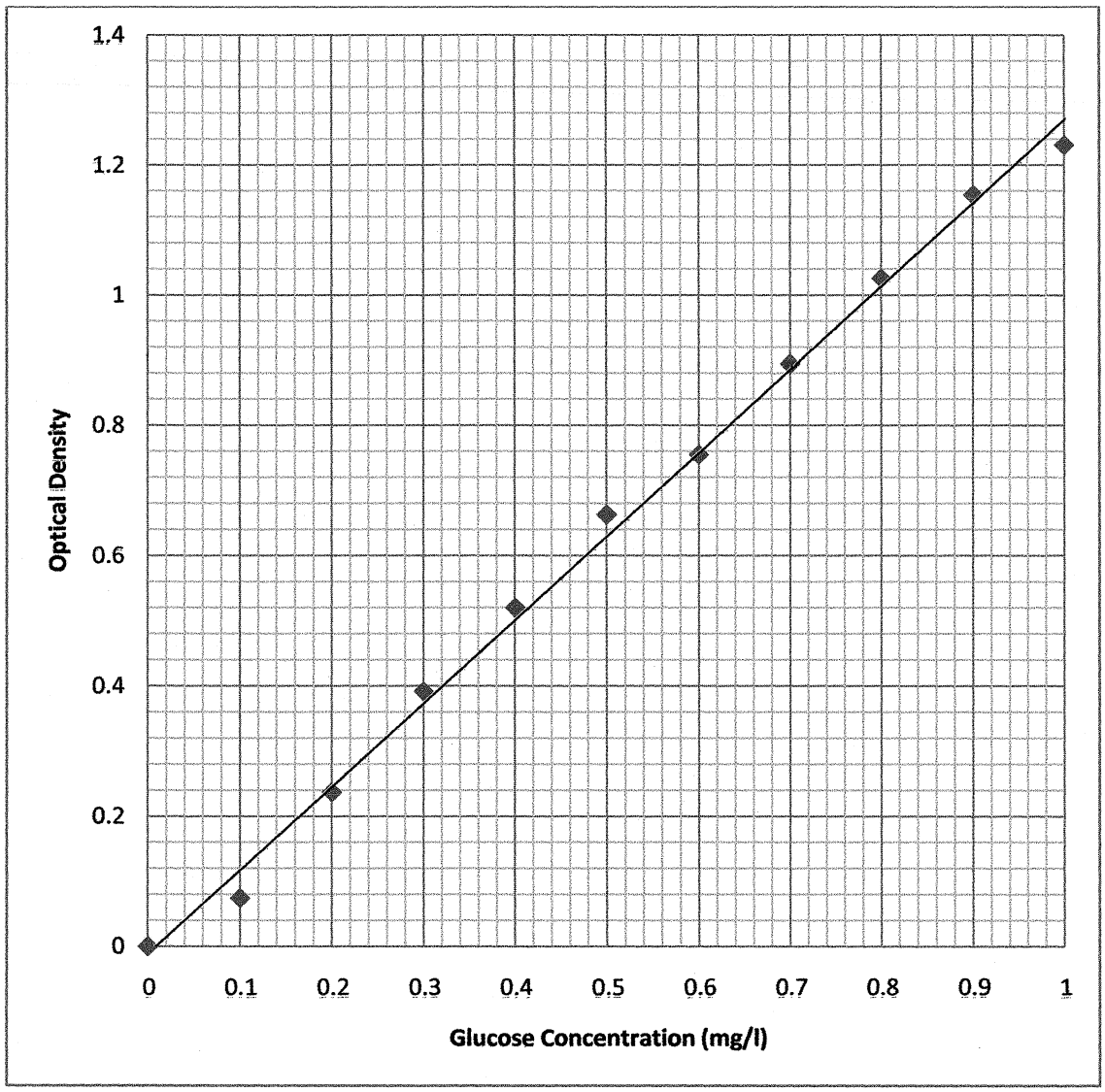
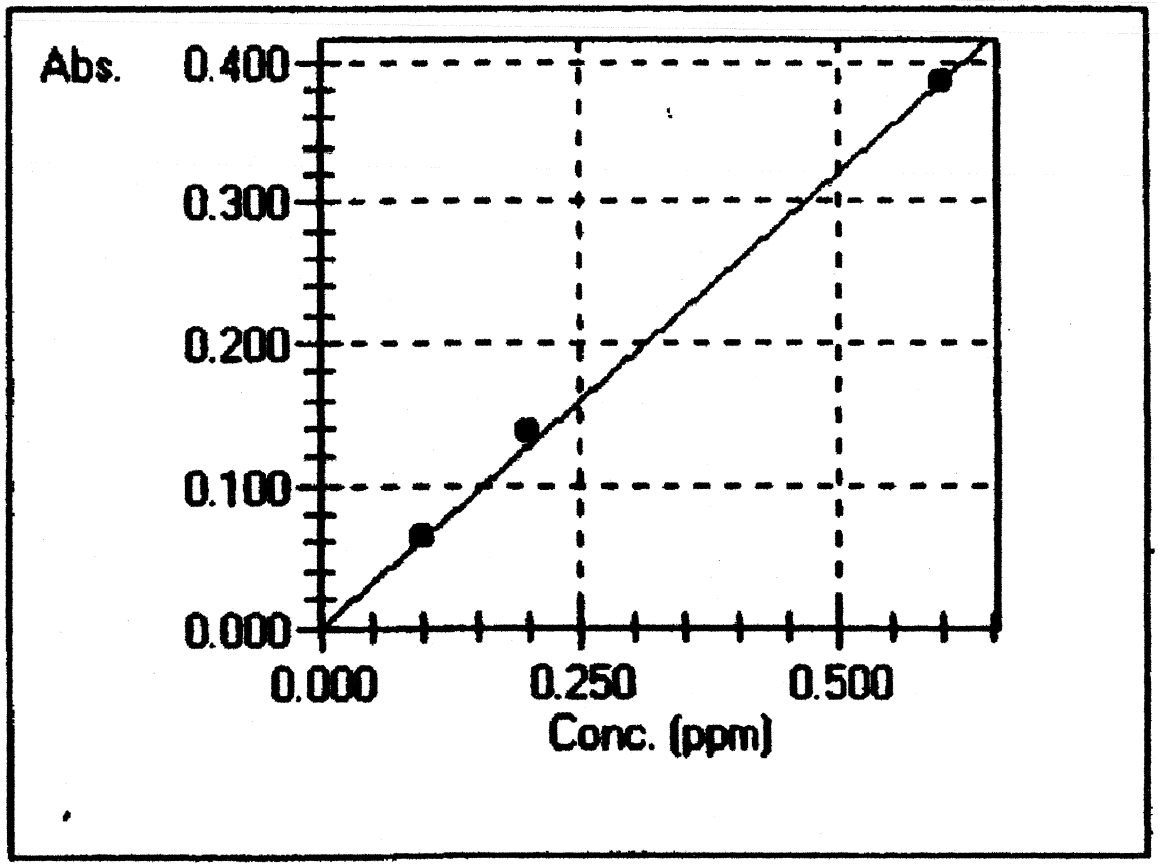


Figure A2: Standard calibration curve of Kalium
(from atomic absorption spectrometer)



APPENDIX C

DATA OF POT EXPERIMENT

Table C1: Pot experiment data (11 January 2009)

Week		Control		Chemical Fertilizer		Organic Fertilizer		BioFertilizer		BioFertlizer +5% KSB		BioFertlizer +10% KSB		BioFertlizer +15% KSB		BioFertlizer +20% KSB	
0		A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
	Colour Of Leaves	light green	light green	light green	light green	light green	light green	light green	light green	light green	light green	light green	light green	light green	light green	light green	light green
	Total Leaves	2	3	2	2	3	3	2	3	2	2	3	2	3	3	2	2
	Leaves Average Diameter (cm)	3.2	3.2	3.2	3.1	3.4	3.12	3.25	3.01	3.3	3.41	3.22	3.23	3.13	3.21	3.2	3.1
	Seedling Height (cm)	3.21	3.36	3.78	4.58	3.33	3.12	3.79	3.56	3.65	4.23	4.66	5.1	4.37	4.36	4.35	5.55
	Potassium Content (ppm)	0.21	0.32	0.12	0.45	0.22	0.31	0.3	0.14	0.2	0.45	0.36	0.11	0.21	0.22	0.34	0.21

2	**Colour Of Leaves**	green	green	green	green	green	green	green	green	green	green	green	green	green	green	green	green
	Total Leaves	4	6	5	4	4	6	6	5	5	7	7	6	6	5	6	7
	Leaves Average Diameter (cm)	2.5	4	5	4	3	3	4	5	5	5	5	6	6	6	6	6
	Seedling Height (cm)	4.2	4.6	6	7	7	6	6	8	8	6	7	7	6	7	6	8
	Potassium Content	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4	**Colour Of Leaves**	green	green	green	green	green	green	green	green	green	green	green	green	green	green	green	green
	Total Leaves	6	9	9	11	13	7	7	12	6	10	9	16	12	12	8	8
	Leaves Average Diameter (cm)	5	3	6.5	6.5	6.5	5	5.5	6	6.5	6.5	7	6	7	7	6	6
	Seedling Height (cm)	5	5	9.5	9.5	8	9.5	12	12	15	7	16	13	8	12	17	11
	Potassium Content	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6	**Colour Of Leaves**	yellow + green	yellow + green	green	green	green	green	green	green	green	green	green	green	green	green	green	green
	Total Leaves	6	9	10	20	13	7	7	12	6	10	9	16	12	12	8	8
	Leaves Average Diameter (cm)	5	3	6.5	6.5	6.5	5	5.5	6	6.5	6.5	7	6	7	7	6	6
	Seedling Height (cm)	5	5	10	11.3	8	9.5	12.2	12	15.3	8	16	13.2	8.7	12.2	17.7	11.5
	Potassium Content (ppm)	0.05	0.12	2.03	2.53	1.69	1.02	1.53	1.98	1.03	2.84	1.98	2.93	2.96	2.45	2.03	2.89