APPLICATION OF LIQUID BIO-FERTILIZER REDUCED THE NEED OF CHEMICAL FERTILIZER IN BLACK GALINGALE (*Kaempferia parviflora*) PRODUCTION

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ABSTRACT

ULYA ZULFA. APPLICATION OF LIQUID BIO-FERTILIZER REDUCED THE NEED OF CHEMICAL FERTILIZER IN BLACK GALINGALE (*Kaempferia parviflora*) PRODUCTION. (Supervised by NURUL KHUMAIDA and SINTHO W. ARDIE)

Kaempferia parviflora Wall ex. Baker (black galingale) is a medicinal plant which has high potential to be developed in Indonesia as medicinal products. Thus, in order to develop appropriate standard operasional procedure in *K parviflora* cultivation, this research was conducted to study the effect of bio-fertilizer applicationon late-vegetative growth and yield of rhizome. The experiment was arranged in Randomize Completely Block Design (RCBD), where the treatment were Control (0 L ha^{-1} of bio-fertilizer and 100% of chemical fertilizer), D1 (15 L ha^{-1} of bio-fertilizer and 50% of chemical fertilizer), D2 (22.5 L ha^{-1} of bio-fertilizer and 50% of chemical fertilizer), and D3 (30 L ha^{-1} of bio-fertilizer and 50% of chemical fertilizer). The 100% chemical fertilizer application were consisted 300 kg ha^{-1} urea, 250 kg ha^{-1} SP-36, and 250 kg ha^{-1} KCl. The block of the experimental design was based on the shading position of raised bed and difference of sun-lighting time.

The result showed that application of 15 L ha^{-1} of bio-fertilizer and 50% of chemical fertilizer resulted in the best late-vegetative growth (i.e. plant height, leaf number, leaf length, and leaf area) of *K. parviflora* up to 29 week after planting (WAP). Moreover, application of 50% of chemical fertilizer with various rate of bio-fertilizer resulted in similar rhizome yield compared to those planted with 100% of chemical fertilizer. Thus, application of bio-fertilizer can be recommended to be applied in *K. parviflora* cultivation since it could reduce the usage of chemical fertilizer (urea, SP 36, and KCl) up to 50%. The potencial productivity from each treatment range from 4.38 to 8.19 ton ha^{-1}. The result also imply that the usage of bio-fertilizer could suppress disease severity of root-knot caused by nematode in the field.

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The thesis is submitted to the Faculty of Agriculture as one of the requirements for achieving Bachelor of Science in Agriculture

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BIOGRAPHY

Author was born at 6 February 1990 in Kendal, Central Java. Author was first child from H.Achmad and Hj.Suminah, with one siblings from Aminatun Nafi'ah. Author spends her childhood in Kendal.

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PREFACE

Praise and thanks are prayed for Allah SWT so the author could finish a research for bachelor degree thesis entitled "Application of Liquid Bio-Fertilizer Reduced the Need of Chemical Fertilizer in Black Galingale (*Kaempferia Parviflora*) Production". Author would like to express her gratitude to these people:

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Bogor, November 2012

Ulya Zulfa

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INTRODUCTION

Background

The demand of medicinal plants in domestic market is relatively high and tends to increase because of increasing awareness of the public to consume natural medicine. The domestic market that absorb upstream agribusiness products (seed) and downstream (simplicia, starch, oils, extracts) of medicinal plants is 1,023 companies, consist of 118 IOT (*Industri Obat Tradisional*) and 905 IKOT (*Industri Kecil Obat Tradisional*). Medicinal plants are used for IKOT, IOT, and the pharmaceutical industry average by 63%, while for exports and household consumption by 14 and 23% (Ministry of Agriculture, 2007).

Moreover, herbal medicine trade in international market also tends to increase. In 2000 the value of trade in herbal medicines reached U.S. $ 40 million, then in 2002 increased to U.S. $ 60 million, and in 2050 is estimated to be U.S. $ 5 billion with an increase of 15% per year, higher compared with an increase in the value of trade in synthetic medicinal that only 3% per year (Ministry of Agriculture, 2007). Galingale (*Kaempferia galanga* L.) became the most widely cultivated medicinal crops in Indonesia, after ginger (*Zingiber officinale*) and galangal (*Alpinia galangal*).

Like galingale (*Kaempferia galanga*), black galingale (*Kaempferia parviflora* Wall ex. Baker) is a medicinal plant that have high potential to be developed as medicinal products (Chivapat *et al.*, 2010). According to the previous research, black galingale rhizome can be used as an anti-inflammatory and anti-HIV (Sookkongwaree *et al.*, 2006), anti-cancer (Patanasethanont *et al.*, 2007), and anti-allergic (Tewtrakul *et al.*, 2008). Traditionally black galingale rhizome is used as a drug for hypertension patients. Research shows that the ethanol extract of rhizome black galingale triggers vasorelaxant effects on rat aorta vascular (Tep-areenan *et al.*, 2010).

Black galingale is a medicinal plant from tropical countries Thailand. These crops are not cultivated yet in Indonesia, thus the adaptation in difference regions of Indonesia have not known yet. Evi (2012) explained that black

galingale showed better growth under shading than full sun. Introduction of black galingale is conducted to determine the suitable agroclimate for these plants so that a black galingale cultivation procedure in Indonesia could be developed. Fertilization is one of important technology in the cultivation of plants. Standard operational procedures for fertilizing galingale (*Kaempferia galanga* L.) in Indonesia are manure 30 ton ha^{-1}, urea 300 kg ha^{-1}, SP-36 250 kg ha^{-1}, and KCl 250 kg ha^{-1}, depending on the fertility of the soil at each specific location (Rostiana and Effendi, 2007). Previous research also refers to this standard operasional procedure for fertilizing galingale. But this standard operasional procedure should be reviewed and corrected according to the type of plants and development of technology, especially for black galingale that only introduced in Indonesia recently.

The management of fertilization must attempt to ensure both an enhanced and safeguarded environment so that a balanced fertilization strategy that combines the usage of chemical and organic fertilizer must be developed and evaluated (Chen, 2006). Using bio-fertilizer is one of the efficient ways to improving plants production by reducing the usage chemical fertilizers (Soleymanifard and Sidat, 2011). The research "Application of Liquid Bio-Fertilizer Reduced the Need of Chemical Fertilizer in Black Galingale (*Kaempferia Parviflora*) Production" is part of attempts to develop a standard operational procedure for black galingale cultivation in Indonesia.

Objective

The research was conducted to:

1. Study the effect of various rate of liquid bio-fertilizer on plant growth and rhizome production of black galingale (*K. parviflora* Wall ex. Baker).
2. Get a recommendation rate of liquid bio-fertilizer on black galingale (*K. parviflora* Wall ex. Baker) cultivation.

Hypothesis

There is a rate of liquid bio-fertilizer that increase plant growth and production of black galingale rhizome (*Kaempferia parviflora* Wall Ex. Baker).

LITERATURE REVIEW

Black Galingale Plant

Kaempferia parviflora Wall. Ex. Baker known as black galingale belong to the family Zingiberaceae. This plant is indigenous herbal plants that can be found in Thailand, India and Burma. In Thailand, *K. parviflora* are widely cultivated in the province of Loei. *K. parviflora* also known as Thai viagra or ginseng. There was a research evaluated mitigative effect of aqueous rhizome extract of *K. parviflora* against testicular toxicity in male rats (Luangpirom and Komnont, 2011). The taxonomy of black galingale is as follow.

Kingdom : Plantae
Sub Kingdom : Viridaeplantae
Phylum : Tracheophyta
Sub Phylum : Euphyllophytina
Class : Liliopsida
Sub Class : Commelinidae
Ordo : Zingiberales
Family : Zingiberaceae
Genus : *Kaempferia*
Species : *Kaempferia parviflora* Wall ex. Baker
Thailand name : Kra-Chai-Dahm, Kra-Chai-Dum, Thai viagra or ginseng
Scientific synonim : *Boesenbergia pendurata* (Roxb.) Holtt.

Rhizome of *K. parviflora* is fleshy. Cross section of *K. parviflora* rhizome is orbicular or ellipse and have a circle line in the center. The flesh color is violet to blackish purple (Evi, 2012). The purplish color of the rhizome leads to the name Kra-chai-dahm in Thailand (Putiyanan *et al.*, 2008). Rhizome contains bud that will grow to individual plant. As dormancy broken, a shoot appear from this bud. The plane of leaf is parallel to rhizomes. This plant has single blade leaf. The leaf is broadly elliptic with rounded base, 8-21 cm long, and 5-11 cm wide. The leaf venation is parallel with dark green color adaxial leaf. Border of leaf is wavy, sometimes with reddish color. The abaxial leaf is variegated between green and red. The color of leaf is vary depend on environment. Abaxial leaf color in high

altitude had more red composition than in lower altitude (Evi, 2012). *K. parviflora* is a ground herb that grows to 90 cm tall. The middle of the stems with light red color is sheath petiole densely covered with speckling on light green (Putiyanan *et al.*, 2008). In *K. parviflora,* inflorescence appears on the terminal of the stem between the leaves. *K. parviflora* have one to four flowers from one inflorescence. Flower of *K. parviflora* are white with purple spot. The flower is irregular or bilaterally symmetrical (the flower can be cut only along one plane to produce equal halves) (Evi, 2012). The flowers have 2 bracts, 6 stamens, anther is close to long, small style, stigma, brass-shape, hairless, dehiscent fruit split into 3 rays when mature. Seeds are quite large (Putiyanan *et al.*, 2008).

Evi (2012) also explained that *K. parviflora* can be distinguished with the *K. galanga* or galingale, the common herbaceous plant from Indonesia, from the absence of purplish color that *K. parviflora* has in its leaf and stem, the color of the rhizome, and also from the flower. Galingale flower is bigger (about 2.5-3 cm) and have ribbon-like petal. The morphology of *Kaempferia parviflora* Wall Ex. Baker is shown in Figure 1.

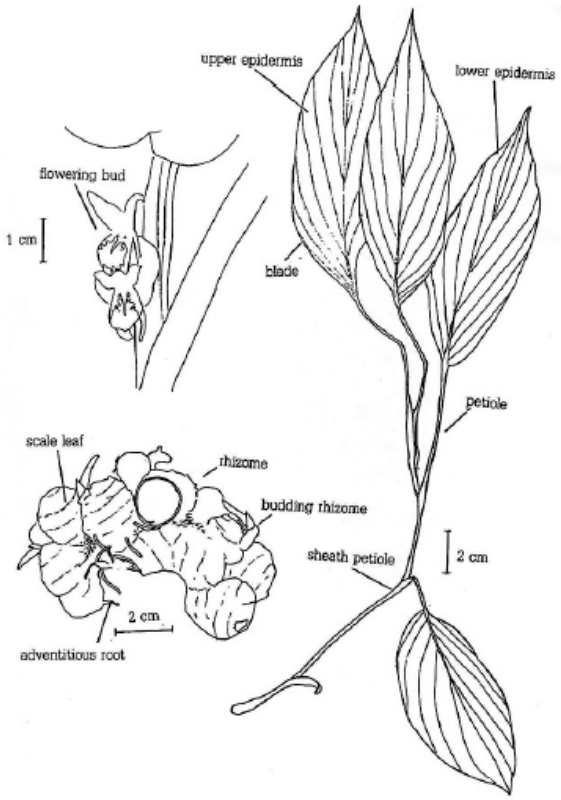

Figure 1. Morphology of *Kaempferia parviflora* Wall Ex. Baker (Putiyanan *et al.*, 2008)

Based on the previous research, rhizome of *K. parviflora* contained some flavonoids, such as 5-hydroxy-3,7-dimethoxyflavone, 5-hydroxy-7-methoxyflavone, 5-hydroxy-3,7,4'-trimethoxyflavone, 5-hydroxy-7,4'-dimethoxyflavone, 3,5,7,4'-tetramethoxyflavone, 5,7,4'-trimethoxyflavone, 5,7,3 '4'-tetramethoxyflavone, and 5-hydroxy-3,7.3'4'tetramethoxyflavone, 3,5,7-trimethoxyflavone (Trisomboon, 2009). Tanasiriwattana *et al.* (1997) in Kummee *et al.* (2008) stated that the essential oil of the *K. parviflora* is borneol (46.41%) and sylvestrene (25.30%). In addition, black galingale rhizome contains a variety of flavonoids such as hydroxyflavone (Daodee *et al.*, 2003; Yenjai *et al.*, 2004) and anthocyanins (Vichitphan *et al.*, 2007). Both flavonoids are anti-HIV (Sookkongwaree *et al.*, 2006), anti-cancer (Patanasethanont *et al.*, 2007), and anti-depressant (Wattanathorn *et al.*, 2007).

Ethanol extract of *K. parvflora* has the highest potential as an anti-allergic than *Kaempferia galanga, Curcuma mango, Zingiber cassumunar, Zingiber oficinale,* and *Zingiber zerumbet* (Tewtrakul *et al*., 2008). Kummee *et al.* (2008) added that the anti-microbial activity of extracts of *K. parviflora* can resist disease agents, such as bacteria, yeast and fungi. Tep-areenan *et al.* (2010) showed that ethanol extract of *K. parviflora* rhizome triggers vasorelaxant effects in rat aorta, so *K. parviflora* rhizome can be used as a hypertension drug. *K. parviflora*'s ethyl acetate extract is now considered to be an invasive enteropathogen because it could significantly inhibit the invasion of both *Helicobacter pylori*, an etiological agent of active cause chronic gastritis and peptic ulcer disease (Chaichanawongsaroj *et al.*, 2010).

Galingale (*Kaempferia galanga* L.) Nutrient Uptake Research in Indonesia

Nutrient requirements of rhizome medicinal plants are quite high. Anorganic fertilizers (urea, TSP/SP-36, and KCl) and organic fertilizer/manure are the common sources of additional nutrient applied on plants (Ruhnayat, 2008). Optimal fertilization can be measured by the efficiency of nutrient uptake by plants or organs. Research of Rosita *et al.* (2007) on galingale (*K. galanga* L.) fertilization showed that nutrient uptake ranged 149.60-415.60 N mg plant^{-1}, P

uptake ranged between 41.50-112.50 mg plant^{-1} and K uptake ranged from 236.10-571.70 mg plant^{-1}.

Phosphorus (P) uptake in rhizomes is less than N and K. This fact similar to research on other rhizome plants, such as *Curcuma aeruginosa* (Djazuli *et al.*, 2001; Rosita *et al.*, 2005a) and ginger (Rosita *et al.*, 2005b). Rosita *et al.* (2007) explained that the need of P on crops is generally less than the others nutrients. Potasium is very important to transport photoshyntate to the storage organs. When the leaves and stems become senescence, nutrients uptake can be seen from the rhizome because the nutrient is translocated to the rhizomes. Rosita *et al.* (2007) analyzed 82.03 g fresh rhizome of promising lines *K. galanga* which planted by giving manure 20 ton ha^{-1} urea + 250 kg ha^{-1} + SP-36 200 kg ha^{-1} KCl + 200 kg ha^{-1}. The result showed that nutrient uptake into the rhizomes is 415.60 mg plant^{-1} N (equivalent to 83.12 kg ha^{-1} N), 112.50 mg plant^{-1} P (equivalent to 22.50 kg ha^{-1} P) and 571.70 mg plant^{-1} K (equivalent to 114.34 kg ha^{-1} K).

In other medicinal rhizome plant research, *Curcuma xanthorrhiza* Roxb., application of urea, SP-36, and KCl with each rate of 100 kg ha^{-1} produced 20.23 ton ha^{-1} rhizome, which absorbed 137.41 kg ha^{-1} N, 15.30 kg ha^{-1} P, and 146.11 kg ha^{-1} K. Application of urea, SP-36, and KCl of 300 kg ha^{-1}, 200 kg ha^{-1}, and 200 kg ha^{-1}, respectively, produced 25.46 ton ha^{-1} rhizome, which absorbed as much as 193.44 kg ha^{-1} N, 21.05 kg ha^{-1} P, and 221.34 kg ha^{-1} K (Rahardjo and Pribadi, 2010). Organic fertilizer should be used in the cultivation of medicinal plants to reduce the usage of anorganic. One of organic fertilizer is bio-fertilizer. Ruhnayat (2008) stated that bio-fertilizers can give positive respond to the growth and rhizome production of medicinal plants.

Biological Fertilizer (Bio-fertilizer)

Bio-fertilizer is fertilizer containing advantageous microorganisms. Simanungkalit *et al.* (2006) stated that bio-fertilizers are inoculants containing living organisms that can increase the availability of nutrients in the soil for plants. Bio-fertilizer usually combined with some type of a particular carrier material as microbial growth media. Bio-fertilizer product with a solid carrier material (granule) is less available in the market than bio-fertilizer product with a

liquid carrier material because process to make granule is difficult and need more cost. Certain bio-fertilizer contains specific microorganisms and the combined microorganisms resulted in specific function (Table 1).

Table 1. Microbial content of some commercial bio-fertilizers in Indonesia (Simanungkalit *et al.*, 2007)

Bio-fertilizer	Content of microbial	Function	Form, color
1	- *Azospirillum lipoverum* - *Aspergillus niger* - *Aeromonas punctata* - *Azotobacter beijerincki*	Fixing N, solving P, improving soil aggregates	Solid granules, gray-white
2	- *Rhizobium* - Solving P bacteria	Fixing N, solving P	Solid peat, dark gray
3	- Solving P bacteria - *Lactobacillus* - *Rhizobium* - *Azotobacter* - *Actinomycetes*	Fixing N, solving P, decomposing organic material	Liquid, brown
4	- *Bacillus* - Yeast - *Azotobacter* - *Lactobacillus* - *Acetobacter*	Fertilizing the soil, improving soil structure, controlling plant diseases	Liquid, brown
5	*Actinomycet* bacteria, yeast, fungi	Decomposing organic material	Solid peat, dark brown
6	- *Trichoderma* sp. - *Aspergillus niger* - *Azotobacter* sp. - *Azospirillum* sp.	Decomposing organic material	Solid peat, dark brown
7	- *Trichoderma pseudokoningii* - *Cytopaga* sp.	Decomposing organic material	Solid peat, dark brown

Note : Trade name of bio-fertilizers are not mentioned

Microorganisms contained in bio-fertilizers have the ability for decomposing organic material, binding heavy metal, supplying most of the N requirement for crops, dissolve phosphate compounds, releasing the compound K from soil colloidal bond, producing plant growth hormone (gibberellin, cytokinin, indole acetic acid) and anti-pathogenic substances for specific microorganisms. Indirectly, bio-fertilizers can increase productivity of crop, improve quality of yield, increase nutrients uptake, reduce the rate of anorganic fertilizer, improve

the physical-chemical-biological structure of soil, control pest and plant disease, and make the balance of flora-fauna in the soil.

Bacteria in Liquid Biological Fertilizer

Generally, liquid biological fertilizer or bio-fertilizer containing various microorganisms that can fix N_2, triggers plant growth, produce plant growth hormone, anti-pest, anti-odor, and decompose cellulose.

1. **N_2 fixing-bacteria and plant growth hormones producing bacteria (*Azospirillum lipoferum* ICBB6088 and *Azotobacter vinelandii* ICBB9098)**

   Azospirillum has a high potential to be developed as bio-fertilizers. There are three known species that have the ability in fixing nitrogen, *A. brasilense, A. lipoferum*, and *A. amazonense*. Mechanical association of these bacteria do not cause changes in root morphology, but increasing the number of hair roots and branching roots to be more intensive in the absorption of nutrients. If there is no activity of fixing nitrogen, mechanical association of bacteria is limited only to increased absorption of nitrogen in the soil.

   Differently, *Azotobacter* spp. is a non-symbiotic bacteria living near plant roots area (rhizosphere). These bacteria were found in all soil types but the population is relatively low. Like *Azospirillum*, *Azotobacter* can also improve plant growth by supplying nitrogen and increasing growth hormones, reducing competition with other microbes in fixing nitrogen, and creating more favorable soil conditions for plant growth. *Azotobacter* inoculation study has been able to increase yield of the variety of plants and accelerate seed germination (Saharan and Nehra, 2011). Because of these reasons, *Azotobacter* as bio-fertilizer can increase yield, reduced the usage of anorganic fertilizers, and increase soil biological activity (Jarak *et al*., 2006).

2. **Antagonistic bacterial with anti-bacterial and anti-pest activities (*Bacillus thuringiensis* ICBB 6095, *Paenobacillus macerans* ICBB8810, and *Saccharomyces cerevisiae* ICBB 8808)**

   Bacillus thuringiensis is a pathogenic bacterium (cause disease) in a particular pest, but do not cause disease in plants. The research showed that *B. thuringiensis* is able to produce a protein that have toxic effect to insects,

particularly insects of the order Lepidoptera, Diptera, and Coleoptera. This protein is soluble and active to be toxic, especially after entering the digestive system of insects. *B. thuringiensis* can be used as biopesticides pest plants. The usage of biopesticides is expected to reduce the negative impact from the usage of chemical pesticides in agricultural products. *B. thuringiensis* strains and new isolates were evaluated for its use as a microbial pesticide (Bahagiawati, 2002). *B. thuringiensis* is known as the most numerous bacteria in the area rhizosfer. Some strains of *Bacillus* can produce plant growth hormone for the plant. Several other strains can also be used as bio-inoculants in the manufacture of bio-fertilizer to increase production of various crops (Saharan and Nehra, 2011).

P. macerans have capability to against *Ralstonia solanacearum* by producing antibacterial activity of protein (Li *et al.*, 2010). *Saccharomyces cerevisiae* can produce chitinase and protease for destroying chitine layer of nematode eggs (Ahmad, 2007).

3. **Cellulose decomposer (*Paenobacillus macerans* ICBB8810, *Lactobacillus* sp. ICBB 6099, *Saccharomyces cerevisiae* ICBB 8808, and *Microbacterium lacticum* ICBB 7125)**

Paenibacillus macerans is the aerobic bacteria that have capability of forming endospores. These bacteria can live in the rhizosphere so the penetration of bacteria in soil can be done easily (Gardener, 2004). *P. macerans* can produce cycloglycosyltransferase enzyme (CGTase), which can decompose cellulose such as ß-cyclodextrin and other carbohydrates. Genus of *Lactobacillus* is a lactic acid bacteria that can be used to accelerate the decomposition process of organic materials and minerals. In its activities produce organic acid that can support plant growth (Hardiningsih *et al*., 2006).

MATERIAL AND METHOD

Place and Time

Research was conducted in November 2011 to June 2012 at Pasir Sarongge Experimental Field, University Farm, Bogor Agricultural University, Cipanas with altitude 1,200 m above sea level (asl) with soil type Andosol. Soil samples was taken before treatment of bio-fertilizer, then analyzed in Indonesian Soil Research Institute Bogor, while the plant tissue analysis was conducted in Plant Analysis and Chromatography laboratory, Department of Agronomy and Horticulture, Bogor Agricultural University. Observation of stomatal density and chlorophyll were conducted at Microtechnique and Molecular Marker Laboratory and Spectrophotometry laboratory, Department of Agronomy and Horticulture, Bogor Agricultural University. After harvesting, the rhizomes also were analyzed in Post Harvest laboratory, Department of Agronomy and Horticulture, Bogor Agricultural University.

Material and Equipment

Plant material used in this research was *K. parviflora* rhizomes which were received from Ogawa Indonesia Company. Rhizomes with one bud ($\pm$ 2 cm length) were cut to approximately 15 g. Rhizomes were then planted at 5 cm depth and 20 cm x 25 cm plant spacing in a raised bed. The raised bed size was 80 cm x 250 cm x 30 cm (width x length x height), thus there were 40 rhizomes planted in one raised bed. *K. parviflora* was planted at 10 June 2011.

Fertilizer were applied following standard procedure of common galingale, that are 30 ton ha^{-1} manure, 300 kg ha^{-1} urea, 250 kg ha^{-1} SP-36, and 250 kg ha^{-1} KCl (Rostiana and Effendi, 2007). Chicken manure 30 ton ha^{-1} was applied at planting day, while half rate of urea, SP-36 and KCl were applied at 18 WAP. Bio-fertilizer used contained *Azospirillum lipoferum* ICBB 6088, *Azotobacter vinelandii* ICBB 9098, *Paenibacillus macerans* ICBB 8810, *Microbacterium lacticum* ICBB 7125**,** *Bacillus thuringensis* ICBB 6095, *Saccharomyces cerevisiae* ICBB 8808, and *Lactobacillus* sp. ICBB 6099, 10^7 cfu ml^{-1}, respectively.

Weeding was done manually every two weeks. Pest controlling was done manually and physically by using trap. Traps are made from yellow plastic covered by sticky glue.

Equipment used for the treatment of liquid bio-fertilizer was measuring glass. Equipment used for the observations are Leaf Area Meter portable type LI-3000C, SPAD portable, light microscopy, stomatal and clorophyll analysis equipment, also soil and plant tissues analysis equipment. Others equipment were tillage tool, ruler, label, camera, weight scale, and oven.

Method

Research was conducted using the Randomize Completely Block Design (RCBD) which the treatment are:

C : 0 L ha^{-1} bio-fertilizer, full rate of urea, SP-36, and KCl (100% chemical fertilizer).

D1 : 15 L ha^{-1} bio-fertilizer, half rate of urea, SP-36, and KCl (50% chemical fertilizer).

D2 : 22.5 L ha^{-1} bio-fertilizer, half rate of urea, SP-36, and KCl (50% chemical fertilizer).

D3 : 30 L ha^{-1} bio-fertilizer, half rate of urea, SP-36, and KCl (50% chemical fertilizer).

Liquid bio-fertilizer was applied 2 times, by watering half rate in soil at 5 and 7 month after planting (MAP). First application of half rate bio-fertilizer at 5 MAP was conducted to promote the late-vegetative growth, while second application of half rate bio-fertilizer at 7 MAP was conducted to promote the rhizome growth. Linear model used was:

$$Y_{ij} = \mu + \alpha_i + \beta_j + \varepsilon_{ij}$$

Where :

Y_{ij} = sum of i fertilizer treatment and j block

μ = average

α_i = i effect of treatment (0 L ha^{-1} bio-fertilizer and 100% chemical fertilizer (C), 15 L ha^{-1} bio-fertilizer and 50% chemical fertilizer (D1), 22.5 L ha^{-1} bio-fertilizer and 50% chemical fertilizer (D2), also 30 L ha^{-1} bio-fertilizer and 50% chemical fertilizer (D3))

β_j = j effect of replication as block (j = shade position with 6 hours of sun-lighting, half shade position with 9 hours of sun-lighting, and full sun position with 12 hours of sun-lighting)

ε_{ij} = treatment error

Obsevation data were tested for normality then tested with F-test at 5% level. Data with significant difference was tested with Duncan Multiple Range Test. All tests were analyzed by SAS V9 software.

Observation

The observation started 22 week after planting (WAP) by taking 5 plants in each unit of experiment. Parameters observed during the research were:

- Height of plants were measured from ground to highest leaf, every two weeks
- Number of leaf per plants were measured every two weeks
- Width and length of specific leaf were measured every two weeks
- Number of shoot per plants
- Leaf area of specific leaf was measured using Portable Leaf Area Meter type LI-3000C at 7, 8, and 9 MAP
- Green color intensity of specific leaf were measured using SPAD at 7, 8, and 9 MAP
- Stomatal density at 5 and 6 MAP was measured by using nail coat which swiped upon leaf surface, dried, taken from leaf surface, then sticked to object glass, and then observed under microscope. Each stomata counted manually, using formula :

$$\frac{\text{n stomata}}{\text{L } (\text{mm}^2)} = \frac{\text{x}}{1\ \text{cm}^2}$$

n stomata = number of stomata that counted

x = stomatal density per cm^2

L = microscope seeing area, was circle area which diameter 0.5 mm^2, so can be counted by using formula :

$$¼\ \pi d^2 = ¼ \text{ x } 3.14 \text{ x } 0.5^2 = 0.19625\ mm^2$$

- Chlorophyll content at 7 and 9 MAP by using Sims and Gamon (2002) method (Appendix 1)

- Character, structure, and color of rhizome were observed under microscope
- Fresh- and dry-weight of shoot at harvesting time
- Fresh- and dry-weight of rhizome per plant at harvesting time
- Potencial and actual productivity

Soil analysis and plant tissue analysis were used to determine the amount of nutrient uptake by plants. Data for temperature and humidity was collected from Meteorology, Climatology and Geophysics Institution (BMKG) for 7 months experiment

In this experiment, diseases intensity were counted by two parameter, diseases incidence of bacterial wilt and diseases severity of root-knot. Diseases incidence of bacterial wilt were measured by formula

$$\text{Disease insidence} = \frac{n}{N} x100\%$$

n = the number of plant which showed the symptom bacterial wilt

N = population of observed plant

After harvesting time, the root-knot were collected and compared with the healthy root of *K. parviflora*. The ratio of root-knot to root (R:K) showed diseases severity counted by formula

$$\text{Diseases severity} = \frac{\Sigma(n \times v)}{N \times Z}$$

n = the number of plant from each category of symptom root-knot

v = score value of each category of symptom root-knot

N = the number of observed plants

Z = score value of the highest category of symptom

According to Kusnanta (2005) research in rust disease patchouli plant (*Pogostemon cablin*) stated that diseases intensity are determined 5 category, there are score 0 (no symptom, 0%), score 1 (mild symptom, 1-25%), score 2 (medium symptom, 26-50%), score 3 (high symptom, 51-75%), and score 4 (severe high symptom, >75%).

RESULT AND DISCUSSION

General Condition

Soil characteristic in the experimental field was analyzed in Indonesian Soil Research Institute Bogor before bio-fertilizer and chemical fertilizer were applied (Table 2). The soil in Pasir Sarongge was categorized as Andosol and was slightly acidic (pH 5.2-5.6), high of C-organic (4.5%), P_2O_5 (19 ppm), high of base saturation Ca^{2+} 16.63 $cmol_c\ kg^{-1}$, Mg^{2+} 2.54 $cmol_c\ kg^{-1}$, K^+ 0.77 $cmol_c\ kg^{-1}$, but low of base saturation Na^+ 0.25 $cmol_c\ kg^{-1}$. Base saturation is used to manage soil Na^+ and can be utilized to determine soil Mg^{2+} availability.

Table 2. Soil analysis of Pasir Sarongge experimental field before treatment (5 MAP)

Parameter	Value	Status
Texture (%)		
a. Sand	44	Loam
b. Silt	43	
c. Clay	13	
pH		
a. H_2O	5.6	Slightly acid
b. KCl	5.2	
C-organic (%)	4.05	High
N total (%)	0.37	Moderately high
C/N ratio	11	Moderately high
P_2O_5 Olsen (ppm)	19	High
K_2O HCl 25% (mg $(100\ g)^{-1}$)	39	Moderately high
CEC ($cmol_c\ kg^{-1}$)	9.58	Low
Cation component:		
a. Exchangeable Ca ($cmol_c\ kg^{-1}$)	16.63	High
b. Exchangeable Mg ($cmol_c\ kg^{-1}$)	2.54	High
c. Exchangeable K ($cmol_c\ kg^{-1}$)	0.77	High
d. Exchangeable Na ($cmol_c\ kg^{-1}$)	0.25	Low
Base saturation (%)	>100	Very high

Base saturation refers to the fraction of the Cation Exchange Capacity (CEC) that is occupied by the basic cations, Ca^{2+}, Mg^{2+}, K^+, and Na^+. Although base saturation was very high, the CEC was low (9.58 $cmol_c\ kg^{-1}$) because the soil

were low in clay (13%). The primary factor determining CEC is the clay and organic matter content of the soil. This experimental field soil had 13% of clay content, but C-organic was 4.05%, categorized as high level. CEC is a measure of the soils ability and capability to hold exchangeable ions. Cation is an ion with a positive electrical charge, while the clay and organic matter of the soil supplies the negative charges. Cations have the ability to be exchanged for another positively charged ion from the surfaces of clay minerals and organic matter. Higher quantities of clay and organic matter may result in higher CEC (Camberato, 2001).

The C/N ratio of soil sample was in high criteria (11:1). C/N ratio for plants growth is 10:1, thus the soil in Pasir Sarongge experimental field had relatively good C/N ratio. Herrmann (2003) showed that C/N ratio below 25:1 indicates that mineralization is higher than immobilization, so NH_4^+ will be released. Soil organic matter is continuously decomposed by a range of soil microorganisms including bacteria, fungi and their predators resulting in the release of ammonium (NH_4^+) (mineralization). While mineralization of soil organic matter provides C (energy) for microbial maintenance and growth.

In the early December 2011, some plant diseases symptoms were found in some spots of raised bed. The analysis result of plant sample in Plant Clinic Laboratory of Bogor Agricultural University showed that *K. parviflora* suffered from bacterial wilt caused by *Ralstonia solanacearum* (Fig. 2A). This soil and water borne bacterium enters the plant roots, through the xylem, collapses the host, and cause the occurance of broken of rhizome (Fig. 2B and discussed in Table 19), then returns to the environment. According to the analysis, the incidence of bacterial wilt disease were 48.68% (Appendix 8) so it can decrease number of healthy plant (Fig. 4B).

R. solanacearum is widespread phytopathogenic bacterium that causes a wilt disease with deadly effects on many economically important crops, such as tomato, potato, tobacco, banana, pepper, and other ornamental plants. It has been reported that before planting *K. parviflora,* the field was planted with tomatoes, the main host of *R. solanacearum.* After destroying the plant, the bacterium can persist in the environment through diverse survival forms up to contact with a new

host. *R. solanacearum* not only has effective pathogenicity determinants to invade and colonize host plants but also exhibits successful strategies for survival in harsh conditions. One of the characteristics of *R. solanacearum* is weak at pH 8, with no growth at pH 4 or 9 (EMPPO, 2004). According to result of the soil analysis, soil sample has pH between 5.2-5.6. It indicates that *R. solanacearum* can survive at the condition and then live with new host, *K. parviflora.* The rainy weather at December also promoted the growth of bacterium because of high humidity.

R. solanacearum can infect over 250 plant species in over 50 families. Many more dicotyl suffer from the disease than do monocotyl. Among the monocotyl host, the order Zingiberales dominates with 5 over 9 families being infected by this bacterium (Denny, 2006).

Figure 2. A) The yellow circle showed *K.parviflora* suffered from bacterial wilt caused by *Ralstonia solanacearum*, and B) The appearance of representative broken rhizome of sample plants at harvesting (11 MAP)

In addition to bacterial wilt, there was another symptom of diseases found in the plant root, called root-knot (Fig. 3). The diseases caused by nematode *Meloidogyne* spp. so that roots will have obvious galls or knot-like swellings. These swellings prevent movement of water and nutrients to the rest of the plant resulting in stunted plant growth. Plants affected by root-knot nematodes are more easily infected by soil-borne diseases caused by *Ralstonia solanacearum* (bacterial wilt). This secondary infection may lead to extensive discoloration of internal stem and root tissue, and rapid plant death (Cerkauskas, 2004).

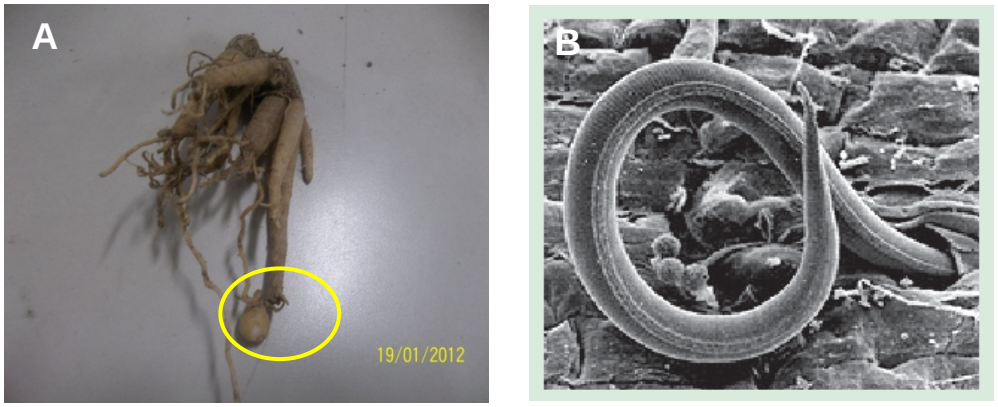

Figure 3. A) Root-knot symptom (indicated by yellow circle), and B) Larva of root-knot nematode, *Meloidogyne incognita*, magnified 500x, shown here penetrating a tomato root (Cerkauskas, 2004)

The ratio root-knot to total root (RK:R) showed the diseases severity of nematode infestation symptom (Appendix 8). Diseases severity of root-knot are 31.57% or medium symptom (Table 3).

Table 3. Diseases severity of root-knot

Score (v)	The number of plant from each category of symptom (n)	(n x v)
0	0	0
1	70	70
2	25	50
3	0	0
4	0	0
$\text{Diseases severity} = \frac{\Sigma(n \times v)}{N \times Z} = \frac{120}{96 \times 4} \times 100\% = 31.57\%$		

Note : n=the number of plant from each category of symptom, v =score value of each category of symptom, N=the number of observed plants, and Z=score value of the highest category of symptom

Bactericide and fungicide was applied to prevent diseases invade other health plants. Mankozeb was used as bactericide and fungicide, while Vandozeb was used as fungicide too. Bactericide and fungicide applied were 20 g L^{-1}, respectively, so that for experimental field area (± 60 m^2) the requirement of liquid bactericide-fungicide of Mandozeb were 3 L, similar to the fungicide Vandozeb needed. But, the application was in different time and frequency. Mankozeb was applied 2 times per week, while Vandozeb was applied 1 times per week. The significant decreasing number of plants was occurred between 24-46 WAP because of diseases effects (Fig. 4B). The disease symptoms were not found

anymore after 12 week of bactericide and fungicide application (Fig. 4A). At harvesting, infestation of nematode (shown by root-knot fresh weight) were found to all plants with no significant level (discussed in page 35, Table 16).

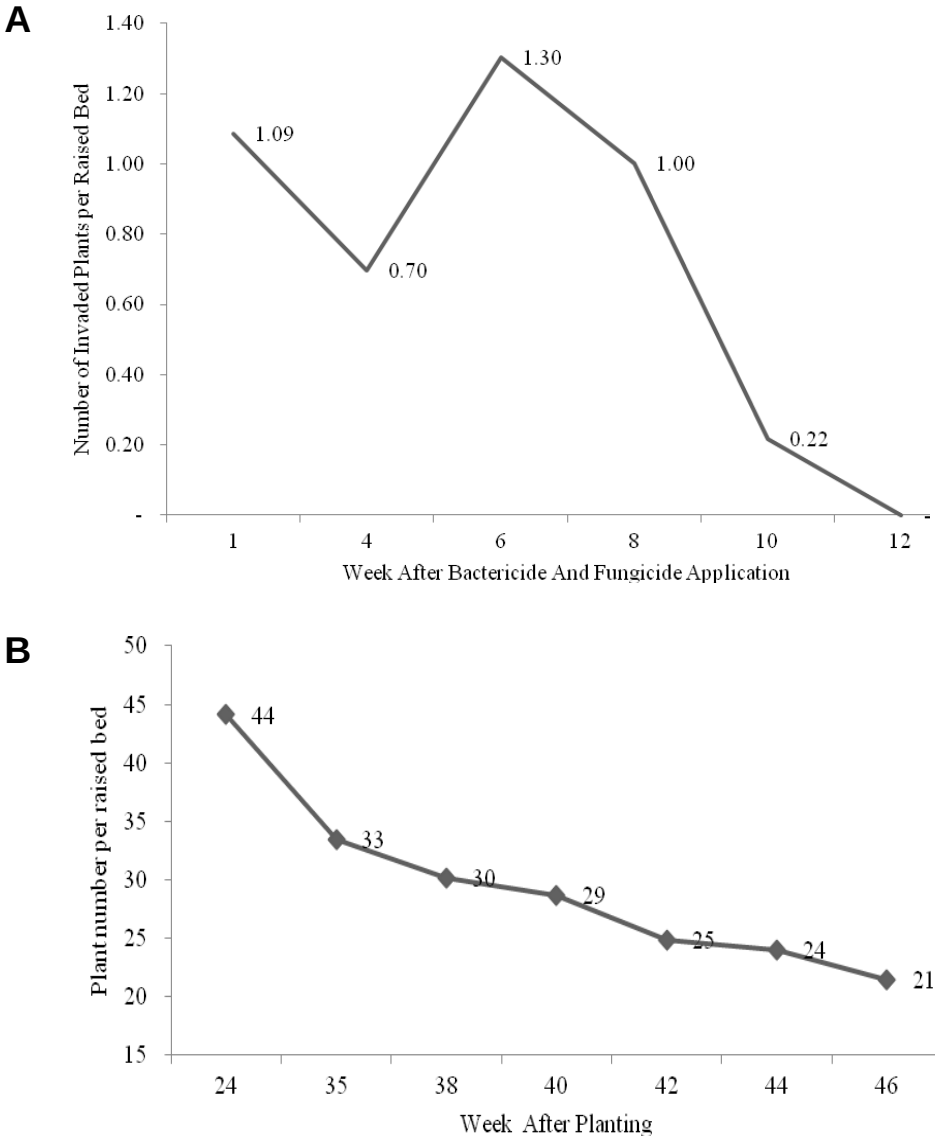

Figure 4. A) Number of invaded plants after bactericide and fungicide application, and B) Plant number decreased

During the research, some weeds and pests have been found and identified around the field. Major weeds found were *Emilia sanchifolia* and *Ageratum conyzoides* (Fig. 5). Manual weeding was done every 2 weeks to control the growth of weeds. Leaves destruction mostly caused by insect and caterpillars. Pests were controlled using trap made from yellow plastic covered by glue.

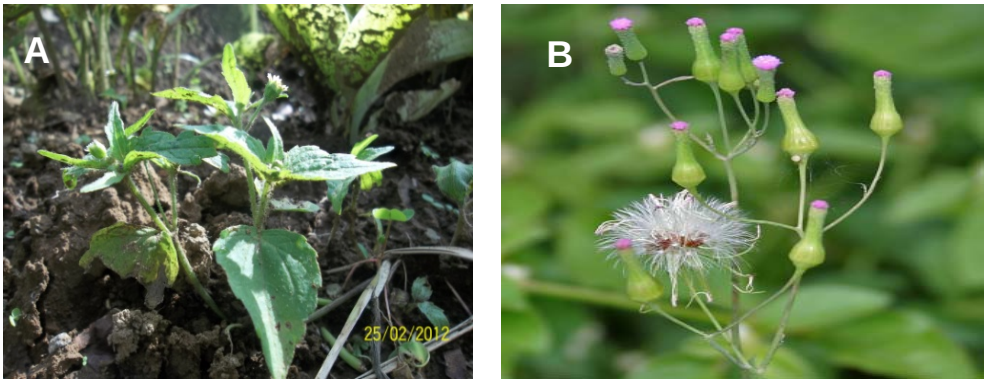

Figure 5. Major weeds found in the experimental field A) *Ageratum conyzoides,* and B) *Emilia sanchifolia*

K. parviflora is cultivated for their rhizomes because rhizome is the storage organ in black galingale. Therefore, deeper observation was done for the root part of this plant. As shown in Figure 6 and Figure 7, the root parts of *K. parviflora* can be divided into (1) green-immature rhizomes, (2) purple-immature rhizomes, (3) mature rhizomes, and (4) fleshy roots. The histology analysis was done in 8 MAP to know the structure of rhizome and root. Figure 6 shows the cross- and longitudinal-section of rhizomes.

In *Curcuma xanthorrhiza* Roxb. the rhizome anatomical structure consisted of the epidermal cells, cortex, endodermal cells, and the central cylinder. Root hairs were formed from the epidermal cells, while cortex and central cylinder composed of parenchymal cells, cell secretion, and the vascular bundles (Kuntorini *et al.*, 2011). Most of the parenchyma cells contain carbohydrates stored in the form of starch grains (Remashree *et al*., 1998). At *K. parviflora* the starch grains are shown by arrow in Figure 6H. Numerous starch grains, varying in shape, size and number, were present in both sides of intermediate zone (Remashree *et al*., 1997). Though *K. rotunda* belongs to monocotyl an obvious demarcation between the outer and inner zone by endodermis like layer is present and it is termed as endodermoidal layer (Sereena *et al.*, 2011).

Similar to ginger, oil cells and canals are also oftenly found in *K. parviflora*. In *K. parviflora* rhizome, there were the secretory cell, identified as oil cell that contained oil component (Fig. 6G). Reddish cell-like structures were also found in the rhizomes. This structure was identified as vacuola that contained by flavonoids (Fig. 6H). Vacuola is the largest part in plants cell

contained organic and anorganic material, such as flavonoids (Fahn, 2000). As plant cell expansion progresses, the tiny provacuoles gradually fuse to form the central vacuole, an enormous acidic compartment which can occupy up to 90% of the cell volume. The nucleus and all the other cytoplasmic organelles are displaced into a narrow, rapidly streaming layer between the tonoplast and the plasma membrane. Many plant cells accumulate water-soluble flavonoid pigments which range in color from orange-red to purple in these vacuola (Taiz, 1992). The fleshy-root was divided in to two parts that were root tip and root base. The fleshy roots also had a few number of vacuola contained flavonoids (showed in the circle) with certain distribution (Fig. 7).

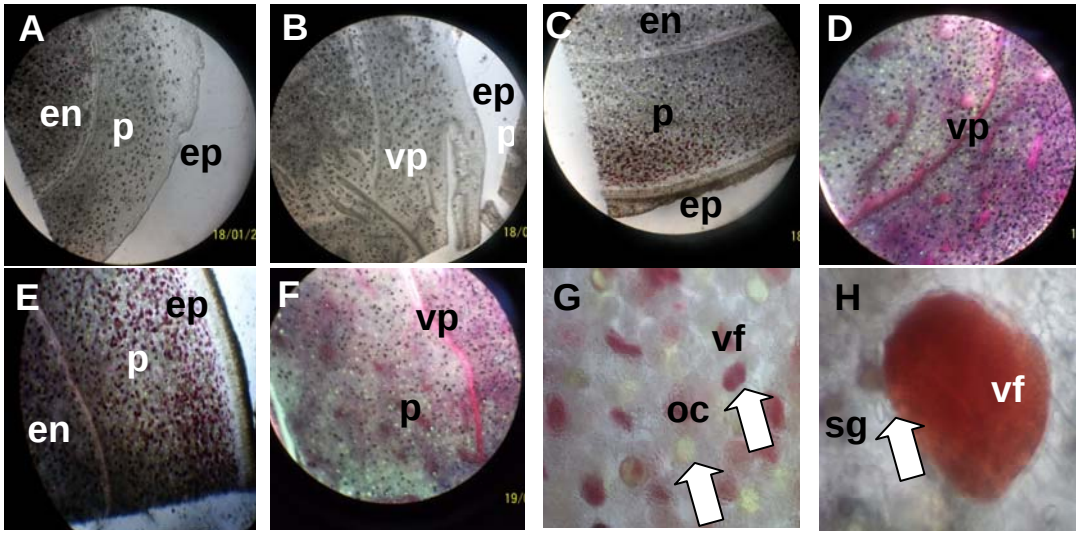

Figure 6. A) Longitudinal section of green-immature rhizome, B) cross section of green-immature rhizome, C) longitudinal-section purple-immature rhizome, D) cross section of purple-immature rhizome, E) longitudinal section mature rhizome, and F) cross section of mature rhizome, in 4x magnification. Larger view of G) mature rhizome in 12x magnification, and h) reddish cell structure, identified as vacuola contained flavonoids. Abbreviation: ep (epidermic), en (endodermic), vf (vacuola contained flavonoids), oc (oil cell), vp (vascular pitch), sg (starch grain), and p (parenchym). Pictures were taken by camera digital Kodak M522.

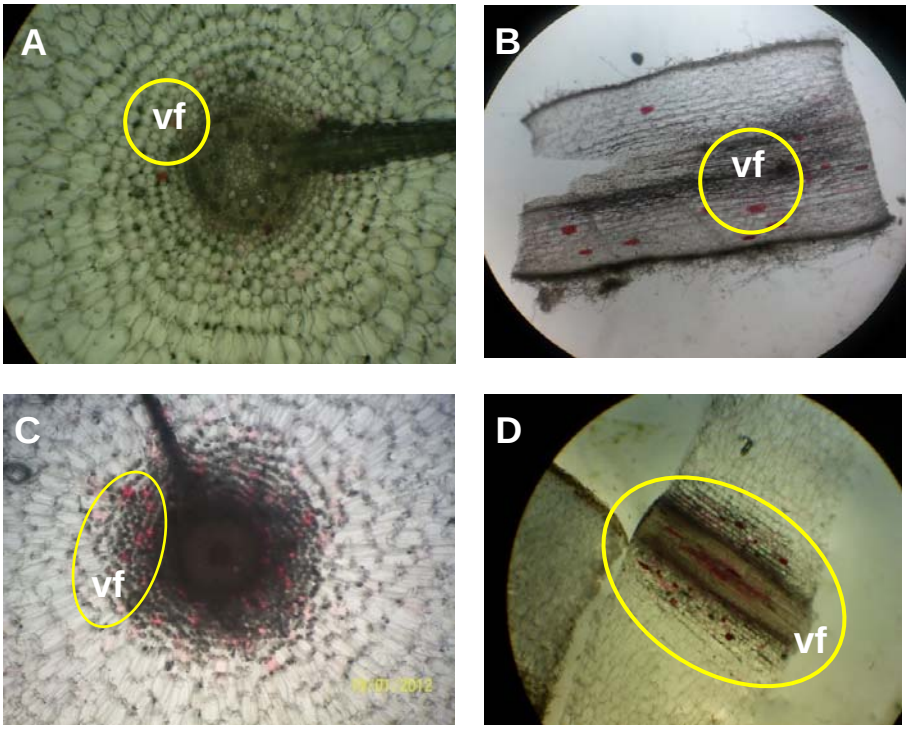

Figure 7. A) Longitudinal-section of root tip, B) cross-section of root tip, C) longitudinal-section of root base, and D) cross-section of root base, vf : vacuola contained flavonoids. Picture were taken using magnification 4x and zoom out by Kodak M22.

Bio-fertilizer Effect on the Late-Vegetative Growth of *K. parviflora*

In this experiment, liquid bio-fertilizer was applied 2 times, by watering half rate bio-fertilizer in soil at 5 and 7 MAP. First application of half rate of bio-fertilizer in 5 MAP was conducted to promote the late-vegetative growth, while second application of half rate of bio-fertilizer in 7 MAP was conducted to promote the rhizome growth. Bio-fertilizer application showed significant effect on late-vegetative growth (plant height, leaf number, leaf length, and leaf width) of *K. parviflora* only 3-6 weeks after the first half rate application of bio-fertilizer. But, the second half rate application of bio-fertilizer did not show significant effect on the late-vegetative growth (Table 4). Plants leaves that start to senescence after 29 WAP so it caused unsiginificant effect of late-vegetative growth after the second bio-fertilizer application. Thus, the late-vegetative growth only affected by the first half rate application of bio-fertilizer for each bio-fertilizer rate. The general obsservation of late-vegetative growth of *K. parviflora* were shown in Appendix 2 up to Appendix 8.

Bio-fertilizer is fertilizer containing advantageous microorganisms. Simanungkalit *et al.* (2006) stated bio-fertilizer is inoculants containing living organisms that can increase the availability of nutrients in the soil for plants. In this experiment, the applied bio-fertilizer contained N_2 fixing-bacteria, *Azospirillum lipoferum* and *Azotobacter vinelandii*. Nitrogen fixation was the first mechanism proposed to explain improved plant growth following inoculation with *Azospirillum* and *Azotobacter*. But these bacterium can also release some plant growth hormones. *Azotobacter* produces B-vitamins, indole acetic acid, gibberellins, cytokinines, also increase the activity of beneficial rhizospere bacteria, while *Azospirillum* penetrates and colonizes the roots as well as the tissues of the plant and release some plant growth hormones which promote crop growth (Kumar *et al.*, 2010).

Table 4. Analysis of variant and coefficient variation (CV) of late-vegetative growth of *K. parviflora*

Time (WAP)	Plant height			Leaf number			Leaf length			Leaf width		
	T	B	CV (%)	T	B	CV (%)	T	R	CV (%)	T	B	CV (%)
24	ns	*	8.29	*	ns	18.13	ns	*	19.20	ns	*	7.90
25	*	*	7.54	*	*	16.91	ns	ns	7.51	ns	*	6.85
27	ns	*	8.67	*	ns	21.05	ns	ns	7.98	*	ns	7.29
29	ns	*	10.40	ns	ns	26.98	ns	ns	9.34	*	ns	8.33
34	ns	*	11.50	ns	ns	39.22	ns	ns	10.75	ns	ns	6.60
36	ns	ns	12.35	ns	ns	32.45	ns	ns	9.68	ns	ns	7.43
38	ns	ns	12.60	ns	ns	35.10	ns	ns	13.75	ns	ns	11.84
40	ns	ns	13.27	ns	ns	25.91[t]	ns	ns	17.50	ns	ns	12.98
42	ns	ns	18.30	ns	ns	27.38[t]	ns	ns	13.91	ns	*	15.41
44	ns	ns	19.48	ns	ns	31.58[t]	ns	*	12.25	ns	*	13.78

Note: Shaded rows showed time of application bio-fertilizer. *) significant, ns) not significant, B) block, T) treatment, t) data were transformed by √x+1...n, and WAP) week after planting.

The parameters observed generally showed that *K. parviflora* which planted by adding 50% chemical fertilizer and 15 L ha^{-1} bio-fertilizer had the best respond of late-vegetative growth. Based on the nutrient uptake analysis at 11 MAP, nutrient uptake of N, P, and K were not significantly different between all treatment (discussed in page 31; Table 13). Therefore, improved vegetative growth after the first half rate application of bio-fertilizer (i.e. plant height at 3

week after first application of bio-fertilizer/WAB[1st], leaf number at 2, 3, and 5 WAB[1st], leaf width at 5 and 7 WAB[1st], and leaf area at 2 WAB[1st]) shown in Table 5 might be because of the capability of *Azospirillum* and *Azotobacter* to produce plant growth hormones (i.e. B-vitamines, indole acetic acid, gibberellins, cytokinines). Kummee *et al.* (2010) stated that *Azotobacter* and *Azospirillum* can release some plant growth which enhanced seed germination, plant height and plant biomass and crop yield of wheat (*Triticum aestivum*). Furthermore, the fact showed that the siginificant vegetative growth improvement was only found at 15 L ha^{-1} bio-fertilizer application suggests that there might be optimum rate of bio-fertilizer.

In this experiment, *K. parviflora* which planted by adding 15 L ha^{-1} of bio-fertilizer had the optimum plant growth than application higher rate, 22.5 L ha^{-1} and 30 L ha^{-1} rate bio-fertilizer. This might be because, in that rate the microorganism population in soil was optimum because both *Azospirillum* and *Azotobacter* could grow well and did not compete in using nutrition and energy from C compound from organic acid and sugars. Thus, they could effectively inoculated the plant and promote plant growth. In other study, the interaction of *Azospirillum* with plant indicated its high affinity for organic acids, mainly malate and succinate, as carbon sources for their energy (Bashan *et al*., 2011). According to the Hindersah and Simarmata (2004), high inoculation of *Azotobacter* in lettuce (*Lacutca sativa* L.) caused high density and nutrition competition of population, so that affect the decrease of its population.

Table 5. The effect of bio-fertilizer rate on late-vegetative growth

Treatment	Plant height (cm)	Leaf number			Leaf width (cm)		Leaf area (cm^2)
	3/25	2/24	3/25	5/27	5/27	7/29	2/24
	……..WAT/WAP…….....						
100%ChemF+no BioF	25.3b	15.7b	18.5b	20.4b	6.8b	6.8ab	89.4ab
50%ChemF+15L ha^{-1} BioF	28.4a	22.6a	28.2a	28.0a	7.6a	7.5a	99.7a
50%ChemF+22.5L ha^{-1} BioF	25.4b	17.1b	20.0b	21.2b	6.9b	6.6b	77.8b
50%ChemF+30L ha^{-1} BioF	24.2b	15.7b	18.6b	20.8b	7.6a	7.4a	96.5a
CV (%)	7.54	18.13	16.91	21.05	7.29	8.33	12.35

Note: Numbers followed by the same letter in the same columns are not significantly different based on DMRT at level α = 5%. ChemF = chemical fertilizer, BioF= bio-fertilizer, WAT= week after treatment, WAP = week after planting. The row contain number 3/2,2/24,...,7/29,2/24 showed WAT/WAP, respectively.

The coefficient of variation (CV) value of leaf number were relatively high (> 40%) at 40, 42, and 44 WAP because of the fluctuation number of young leaves and old senescence leaves. Young leaves develops (Fig. 8A) while old leaves are senescing and finally dead (Fig. 8B). Leaf senescence is the final stage of leaf development and indicates nutrient relocation from leaves to reproducing organ or to other growing organs, like rhizome (Lim *et al.*, 2007). In addition, many leaves were destructed by insect and caterpillars.

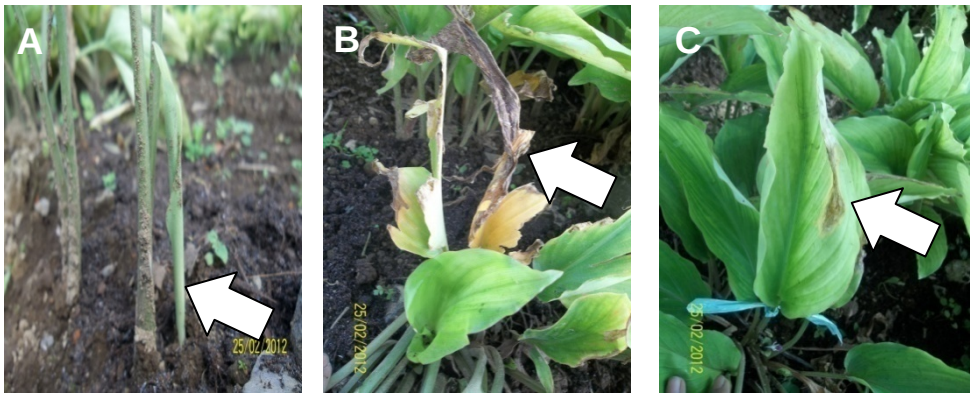

Figure 8. Arrows showed a) new young leaf, b) old senescence leaf, and c) senescence of specific leaf

Full open mature leaves were chosen as specific leaf that would be measured. During the experimental the specific leaf was senesced after 12 week after full open mature (WAF) (Fig. 8C), so the mean of length and width of specific leaf tend to decrease (Fig. 9).

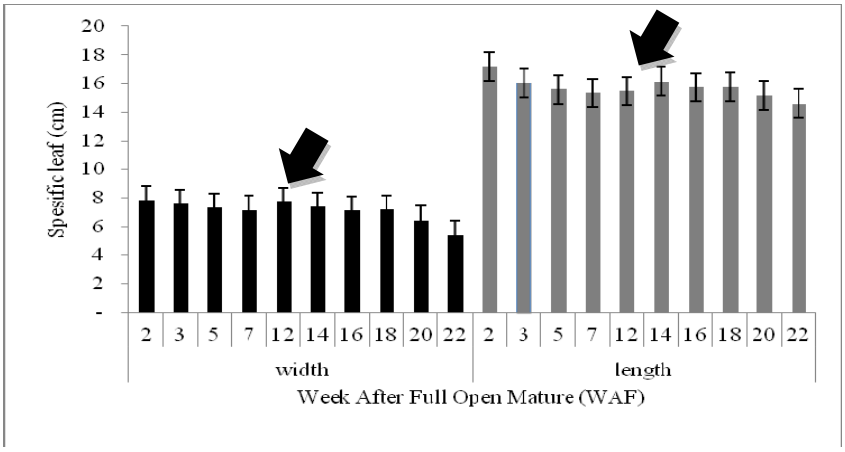

Figure 9. Growth of spesific leaves of *K. parviflora* (arrows indicate the time of leaves senenscence).
Width of spesific leaf
Length of spesific leaf

Number of shoot were observed to predict the yield of rhizome before harvesting. Based on statistical analysis, there was no significant different between treatment of fertilizer (Table 6). Number of shoot was 4.17 per plant at final observation (data not shown). Actually, there is no correlation between number of shoot and yield of rhizome so number of shoot can not be used for predicting the yield of rhizome.

Table 6. Analysis of variant and coefficient variation (CV) of shoot number

Time (WAP)	Number of shoot		
	Treatment	Block	CV (%)
36	ns	ns	27.09
38	ns	ns	38.05
42	ns	ns	34.00
44	ns	ns	30.09[t]

Note : ns) not significant, t) data were transformed by √x+1...n , WAP) week after planting.

Green color intensity were measured by SPAD meter in 7, 8, and 9 MAP. Based on statistical analysis, the green color intensity were not significant for all treatment applied (Table 7), with value ranged 18.55-20.00 ± 1.06 unit (Fig. 10). While chlorophyll content analysis were conducted in 7 and 9 MAP by using destructive analysis (Sims and Gamon, 2002) and determined per dry weight of leaf sample (mmol g^{-1}). Based on statistical analysis all photosynthesis pigment contents were also not significantly different among treatments.

The value of chlorophyll a, chlorophyll b, anthocyanin, and carotenoids content are shown in Table 8. Although the value of all photosynthesis pigment contents seems not significant for all treatment, but parameter leaf number (2, 3, and 5 WAB^{1st}), leaf width (5 and 7 WAB^{1st}), shown in Table 5, and leaf area (2 WAB^{1st}), shown in Table 7, were significantly different and showed that the best respond of late-vegetative growth of *K. parviflora* was from treatment of 50% chemical fertilizer and 15 L ha^{-1} bio-fertilizer. Dry weight of leaf tend to increase by the increasing leaf area, so it can be implied that the value photosynthesis pigment per leaf area similar to value photosynthesis pigment per dry weight of leaf. Higher leaf area and leaf number of *K. parviflora* which planted by treatment

of 50% chemical fertilizer and 15 L ha^{-1} bio-fertilizer caused the higher mechanism of photosynthesis.

Table 7. Analysis of variant and coefficient variation (CV) of green color intensity and leaf area

Time (MAP)	Green intensity			Leaf area		
	Treatment	Block	CV (%)	Treatment	Block	CV (%)
7	ns	ns	11.57	*	*	12.35
8	ns	ns	13.55	ns	ns	13.07
9	ns	ns	14.43	ns	ns	26.65

Note: *) significant different at $P < 0.05$; ns = not significant; MAP = month after planting.

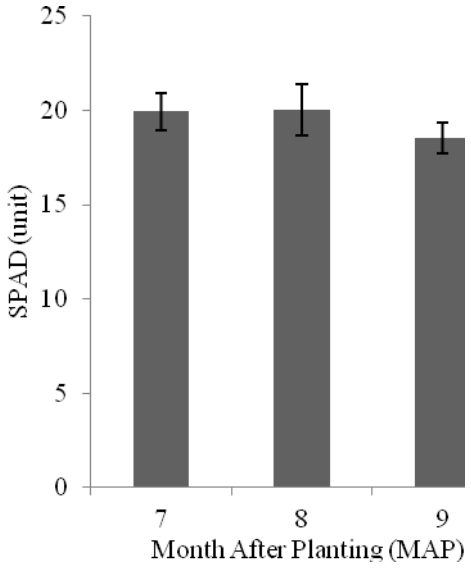

Figure 10. Green color intensity in 7, 8, and 9 MAP

Table 8. The effect of bio-fertilizer rate on photosynthesis pigment content

Treatment	7 MAP					9 MAP				
	Chl a	Chl b	Total Chl	Anth	Car	Chl a	Chl b	Total Chl	Anth	Car
	.....mmol g^{-1}.....									
100% ChemF+no BioF	0.28	0.16	0.44	0.31	0.23	0.24	0.13	0.37	0.25	0.20
50% ChemF+15L ha^{-1} BioF	0.23	0.13	0.36	0.25	0.25	0.20	0.14	0.33	0.34	0.20
50% Chem+22.5L ha^{-1} BioF	0.27	0.15	0.43	0.29	0.28	0.18	0.11	0.30	0.20	0.18
50% ChemF+30L ha^{-1} BioF	0.20	0.09	0.29	0.13	0.21	0.19	0.14	0.33	0.35	0.18
Mean	0.25	0.14	0.38	0.25	0.24	0.20	0.13	0.33	0.28	0.19
Stdev	0.03	0.03	0.06	0.08	0.03	0.03	0.01	0.03	0.07	0.01
CV (%)	39.6	22.5	32.4	24.9[t]	21.3	29.4	24.7	26.7	37.5	24.3

Note: ns = not significant; t = data were transformed by √x+1...n; MAP = month after planting, ChemF = chemical fertilizer, BioF = bio-fertilizer. Chl a = Chlorophyll a, Chl b = Chlorophyll b, Chl Total = Chlorophyll Total, Anth = Anthocyanin, and Car = Carotenoids.

The SPAD meter is used for associating the relative chlorophyll content of leaves with the one-dimensional values established by the meter (green color intensity). The correlation between SPAD meter measurements and photosynthesis pigment contents were not found (Table 9) so in conclusion SPAD meter can not be used for predicting the physiological status in *K. parviflora.*

Table 9. Correlation between green color intensity index and photosynthesis pigment

Photosynthesis pigment	Green intensity (7 MAP)		Green intensity (9 MAP)	
	p-value	R	p-value	R
Chl a	ns	0.13	ns	-0.37
Chl b	ns	0.29	ns	-0.51
Chl Total	ns	0.19	ns	-0.44
Anth	ns	0.31	ns	-0.29
Car	ns	0.80	ns	-0.25

Note: ns = not significant; R = coefficient of correlation, Chl a = Chlorophyll a, Chl b = Chlorophyll b, Chl Total = Chlorophyll Total, Anth = Anthocyanin, and Car = Carotenoids.

In this experiment, the block of each treatment was determined according to the shading position of raised bed and difference of sun-lighting time. First block were in shade position with 6 hours of sun-lighting, second block were in half shade position with 9 hours of sun-lighting, and the last block were in full sun position with 12 hours of sun-lighting. These different condition showed significant mean of plant height (24-29 WAP), leaf number (25 WAP) (Table 11), leaf width (24, 25, and 42 WAP), leaf length (22 and 44 WAP), and leaf area (24 WAP) (Table 10).

Shading affects the early vegetative growth of *K. parviflora* at plant height, leaf number, and size. Plants grown under different shading conditions had different leaf width and length, which resulted in different leaf area. Plants grown under natural shading by canopy tree had the longest leaf and the biggest leaf area (Evi, 2012). According to the Table 10 and Table 11, the highest value of each parameter observed was from shading condition and 6 hours sun-lighting. It means that shading also affects the late-vegetative growth of *K. parviflora.* Leaf of the plant grown under shade was bigger in order to enhance the light

absorption. Difference characters at leaf area and leaf thickness is shade avoidance response from plant that grown under low light intensity is aimed to maximize light absorption thus more efficient photosynthesis (Taiz and Zeiger, 2003).

Table 10. Affect of shading position and sun-lighting time on growth parameter

Position and sun-lighting time	Plant height (cm)				Leaf number (unit)
	24	25	27	29	25
	.....WAP.....				
S + 6 HSL	26.96a	28.29b	28.82b	29.77b	24.00c
HS + 9 HSL	23.45a	23.55a	24.78a	25.50a	17.40c
FS + 12 HSL	25.20a	25.02a	25.89a	26.10a	21.14c

Note: Shaded rows showed that FS + 12 HSL is the control for t-Dunnett test. Numbers followed by the same letter in the same columns are not significantly different based on t-Dunnett at level α = 5%, S + 6 HSL: Shading + 6 hours of sun-lighting, HS + 9 HSL: Half shading + 9 hours of sun-lighting, and FS + 12 HSL: Full sun + 12 hours of sun-lighting.

Table 11. Affect of shading position and sun-lighting time on spesific leaf growth

Position and sun-lighting time	Leaf width (cm)				Leaf length (cm)		Leaf area (cm^2)
	24	25	42	44	24	44	24
	.....WAP.....						
S + 6 HSL	8.23b	7.84a	7.60b	7.74b	16.97a	17.48b	98.56a
HS + 9 HSL	6.77a	6.81a	6.34a	5.30a	14.26a	14.48a	90.77a
FS + 12 HSL	7.91a	7.76a	5.78a	4.74a	16.03a	13.48a	76.23a

Note: Shaded rows showed that FS + 12 HSL is the control for t-Dunnett test. Numbers followed by the same letter in the same columns are not significantly different based on t-Dunnett at level α = 5%, S + 6 HSL: Shading + 6 hours of sun-lighting, HS + 9 HSL: Half shading + 9 hours of sun-lighting, and FS + 12 HSL: Full sun + 12 hours of sun-lighting.

Previous studies in other Zingiberaceae showed that increasing shade intensity can increase leaf number, stem number, and dry weight of ginger plants. These Zingiberaceae was type of plant that quite tolerant to high shade intensity, being suitable for a multiple cropping system with other plants with 25 to 50% shade intensity. Gardner *et al.* (1985) stated that high light intensity resulted in slow plant growth because auxin was reduced and destroyed. In addition, Soenanto (2001) stated that high temperature increase evapotransporation and

destroy leaf directly (Fig. 11A). The development of leaves in ginger rhizomes are clockwise directions and it encircled the shoot apex (Remashree *et al*., 1998), similar to *K. parviflora* (Fig. 11B).

Figure 11. The different condition of *K. parviflora* in A) full sun, and B) shading, also showed clockwise directions phylotaxis of leaves

Stomatal density was observed below microscope with magnification 400x. F-test showed that there was no significant different in stomatal density cm^{-2} in 5 and 6 MAP that showed significant different at 6 MAP (Table 12). In open place, plant are exposed to higher fluence rate of photosyntetically active radiation and to higher red to far-red ratios than under the shade. Thus, the stomatal density of leaves plant in open place are higher than under the shade plant.

Table 12. Analysis of variant and coefficient variation (CV) of stomatal density in spesific leaf

Position and sun-lighting time	Stomatal density	
	5 MAP	6 MAP
	.....(unit cm^{-2}).....	
S + 6 HSL	3,431.0	3,335.5b
HS + 9 HSL	4,280.3	3,898.1a
FS + 12 HSL	3,796.2	4,025.5a
F-test	0.091^{ns}	0.031*
CV (%)	7.53	5.43

Note: Shaded rows showed that FS + 12 HSL is the control for t-Dunnett test. Numbers followed by the same letter in the same columns are not significantly different based on t-Dunnett at level α = 5%, S + 6 HSL: Shading + 6 hours of sun-lighting, HS + 9 HSL: Half shading + 9 hours of sun-lighting, and FS + 12 HSL: Full sun + 12 hours of sun-lighting.

Boccalandro *et al.* (2009) showed that higher red to far-red ratios increase stomatal density in Arabidopsis. This was goes along with The result which

showed that without shading (high red-far red ratio) had higher stomatal density. Lower stomatal density could compensate CO_2 assimilation by increasing stomatal aperture and conversely, increased stomatal density could compensate CO_2 assimilation by reducing stomatal aperture (Bussis *et al.*, 2006). In monocotyledons the stomata is surrounded with two guard cells with bulbous ends straight in the middle, walls are thick in the middle and thin at the ends (Fig. 12).

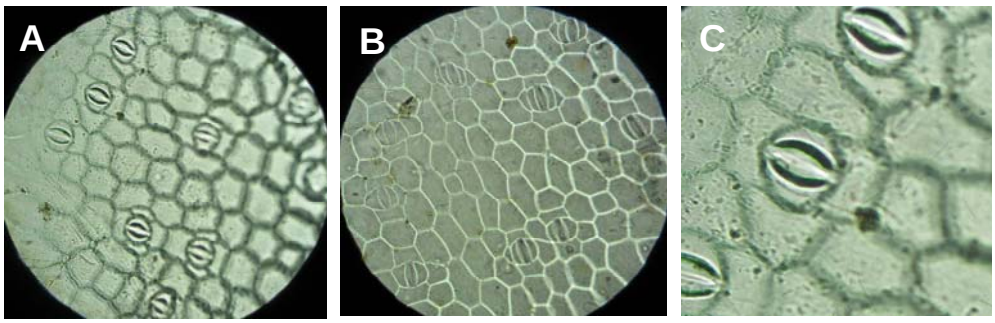

Figure 12. Stomatal density by magnification 400x in a) 5 MAP, b) 6 MAP, and c) zoom in stomata of adaxial epidermis of *K.parviflora* leaves. Picture was taken by camera digital Kodak M522.

Nutrient Uptake Analysis

Nutrient uptake analysis were conduted in 11 MAP, both on upground canopy (leaf and stem) and underground structures (rhizome and roots). Based on the statistical analysis, application of bio-fertilizer rate and 50% chemical fertilizers was not significantly different from full rate of chemical fertilizers for uptake value of N, P, and K (Table 13). It shows that bio-fertilizer could increase nutrient uptake, especially for N uptake, because bio-fetilizer applied contained N_2 fixing bacteria.

Table 13. Analysis of variant and coefficient variation (CV) of nutrient uptake

Nutrient Uptake Analysis	N			P			K		
	T	B	CV (%)	T	B	CV (%)	T	B	CV (%)
Uptake of leaf and stem	ns	ns	22.23^t	ns	ns	40.67^t	ns	ns	33.49^t
Uptake of rhizome & roots	ns	ns	26.98^t	ns	ns	22.83^t	ns	ns	20.33^t

Note: ns) not significant, B) block, T) treatment, t) data were transformed by $\sqrt{x+1}$...n

Nitrogen fixation was the first mechanism proposed to explain improved plant growth following inoculation with *Azospirillum* and *Azotobacter*. This was mainly because of an increase in the number of nitrogenous compounds and the nitrogenase activity in inoculated plants (Bashan and Holguin, 1997). Maximum grain yield of rice (8.43 ton ha^{-1}) was recorded with *Azospirillum* at 100 N kg ha^{-1}, 1.34 ton ha^{-1} higher than the yield obtained with only 120 kg N kg ha^{-1} applied, suggesting the benefit of reducing N application by 20 kg ha^{-1} with extra yield. Similarly, *Azotobacter* showed the potential to save 20 N kg ha^{-1} with additional yield of 1.24 ton ha^{-1}. Mixed cultures (*Azospirillum* and *Azotobacter*) were statistically similar to individual cultures. In the case of rice, grain yield increased significantly (Sattar *et al*., 2006). Interaction of N and bio-fertilizer affects yield. Using *Azotobacter* and *Azospirillum* caused to increasing 35 and 21% in yield, while getting a suitable yield in safflower by loss 50% N in compared with 60 kg ha^{-1} (standard operational procedure of chemical fertilizer). Decreasing N chemical fertilizer help to reduce environment pollution and developing sustainable agriculture (Soleymanifard and Sidat, 2011). In addition, the usage of agrochemicals was seriously disturbing plant biotic characters and soil ecology (Martin *et al*., 2011).

Rosita *et al.* (2007) showed that nutrient uptake of P in rhizome *Kaempferia galanga* L., was smaller than N and K. Generally requirement of P in plant was smaller than N and K. In contrast, nutrient uptake of K in rhizome was higher than N and P, because K was important nutrient for transporting photosyntate to the storage organ. This cases refers to previous research in *Curcuma aeruginosa* Roxb. (Djazuli *et al*., 2001), *Zingiber pupureum* Roxb. (Rosita *et al*., 2005a), and *Zingiber officinale* Rocs. (Rosita *et al.,* 2005b).

Percentage of N, P, and K showed that in canopy, nutrient K was the highest value, following by N and P, while in the rhizome and roots nutrient N was the highest, following by P and K (Table 14). The result was different with Rosita *et al.* (2007) and previous research in some Zingiberaceae. Potassium in canopy was still high while in rhizome it showed the lowest percentage, because K keep formed in ion K^+ so it can mobile and translocate to the needed organ. Generally, percentage of N was high in both canopy and rhizome and roots

because plants need much N for forming protein and nucleic acid (Soleymanifard and Sidat, 2011).

The nutrients uptake of leaf and stem were 18.26-23.80 mg plant^{-1} of N, 6.17-9.39 mg plant^{-1} of P, and 21.09-32.71 mg plant^{-1} of K nutrients. While the nutrients uptake of rhizome and roots were 361.75-473.59 mg plant^{-1} of N, 134.22-193.88 mg plant^{-1} of P, and 122.05-131.66 mg plant^{-1} of K (Table 14). Generally this result was different from Rosita *et al.* (2007) experiment that showed the nutrients uptake of the promising lines *Kaempferia galanga* L. to the applied fertilizer technology package (manure 20 ton ha^{-1}, urea 250 kg ha^{-1}, SP-36 200 kg ha^{-1}, and KCl 200 kg ha^{-1}) were 149.60-415.60 mg plant^{-1} of N, 41.50-112.50 mg plant^{-1} of P, and 236.10-571.70 mg plant^{-1} of K nutrients, only measured on rhizome. But K uptake of rhizome and roots seems lower than Rosita *et al.* (2007) experiment.

Table 14. The effect of bio-fertilizer rate on nutrient uptake

Bio-fertilizer rate	Percentage (%)						Nutrient uptake (mg plant^{-1})*					
	Canopy			Rhizome& Root			Canopy			Rhizome& Root		
	N	P	K	N	P	K	N	P	K	N	P	K
C	1.94	0.71	2.76	2.10	0.90	0.83	20.0	9.4	30.1	473.6	193.9	131.7
D1	2.43	0.57	2.83	2.09	0.81	0.68	23.8	6.5	32.7	378.5	148.1	122.0
D2	2.16	0.74	3.60	2.14	0.79	0.77	18.3	6.2	32.3	361.8	134.2	131.0
D3	2.33	0.74	2.57	2.01	0.84	0.65	20.7	7.3	21.1	389.3	162.0	128.1

Note : *) Nutrient uptake (mg plant^{-1}) were determined by percentage (%) x dry weight (mg plant^{-1}), C: Control (100% chemical fertilizer), D1: 50% chemical fertilizer + 15 L ha^{-1} Bio-fertilizer, D2: 50% chemical fertilizer + 22.5 L ha^{-1} Bio-fertilizer, and D3: 50% chemical fertilizer + 30 L ha^{-1} Bio-fertilizer.

Table 15 and Figure 13 showed the total nutrient uptake that used by population plant in 10,000 m^2 (ha) compared with nutrient given from chemical fertilizer (urea contained 46% N, SP-36 contained 36% P_2O_5, and KCl contained 60% K_2O). Ratio nutrient uptake to nutrient given of application 50% chemical fertilizer and its combination with bio-fertilizer were relatively higher than the aplication of full rate urea, SP-36, and KCl so the combination with bio-fertilizer can be applied to increase the nutrient uptake of plants. Nutrient uptake of N and P were more than 100% might be caused by residual N and P from previous fertilizer in soil. This residual N and P are shown by the result of soil analysis

before experiment which shown that N total in soil was in moderately high and P_2O_5 Olsen was in high category (Table 2).

Table 15. The effect of bio-fertilizer rate on nutrient uptake percentage

Treatment	Nutrient given from chemical fertilizer (kg ha^{-1})			Total nutrient uptake by plants ** (kg ha^{-1})			Ratio nutrient uptake to nutrient given *** (%)		
	N	P	K	N	P	K	N	P	K
100% ChemF+no BioF	138	39.28	125	98.73	40.65	32.35	71.54	103.49	25.88
50% ChemF+15L ha^{-1} BioF	69	19.64	62.5	80.46	30.92	30.95	116.61	157.43	49.52
50% Chem+22.5L ha^{-1} BioF	69	19.64	62.5	76	28.08	32.66	110.14	142.97	52.26
50% ChemF+30L ha^{-1} BioF	69	19.64	62.5	82	33.87	29.86	118.84	172.45	47.78

Note : **) Total nutrient uptake (kg ha^{-1}) were determined by nutrient uptake (mg plant^{-1})* (Table 14) x population (plant ha^{-1}), ***) Ratio nutrient given to nutrient uptake were determined by (total nutrient uptake by plants (kg ha^{-1}) : nutrient given from chemical fertilizer (kg ha^{-1})) x 100%, ChemF: chemical fertilizer, BioF: bio-fertilizer.

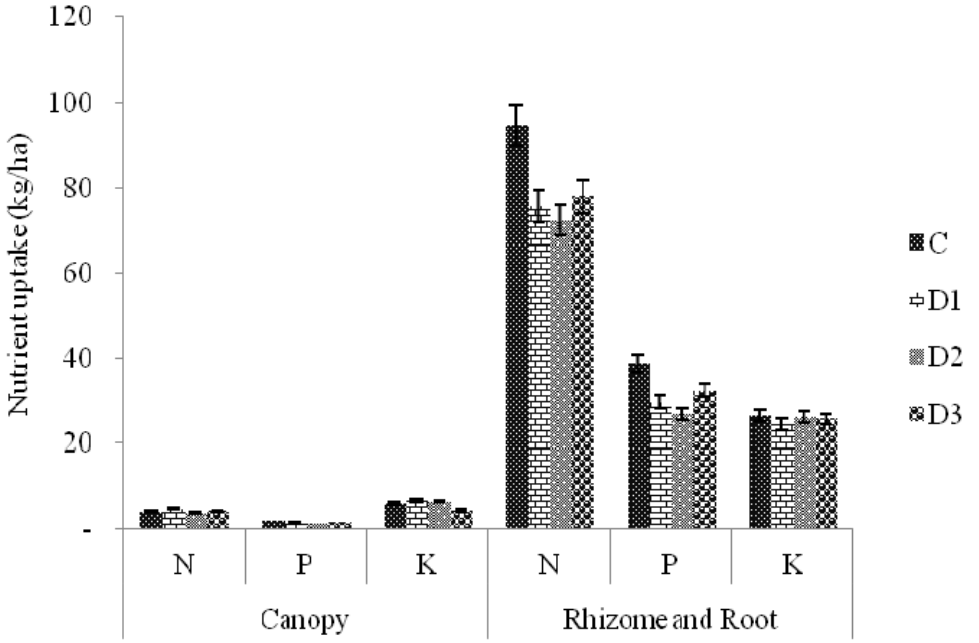

Figure 13. Mean of nutrient uptake of *K. parviflora* at 11 MAP. C: Control (100% chemical fertilizer), D1: 50% chemical fertilizer + 15 L ha^{-1} Bio-fertilizer, D2: 50% chemical fertilizer + 22.5 L ha^{-1} Bio-fertilizer, and D3: 50% chemical fertilizer + 30 L ha^{-1} Bio-fertilizer.

Bio-fertilizer Effect on the Harvest Component and Rhizome of *K. parviflora*

The climate condition in 11 MAP was dry season, with low intensity of rain so that leaves got senescence. Harvest was conducted when 80% of leaves became senescens, as showed in Figure 14B. All harvest parameter showed that

there were no significant different between the treatment applied (Table 16), but positive correlation were found between infestation of nematode (root-knot) with weight of root, also between weight of root with weight of rhizome. It means that the increasing of root affected the increasing of nematode infestation to the roots (Table 17), this similar to the increasing of root affected the increasing of rhizome.

Table 16. Analysis of variant and coefficient variation (CV) of harvest component parameter

Parameter	T	B	CV (%)
Fresh canopy weight per plant (g)	ns	ns	19.33[t]
Dry canopy weight per plant (g)	ns	ns	20.18 [t]
Fresh rhizome weight per plant (g)	ns	ns	19.91 [t]
Dry rhizome weight per plant (g)	ns	ns	20.27 [t]
Fresh roots weight per plant (g)	ns	ns	19.86 [t]
Dry roots weight per plant (g)	ns	ns	19.04 [t]
Knot-roots weight per plant (g)	ns	ns	20.86 [t]
Number of individual rhizome per plant	ns	ns	19.13 [t]
Fresh rhizome weight per plant (g)	ns	ns	19.26 [t]
Rhizome length per plant (cm)	ns	ns	22.20 [t]
Rhizome width per plant (cm)	ns	ns	14.55 [t]
Diameter of individual rhizome (cm)	ns	ns	4.56
Fresh canopy weight per plot (g)	ns	ns	22.15 [t]
Dry canopy weight per plot (g)	ns	ns	19.83 [t]
Fresh roots weight per plot (g)	ns	ns	23.01 [t]
Fresh rhizome weight per plot (g)	ns	ns	21.06 [t]

Note: ns = not significant; B = block; T = treatment; t = data were transformed by √x+1...n. canopy were consist of stem and leaf

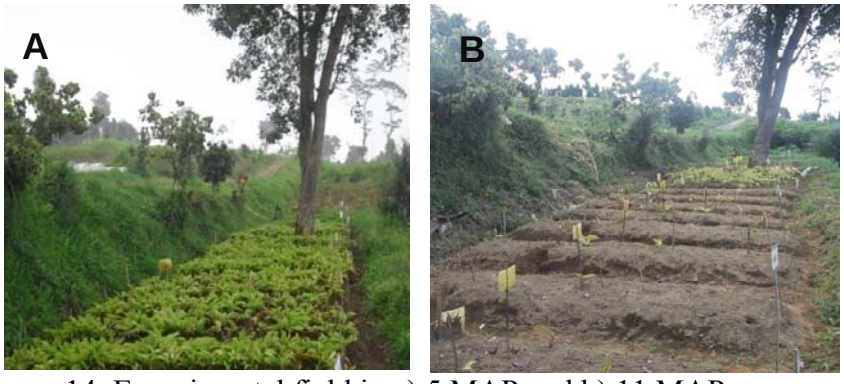

Figure 14. Experimental field in a) 5 MAP and b) 11 MAP

Table 17. Correlation between number of rhizome, roots, and root-knot

Correlation	Rhizome			Roots		
	p-value	r (Pearson)	R-square	p-value	r (Pearson)	R-square
Root-knot	0.0127*	0.56	0.31	<.0001*	0.80	0.65
Roots	<.0001*	0.82	0.67			

Note: *) significant, The formula which can be used to predict yield of rhizome was Y=24.63g +1.93X, where Y are fresh weight of rhizome (g) and X are fresh weight of root (g).

Table 18 showed the effect of bio-fertilizer rate on potential and actual productivity. Based on statistical analysis, potencial productivity showed not significant value, so rhizome yield of application 50% chemical fertilizer and bio-fertilizer rate were similar to hizome yield of application 100% chemical fertilizer. Aplication of chemical fertilizer and its combination with bio-fertilizer resulted in rhizome fresh weight of 29.48-45.66 g plant^{-1}. This result were lower than Rosita *et al.* (2007) result of fresh weight of promising lines *Kaempferia galanga* L. rhizomes, that ranged from 62.27-70.22 g plant^{-1}. Potencial productivity of *K.* parviflora, 4.38-8.19 ton ha^{-1}, were also lower than Rosita *et al.* (2007) experiment ranged from 9.52-11.1 ton ha^{-1}.

Table 18. The effect of bio-fertilizer rate on potential productivity and actual productivity

Treatment	Rhizome FW (g plant^{-1})	Rhizome FW (g (2 m^2)$^{-1}$)	Potential productivity (ton ha^{-1})*	Actual Productivity (ton ha^{-1})**
100% ChemF+no BioF	29.48	325.60	4.38 (100%)	1.30
50% ChemF+15L ha^{-1} BioF	45.66	765.25	8.19 (187%)	3.06
50% Chem+22.5L ha^{-1} BioF	37.42	392.80	5.73 (131%)	1.57
50% ChemF+30L ha^{-1} BioF	33.98	742.67	5.35 (122%)	2.97
Mean	36.64	556.58	7.33	2.23
St-dev	5.04	174.79	1.01	0.70

Note: FW = fresh weight; *) Potential productivity was determined by rhizome fresh weight per plant (g plant^{-1}) x population (plant ha^{-1}) x 80%, percentage in brackets were compared with treatment Control as 100%; **) Actual productivity was determined by (10,000 m^2 (2 m^2)$^{-1}$ x fresh rhizome weight g plot^{-1} (2 m^2) x 80%.

Percentage of potential productivity shown in bracket (Table 18) have the tendency that rhizome yield from application of bio-fertilizer were relatively

higher than rhizome yield from the absence of bio-fertilizer. The actual productvity seems to be very different with potencial productvity because in this experimental field found some diseases, namely root-knot and bacterial wilt (Table 3, Fig. 2, and Fig. 3), that cause decreasing almost 50% number of plant per raised bed (Fig. 4B).

The weight of root-knot, ratio root-knot to total root (RK:R) and the broken rhizome tend to decrease by bio-fertilizer application (Table 19). The underlying mechanism might be related to the content of the bio-fertilizer. Bio-fertilizer applied in this experiment contains *Saccharomyces cerevisiae* and *Paenibacillus macerans*. *Saccharomyces cerevisiae* can produce chitinase and protease for destroying chitine layer of nematode eggs (Ahmad, 2007), while *Paenibacillus macerans* have capability to against *Ralstonia solanacearum* (Li *et al.*, 2010).

Table 19. The effect of bio-fertilizer application on root-knot disease in *K. parviflora*

Bio-fertilizer rate	Weight of root-knot (RK) (g plant^{-1})	Weight of root (R) (g)	Ratio RK:R (%)	Broken rhizome weight* (g plot^{-1})
100% ChemF+no BioF	7.04	26.36	26.70	14.77
50% ChemF+15L ha^{-1} BioF	7.10	32.39	21.93	16.83
50% Chem+22.5L ha^{-1} BioF	6.21	30.30	20.49	10.46
50% ChemF+30L ha^{-1} BioF	3.91	22.70	17.23	0

Note: R) Root consisted of fleshy root and adventitous roots, *) Broken rhizome which caused by bacterial wilt and nematode.

The rhizome dimension were classified as small, medium, and large rhizome based on fresh weight per clump (Table 20). As showed in Table 21, all treatment tend to produce small and medium rhizomes, while large rhizome were rarely formed.

Table 20. Classification of rhizome dimension

Dimension of rhizome	Rhizome grade (interval, mean)		
	Small	Medium	Large
Fresh Weight (g)	(18.71-19.61, 19.18)	(20.45-39, 28.55)	(41.14-63.19, 55.54)
Length (cm)	5.39	6.95	8.18
Width (cm)	3.93	4.71	6.00
Diameter (cm)	1.59	1.6	1.62

Note: Classification was based on rhizome fresh weight, small < 20 g, medium 20 g $\leq$ 40 g, and large > 40 g.

Table 21. The effect of bio-fertilizer rate on grade of rhizome

Treatment	Number of rhizome according to grade		
	Large	Medium	Small
	.....clump 2 m^{-1}.....		
100% ChemF+no BioF	5	4	17
50% ChemF+15L ha^{-1} BioF	5	8	8
50% Chem+22.5L ha^{-1} BioF	5	10	11
50% ChemF+30L ha^{-1} BioF	5	8	10

Table 22. Analysis of different fertilizing standard operasional procedure of *K. parviflora* per ha

Type of Cost	No. of unit	Unit	Price $unit^{-1}$	Total
.....100% chemical fertilizer.....				
*Income				
Yield	1,300	kg	18,000	23,400,000
*Input				
Bio-fertilizer	0	liter	0	0
Urea	300	kg	2,300	690,000
SP 36	250	kg	2,300	575,000
KCl	250	kg	12,000	3,000,000
Total cost				4,265,000
.....50% chemical fertilizer and 15 L ha^{-1} bio-fertilizer.....				
*Income				
Yield	3,060	kg	18,000	55,080,000
*Input				
Bio-fertilizer	15	liter	50,000	750,000
Urea	150	kg	2,300	345,000
SP 36	150	kg	2,300	345,000
KCl	125	kg	12,000	1,500,000
Total cost				2,940,000

Economically, the application of bio-fertilizer in *K. parviflora* cultivation could reduce the usage of chemical fertilizer up to 50% so that it can save the cost for chemical fertilizer. According to the above analysis, the comparison were only between treatment 100% chemical fertilizer to 50% chemical fertilizer and 15 L ha^{-1}. Total cost of the usage of 100% chemical fertilizer was IDR.4,265,000, while total cost of the usage 50% chemical fertilizer and 15 L ha^{-1} bio-fertilizer was

lower, IDR.2,940,000, saving IDR.1,325,000. In addition, rhizome yield of *K. parviflora* from the application of bio-fertilizer also relatively higher than yield of 100% chemical fertilizer. The usage of 100% chemical fertilizer resulted yield 1,300 kg and income IDR.23,400,000, while the usage 50% chemical fertilizer and 15 L ha^{-1} bio-fertilizer resulted higher yield and income, 3,100 kg and income IDR.55,080,000 (Table 22).

CONCLUSION AND SUGGESTION

Conclusion

The conclusion of this research is that the usage of bio-fertilizer could reduce the need of chemical fertilizer (i.e. urea, SP-36, and KCl) up to 50% without significant difference in rhizome yield. Application rate of 15 L ha^{-1} bio-fertilizer and 50% chemical fertilizer also could promote late-vegetative growth of *K. parviflora* better than other rate or without bio-fertilizer up to 29 WAP. From economic point of view, the usage of bio-fertilizer can save the cost for buying chemical fertilizer so that the benefit become higher. The result also imply that the usage of bio-fertilizer could suppress disease severity of root-knot caused by nematode in the field.

Suggestion

The suggestion can be given from this research is to cultivate *K. parviflora* by adding bio-fertilizer because it can reduce the usage of chemical fertilizer. But, the bio-fertilizer need to be applied at early vegetative growth up to the growth of rhizome. Diseases and pest control need to be concerned at early stage of infestation, because they can decrease the rhizome yield. Before it is recommended to massive production and application it is necessary to implementing and replicating these experiments in different region.

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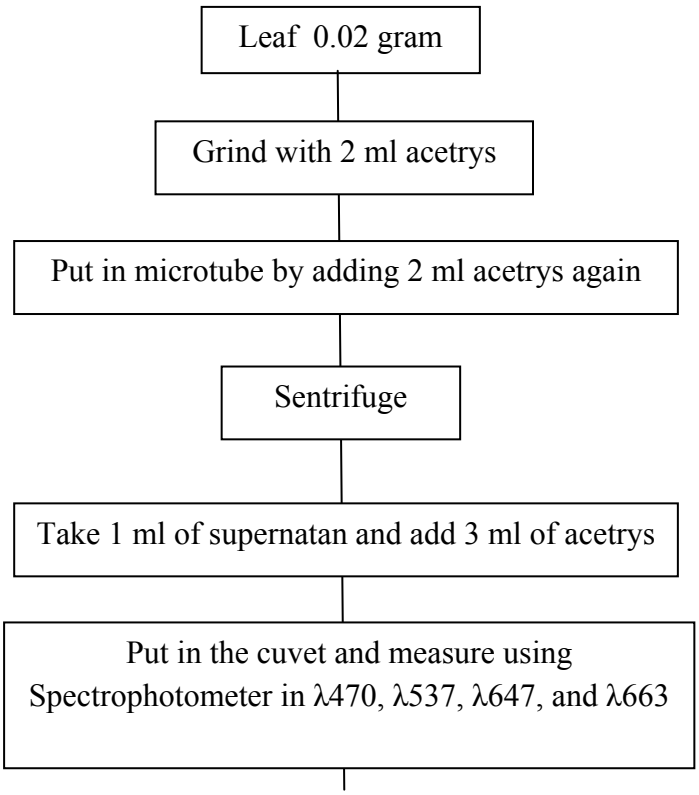
APPENDIX

Appendix 1. Method of Chlorophyll Content Analysis (Sims and Gamon, 2002)



$$\text{Chlorophyll a} = \frac{\{(0{,}01373 \times \lambda 663) - (0{,}00697 \times \lambda 537) - (0{,}003046 \times \lambda 647)\} \times 8}{\text{Leaf fresh weight}}$$

$$\text{Chlorophyll b} = \frac{\{(0{,}02405 \times \lambda 647) - (0{,}004305 \times \lambda 537) - (0{,}005507 \times \lambda 663)\} \times 8}{\text{Leaf fresh weight}}$$

$$\text{Anthocyanin} = \frac{\{(0{,}08173 \times \lambda 537) - (0{,}00697 \times \lambda 647) - (0{,}002228 \times \lambda 663)\} \times 8}{\text{Leaf fresh weight}}$$

$$\text{Carotenoids} = \frac{\left\{ \begin{array}{c} (\lambda 470 - (17{,}1 \times ((0{,}01373 \times \lambda 663) - (0{,}000897 \times \lambda 537) - (0{,}003046 \times \lambda 647)) + ((0{,}02405 \times \lambda 647) - \\ \underline{(0{,}004305 \times \lambda 537) - (0{,}005507 \times \lambda 663)})) - (9{,}479 \times ((0{,}08173 \times \lambda 537) - (0{,}00697 \times \lambda 647) - (0{,}002228 \times \lambda 663)))) \\ 119{,}26 \end{array} \right\} \times 6}{\text{Leaf fresh weight}}$$

Appendix 2. Mean of plant height per treatment

Bio-fertilizer rate	2/24	3/25	5/27	7/29	12/34	14/36	16/38	18/40	20/42	22/44
	……WAT/WAP……									
100% ChemF + no BioF	24.04	25.30	26.10	26.36	29.41	30.23	30.14	29.24	28.35	28.46
50% ChemF + 15L ha^{-1} BioF	27.55	28.44	29.18	29.53	29.99	30.76	29.99	28.86	29.13	28.52
50% ChemF + 22.5L ha^{-1} BioF	25.37	25.48	26.36	27.43	28.72	27.88	27.74	27.40	26.59	25.21
50% ChemF + 30L ha^{-1} BioF	24.85	24.29	25.30	25.66	26.94	26.74	26.35	26.05	24.89	26.44

Appendix 3. Mean of leaves number per treatment

Bio-fertilizer rate	2/24	3/25	5/27	7/29	12/34	14/36	16/38	18/40	20/42	22/44
	……WAT/WAP……									
100% ChemF + no BioF	15.76	18.56	20.44	21.41	24.13	26.00	25.50	23.79	19.83	19.02
50% ChemF + 15L ha^{-1} BioF	22.63	28.27	28.03	27.48	31.69	27.80	25.44	20.21	19.98	21.27
50% ChemF + 22.5L ha^{-1} BioF	17.13	20.00	21.23	25.28	28.10	25.69	26.14	26.12	25.89	21.48
50% ChemF + 30L ha^{-1} BioF	15.77	18.63	20.80	22.13	24.23	21.90	20.60	19.87	17.27	17.21

Appendix 4. Mean of leaf length per treatment

Bio-fertilizer rate	2/24	3/25	5/27	7/29	12/34	14/36	16/38	18/40	20/42	22/44
	……WAT/WAP……									
100% ChemF + no BioF	16.85	15.64	15.04	14.70	16.30	16.56	16.34	15.67	15.74	15.55
50% ChemF + 15L ha^{-1} BioF	19.17	17.18	16.79	16.57	15.59	16.60	15.72	17.58	15.60	15.39
50% ChemF + 22.5L ha^{-1} BioF	15.10	15.62	14.97	14.70	15.75	16.18	15.88	15.59	15.16	13.86
50% ChemF + 30L ha^{-1} BioF	17.70	15.71	15.56	15.46	14.28	15.28	15.00	14.24	14.22	13.59

Appendix 5. Mean of leaf width per treatment

Bio-fertilizer rate	2/24	3/25	5/27	7/29	12/34	14/36	16/38	18/40	20/42	22/44
	…… WAT/WAP……									
100% ChemF + no BioF	7.34	7.15	6.95	6.95	8.09	7.39	7.33	7.16	7.11	6.64
50% ChemF + 15L ha^{-1} BioF	8.17	8.06	7.73	7.64	7.73	7.54	7.25	8.18	6.06	5.60
50% ChemF + 22.5L ha^{-1} BioF	7.57	7.25	6.91	6.59	7.57	7.40	7.18	6.95	6.71	4.38
50% ChemF + 30L ha^{-1} BioF	8.10	7.84	7.58	7.36	7.41	7.21	6.74	6.47	5.85	4.87

Appendix 6. Mean of number of shoot per treatment

Bio-fertilizer rate	12/34	16/38	18/40	22/44	24/46
	……...WAT/WAP…….				
100% ChemF + no BioF	4.32	3.80	2.82	3.11	3.46
50% ChemF + 15L ha^{-1} BioF	4.43	3.99	3.13	2.73	4.47
50% ChemF + 22.5L ha^{-1} BioF	4.37	3.69	3.24	2.96	4.71
50% ChemF + 30L ha^{-1} BioF	3.77	3.40	3.70	3.12	4.06

Appendix 7. Mean of green color intensity index and leaf area per treatment

Bio-fertilizer rate	Green color (unit)			Leaf area (cm^2)		
	2/24	12/34	18/40	2/24	12/34	18/40
	…..... WAT/WAP….....					
100% ChemF + no BioF	89.43	98.18	85.83	20.35	19.62	17.80
50% ChemF + 15L ha^{-1} BioF	99.70	88.54	87.66	21.16	21.48	18.47
50% ChemF + 22.5L ha^{-1} BioF	77.85	89.88	80.22	19.08	20.59	19.71
50% ChemF + 30L ha^{-1} BioF	96.58	77.86	68.69	19.18	18.31	18.20

Appendix 8. Result of plant diseases analysis

In this experiment, diseases intensity were counted by two parameter, diseases incidence of bacterial wilt and diseases severity of root-knot. Diseases incidence of bacterial wilt were measured by formula

$$\text{Disease insidence} = \frac{n}{N} x100\%$$

$$\text{Disease insidence} = \frac{408}{838} x100\% = 48.68\%$$

n = the number of plant which showed the symptom bacterial wilt

N = population of observed plant

This is the observation of root-knot analysis that showed nematode infestation.

T	Block	RK : R	Score (v)	T	Block	RK : R	Score (v)
C	S + 6 HSL	30.14	2	D2	FS + 12 HSL	6.17	1
C	HS + 9 HSL	40.38	2	D2	FS + 12 HSL	20.32	1
C	FS + 12 HSL	7.02	1	D2	S + 6 HSL	17.82	1
C	S + 6 HSL	19.18	1	D2	FS + 12 HSL	35.05	2
C	FS + 12 HSL	16.85	1	D3	S + 6 HSL	44.96	2
D1	HS + 9 HSL	27.48	2	D3	HS + 9 HSL	9.99	1
D1	FS + 12 HSL	7.66	1	D3	FS + 12 HSL	5.07	1
D1	FS + 12 HSL	22.32	1	D3	FS + 12 HSL	8.36	1
D1	FS + 12 HSL	9.00	1	D3	FS + 12 HSL	13.23	1
D2	HS + 9 HSL	18.71	1				

While diseases intensity of root-knot were counted by this formula

$$\text{Diseases severity} = \frac{\Sigma(n \times v)}{N \times Z}$$

$$\text{Diseases severity} = \frac{\Sigma(n \times v)}{N \times Z} = \frac{120}{95 \times 4} x100\% = 31.57\%$$

n = The number of plant from each category of symptom

v = Score value of each category of symptom

N = The number of observed plants

Z = Score value of the highest category of symptom

According to Kusnanta (2005) research in rust disease patchouli plant (*Pogostemon cablin*) stated that diseases severity are determined 5 category, there are score 0 (no symptom, 0%), score 1 (mild symptom, 1-25%), score 2 (medium symptom, 26-50%), score 3 (high symptom, 51-75%), and score 4 (severe high symptom, >75%).